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The Role of Stress during *in Vivo* and *in Vitro* Plant Reproductive Development: Implications for Cropping Systems and Germplasm Enhancement in Canada

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ABSTRACT

High-yielding cereal and oilseed cultivars are integral components of modern cropping systems in Canada. Climate change occurring at both regional and global scales, along with increased frequency of extreme weather events, has resulted in greater emphasis upon yield stability or safety in local breeding programs. The reproductive development of crop and model plant species is particularly sensitive to environmental stress with undesirable reductions in seed yield linked to pollen sterility and ovule abortion along with defects in embryogenesis, storage product accumulation and seed maturation. In contrast to the detrimental role environmental stress plays in reducing harvestable yields, plants also employ controlled 'stress' programs at various checkpoints throughout the plant lifecycle. Modern *in vitro* tissue culture techniques which support breeding programs also employ stress as a means of reprogramming plant development. The following review covers recent molecular and physiological studies that have improved our understanding of the mechanism(s) through which both model and crop species cope with environmental or imposed stress during *in vitro* and *in vivo* reproductive development. Through approaches such as germplasm screening or genetic engineering plant biologists can utilize the information provided to enhance the stress tolerance of species of importance to the Canadian agriculture and forestry sectors.

Keywords: abscisic acid, androgenesis, embryogenesis, reproductive development, stress

CONTENTS

INTRODUCTION

In the last decade it has become apparent that human activities have contributed significantly to global climate change. For the Canadian agriculture sector rising mean temperatures, regional changes in annual precipitation and increased frequency of weather extremes (drought, heat, excess water, cold temperatures or frost) are specific challenges that producers, agronomists and plant breeders will encounter in coming years (Porter and Semenov 2005; Motha and Baier 2005; Schindler and Donahue 2006). Forecasted climate changes also present opportunities for introgession of early maturing warm-season crops (e.g. maize, soybean, dry beans) to regions previously unable to support such crops (Cutforth *et al*. 2007).

The aforementioned changes to Canadian cropping systems also occur within the context of worldwide population growth predicted to reach 9-10 billion by the year 2050 (Rothstein 2007). Issues of food scarcity are further heightened by competing demands from the biofuel sector as well as a disproportionate increase in production of primary grain and oilseeds to support dietary trends in developing countries (Naylor *et al*. 2005). In any given growing season Canadian cropping systems encounter resource limitations or stress conditions that prevent producers from attaining the true genetic yield potential of modern cultivars. In the age of rapid climate change, however, yield stability has emerged as a focus of modern breeding programs (Porter and Semenov 2005). The following review examines how *in vivo* and *in vitro* plant reproductive development is affected by environmental or imposed stress. Recent molecular and physiological studies conducted in both model systems and crop species have provided critical insights into aspects of carbohydrate transport and assimilation, phytohormone metabolism or signaling, heat shock protein (HSP) production and reactive oxygen species (ROS) metabolism which affect the success or failure of plant reproductive development in the face of environmental stress.

Although the following review explores fundamental molecular, physiological and developmental aspects of *in vivo* and *in vitro* plant reproduction, we emphasize areas where additional efforts may be focused or integrated into

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Invited Review

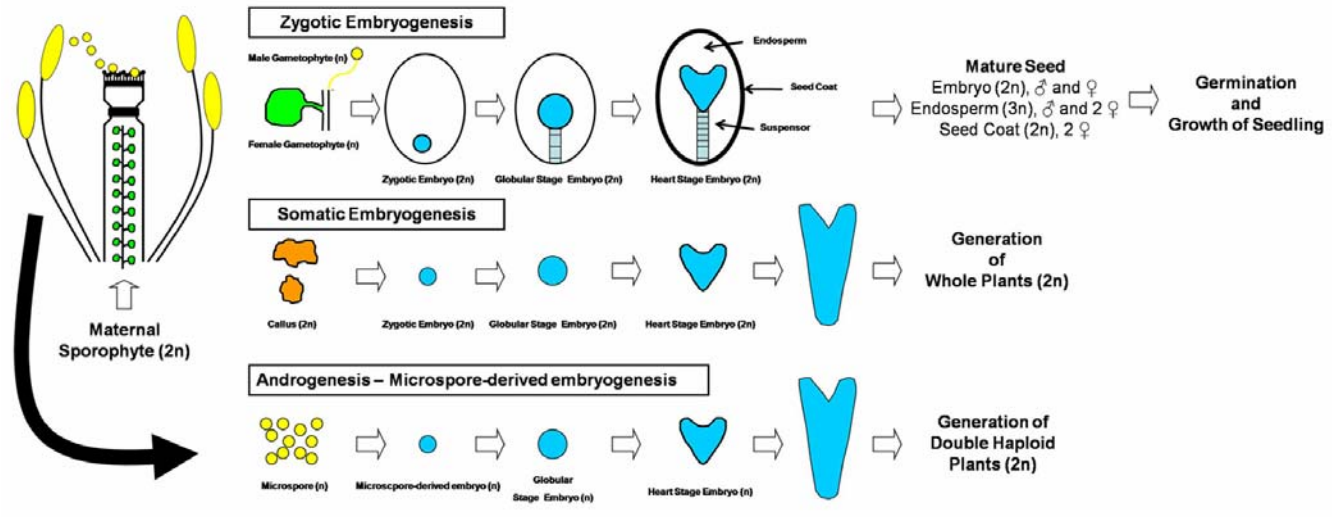

Fig. 1 Schematic representation of *in vivo* and *in vitro* plant reproductive development. The maternal sporophyte (2n) provides support and nourishment to developing haploid (n) male and female gametophytes. Pollination and double fertilization of the female gametophyte gives rise to the zygotic embryo (2n), triploid endosperm (3n) and diploid seed coat (2n). Stress can negatively affect *in vivo* reproductive development from early stages of male and female gamete development through to late stages of seed development and maturation. During somatic embryogenesis embryogenic tissue is often obtained in the presence of auxins and cytokins. In the case of white spruce, embryo development requires applications of abscisic acid and it is favoured by an imposed water stress generated by inclusions of osmoticum agents in the culture medium. Oxidative stress also enhances embryonic growth and quality (see text for details). During androgenesis haploid microspores are exposed to a stress treatment which redirects the gamethophytic pathway towards an embryogenic fate. Through applications of colchicine, haploid (n) microspore-derived embryos undergo chromosome doubling and this leads to the production of fertile double haploid plants. Heat and oxidative stresses play a key role during the development of *Brassica* microspore-derived embryos (see text for details). The figure was adapted from Zimmerman (1993; *Plant Cell* **5**, 1411-1423).

breeding programs to enhance the stress tolerance and yield stability of cropping systems. The reader is also directed to reviews covering related aspects of plant reproductive development (Saini and Westgate 2000; Liu *et al*. 2005; Barnabás *et al*. 2008; Hedhly *et al*. 2008; Donahue *et al*. 2009; Thakur *et al*. 2010).

MICROSPOROGENESIS, MICROGAMETOGENESIS AND POLLEN DEVELOPMENT

Male reproductive development in angiosperms commences with stamen primordia formation within floral organs and generation of a filament-supported anther housing specialized meiotic male cells (microspore mother cell = MMC) (Ma 2005). Maternal sporophytic tissues, including the epidermis, endothecium, middle layer and tapetum, support and nourish the MMCs. Interior to the tapetum MMCs undergo meiosis and subsequently dissociate from the tapetum wall forming the locule, an intercellular space. At this stage of development microsporogenesis is complete and microgametogenesis initiates with two rounds of meiosis to generate a tetrad of haploid microspores (Scott *et al*. 2004; Ma 2005) (**Fig. 1**). Microspores then undergo asymmetric mitotic cell division and differentiation to form pollen grain containing a vegetative cell and two sperm cells. With subsequent pollen coat formation, anther dehiscence and pollen release, viable pollen grains are capable of germinating following contact with compatible stigma. Following pollen tube germination and growth within the transmitting tract, sperm cells are delivered to the female gametophyte during double fertilization (Kandasamy *et al*. 1994; Berger *et al*. 2008; Crawford and Yanofsky 2008).

The capacity for environmental stress to reduce pollen fertility and subsequent seed sets has been known for some time (reviewed in Barnabás *et al*. 2008). However, recent physiological and molecular studies of drought or cold-induced pollen sterility in wheat (Dorian *et al*. 1996; Koonjul *et al*. 2005) or rice (Oliver *et al*. 2005, 2007), respectively, revealed commonalities in the underlying mechanism through which environmental stress triggers pollen stertility. The young microspore stage (tetrad to uninucleate) of pollen development was noted for being particularly vulnerable to applied stresses, with reduced invertase activity, impaired sucrose transport or loading, and elevated abscisic acid (ABA) contents serving as correlative markers of reduced pollen viability or abortion (Koonjul *et al*. 2005; Oliver *et al*. 2007).

At the young microspore stage of pollen development the tapetum functions at maximum capacity to support and nourish symplastically-isolated pollen by loading carbohydrates and proteins into the locules (Oliver *et al*. 2007). An apoplastic unloading pathway supports this process, with sucrose transporters exporting sucrose from phloem sieve cells and extracellular invertases hydrolysing transported sucrose to monomers of glucose or fructose (Roitsch and Gonzàlez 2004). In turn sink cells utilize high-affinity hexose transporters to enhance monomer uptake and create a localized concentration gradient, thus increasing the combined sink strength of actively growing tissues (Roitsch and Gonzàlez 2004).

Antisense repression and RNAi silencing of invertase genes in tobacco (*Nin88*) and tomato (LIN5), respectively, result in male sterility or severe defects in pollen viability (Goetz *et al*. 2001; Žanor *et al*. 2009). These independent studies provide direct evidence that pollen abortion is linked to impaired invertase activity and sucrose cleavage. In comparing pollen sterility between cold-resistant (*R31*) and cold-sensitive (*Doongra*) rice cultivars, Oliver *et al*. (2007) identified ABA as a regulatory signal that could phenocopy cold-induced pollen sterility and similarly modify invertase and monosaccharide transporter gene expression. Further probing of cultivar-dependent differences in pollen sterility between *R31* and *Doongra* revealed anthers of the former maintained lower endogenous levels of ABA following exposure to cold stress. Lower endogenous ABA levels in *R31* also correlated with the activity of genes involved in ABA metabolism (Oliver *et al*. 2007). Similar genotype-dependent alterations in ABA appear to underlie pollen sterility in reproductive structures of *Brassica napus*, with maximal ABA contents of GMS (genic male sterile) lines observed in mature stamens (Singh and Sawhey 1992; Shukla and Sawhey 1994). Correlative evidence of a role for ABA in pollen sterility was first described for wheat where ABA could phenocopy the reduced seed set and

altered pollen morphology of drought-stressed wheat plants (Morgan 1980). However, several lines of evidence, including localization of enzymes involved in ABA biosynthesis, reduced fertility of ABA-deficient mutants, and immunolocalization of ABA to distinct male gametophytic tissues, all lend support to a promotive role for basal ABA levels in facilitating male gametophyte development (Cheng *et al*. 2002;Tan *et al*. 2003; Peng *et al*. 2006).

Biosynthesis and signaling of the phytohomone methyl jasmonate has also been implicated in defective male gametophyte development which contributes to reduced seed sets, with a possible feedback mechanism proposed to operate between jasmonate and ABA signaling pathways (Cippolini 2007; Kim *et al*. 2007). In the case of rice plants, overexpression of jasmonic carboxyl methyltransferase (JMT) and exogenous methyl jasmonates treatments enhanced the ABA content of reproductive structures. In turn, ABA biosynthesis and signaling appears to serve as a prerequisite for jasmonate signalling (Adie *et al*. 2007). For plant biologists seeking to enhance the stress tolerance of reproductive processes and elucidate the physiological basis of natural variation for related traits, it may be advantageous to expand the complement of phytohormones assayed (e.g. ethylene, GA, auxin, brassinosteroids, etc). Developmental programs that underlie reproductive processes are particularly dynamic with respect to the recruitment of phytohormone biosynthesis and signaling.

Heat shock proteins (HSPs) also accumulate during pollen development with expression patterns appearing complex and sometimes weak in contrast to the heat shock response of vegetative tissues (Crone *et al*. 2001; Young *et al*. 2004; Volkov *et al*. 2005; Frank *et al*. 2009). From these observations pollen is proposed to carry a subset of HSPs induced by a developmental program and an additional subset responsive to heat stress (Volkov *et al*. 2005). It is also noteworthy that higher basal levels of HSP expression may underlie natural variation for thermotolerance amongst tomato cultivars (Frank *et al*. 2009). Similar profiling of HSPs has occurred for *Brassica napus*, although it has yet to be determined if natural variation in thermotolerance amongst *Brassica* cultivars can be linked to differential expression or activity of HSPs (Young *et al*. 2004).

Microspore maturation is also associated with activation of genes involved in scavenging reactive oxygen species (ROS) (Frank *et al*. 2009). However, in rice anthers undergoing programmed cell death (PCD) and pollen abortion in response to drought stress, hydrogen peroxide (H_2O_2) levels rise with a concomitant down-regulation of antioxidant transcripts and depletion of ATP pools (Nguyen *et al*. 2009). Therefore, additional work is required to determine whether genotypic variation in stress tolerance can be attributed to the capacity of male gametophytic tissues to support or repair oxidative damage.

TdT-mediated dUTP nick-end labelling (TUNEL) assays that detect fragmentation of genomic DNA indicate PCD is developmentally controlled during *in vivo* male gametophyte development (Wang *et al*. 1999; Vizcay-Barrena and Wilson 2006). However, similar TUNEL assays indicate endogenous and exogenous ABA suppresses DNA fragmentation of barley microspores, leading to enhanced microspore viability during androgenesis. From these observations enhanced synthesis or impaired catabolism of ABA is proposed to function towards improving microspore viability and suppressing cell death programs which ultimately lead to pollen abortion (Wang *et al*. 1999).

To date carbohydrate metabolism and transport, phytohormone synthesis and signaling, antioxidant status and programmed cell death have emerged as mechanism(s) which underlie the success or failure of male gametophyte development following imposed drought, heat or cold temperature stresses. In translating this knowledge to improved stress tolerance of modern cultivars, the approach taken by Oliver *et al.* (2007) is particularly telling as to the utility of classical breeding and natural variation to identify trait of interests at the field level. Gene expression and physiological studies with available genomic tools can subsequently be applied to provide a mechanistic model of the genes, proteins and metabolites that correlate with stress-tolerant or stress-sensitive phenotypes. Even when genomic tools are comparatively less developed for a crop of interest and complex gene by environment interactions must be considered, the natural variation that exists for stress-related phenotypes (e.g. thermotolerance of floral bud abortion) in model species can serve as a starting point towards revealing the genetic basis of a differential physiological response (Warner and Erwin 2005). In turn, this knowledge can be applied towards species of regional interest (e.g. *Brassica*, *Linum*) which are profiled for stress sensitivity of particular tissues or stages of reproductive development (Angadi *et al*. 2000; Cross *et al*. 2003; Young *et al*. 2004).

POLLINATION AND PRE-ZYGOTIC DEVELOPMENT

Provided male gametophyte development yields pollen of adequate quantity and quality to target unfertilized ovules, pollen germination and vigor, stigma receptivity, pollen tube growth and recognition by female gametophytic tissues are subsequent stages of development at which environmental stress may limit seed sets (Shivanna *et al*. 1991; Crawford and Yanofsky 2008; reviewed in Hedhly *et al*. 2009). Transcriptional profiling of stigmas, pistils and ovaries indicate rapid and dynamic changes in hormone metabolism/signaling and cell-to-cell communication accompany pollination events (Tung *et al*. 2005; Li *et al*. 2007; Vriezen *et al*. 2007). Stigmatic cells further present an over-representation of stress-related transcripts suggesting that genetic programs for pollen recognition overlap with stress responses to some extent (Lan *et al*. 2005). The synchrony of anther dehiscence with stigma receptivity during reproductive development has also recently been considered in the context of climate change (Hedhly *et al*. 2009). In general increasing temperatures accelerate male and female gametophyte development, with low temperature prolonging stigmatic receptivity and longevity of ovules.

For several crop species (e.g. soybean, canola, cotton) *in vitro* pollen-based assays have been applied towards isolating thermotolerant genotypes (Liu *et al*. 2006; Salem *et al*. 2007; Singh *et al*. 2008). The polyamine titre of mature pollen grains was also reported to correlate with rates of pollen germination, depth of pollen tube growth, and enhanced pollen tube growth during high temperature stress (Chibi *et al*. 1994; Song *et al*. 1999). From these results it is readily apparent that pollen-based assays have proven their utility in isolating genotypes with thermotolerance during reproductive development. However, some authors have advocated the use of alternative assays or screens to isolate genotypes with thermotolerant vegetative tissues (Salem *et al*. 2007).

At the molecular level NADPH oxidase-mediated reactive oxygen species (ROS) production, in addition to reactive nitrogen species (RNS), have been implicated as signal molecules in pollen-stigma interactions, pollen tube guidance and ovule targeting (McInnis *et al*. 2006; Potocky *et al*. 2007; Prado *et al*. 2008). Genetic lesions which impair ROS/RNS generation during pollen tube growth and ovule targeting are similar to those which operate during ABA-mediated stomatal closure (Kwak *et al*. 2003; Bright *et al*. 2006). Mutant or transgenic plants altered in gibberellin (GA) metabolism or signaling also highlight GA as a regulator of pollen tube growth (Singh *et al*. 2002; Swain *et al*. 2004; Hu *et al*. 2008). GA-deficiency or overdose are similarly linked with seed sets and localized promotion of fruit development (Vivian-Smith *et al*. 1999; Cox and Swain 2006; Hu *et al*. 2008). The localization of ABA-receptor complexes and signaling intermediates to pollen grains, growing pollen tubes and developing seeds/siliques (Brocard *et al*. 2002; Ma *et al*. 2009) also invites speculation that mechanisms of ABA-GA antagonism which have been well characterized for seed development or dormancy might

also be operative during intermittent aspects of sexual plant reproduction (Seo *et al*. 2006; Piskurewicz *et al*. 2008; Toh *et al*. 2008). The versatile nature of ROS/RNS as intermediates in hormonal signaling, plant development and programmed cell death also warrants consideration when aspects of reproductive development are superimposed by conditions of environmental stress.

FEMALE GAMETOPHYTE DEVELOPMENT

In both eudicot and monocot species, ovules are specialized structures that develop from the ovary wall and consist of three basic structures: the nucellus, one or two integuments and the funiculus (Reisner and Fischer 1993). Within ovules the nucellus gives rise to a megasporocyte and subsequently generates the embryo sac, or female gametophyte, during the process of megagametogenesis. At the completion of megagametogenesis species with a *Polygonum*-type embryo sac consist of one (1) egg cell, one (1) central cell, three (3) antipodal cells and two (2) synergids. Following double fertilization of the egg cell and central cell, endosperm (central cell) nuclei proliferate and facilitate transport of nutrients and signal molecules from the maternal sporophyte (Berger *et al*. 2008).

In *Arabidopsis* embryo growth initially proceeds at a very slow rate immediately following fertilization, with rapid embryo development (globular → heart → torpedo) occurring after endosperm replication has ceased. At the torpedo stage the embryo undergoes rapid cell expansion and crushes the endosperm. Although differences in seed development and anatomy are apparent amongst *Arabidopsis*, legumes and cereals (Chaudhury *et al*. 2001; Weber *et al*. 2005; Sabelli and Larkins 2009) the basic steps in endosperm development appeared to be conserved between eudicots and monocots, with cell divisions and differentiation in the developing embryo invariably delayed relative to endosperm.

Cell biology studies examining the movement of GFP variants in *Arabidopsis thaliana* have determined the outer integument serves as a symplastic extension of the funicular phloem (Stadler *et al*. 2005). However, the adjacent cells of outer and inner integument are symplastically isolated from one another with little or no plasmodesmatal connections. Developing embryos are also symplastically isolated from the endosperm with the suspensor serving as the conduit for delivery of assimilates, hormones or other signaling molecules (Stadler *et al*. 2005). Therefore apoplastic barriers reside between outer and inner integuments, between the inner integument and endosperm and between the endosperm and embryo. Similar approaches using phloem-mobile dyes indicate ovule abortion in maize is accompanied by decreased delivery of carbohydrates through a post-phloem unloading pathway (Makela *et al*. 2005).

In the context of environmental stress, there exist numerous stages at which the female gametophyte or developing zygote can abort or senesce. For *Arabidopsis*, a number of publications have described in detail the anatomical changes which accompanying stress-induced abortion of *Arabidopsis* ovules (Sun *et al*. 2004, 2005; Hauser *et al*. 2006). TUNEL assays identified maternal nourishing tissues (integuments, chalaza) as sites of DNA fragmentation during stress-induced abortion of female gametophytes (Sun *et al*. 2004). In comparison, developing embryo and endosperm tissue did not undergo significant DNA fragmentation. Changes in mitochondrial membrane potential, callose deposition, ROS generation and downregulation of ROS detoxification genes were also identified as correlative markers of ovules which had committed to abort (Sun *et al*. 2005; Hauser *et al*. 2006).

Although the seed anatomy of *Arabidopsis* differs from that of cereals or legumes, numerous studies reinforce the concept that pre- and post-fertilization abortion of reproductive sinks is tied to decreased non-structural carbohydrate delivery (Patrick and Offler 2001; Makela *et al*. 2005). Furthermore, in line with the putative role proposed for invertase(s) in mediating pollen viability and abortion, the abortion of female reproductive tissues has also been associated with changes in invertase gene expression and activity (Anderson *et al*. 2002; reviewed in Boyer and McLaughlin 2007).

Two recent and independent studies conducted in tomato have highlighted the role of invertase inhibitors as putative intermediaries coordinating ABA and invertase activities during male and female reproductive development (Jin *et al*. 2009; Zanor *et al*. 2009). Proteinaceous invertase inhibitors have previously garnered attention as regulators of sucrose transport or metabolism although their exact physiological role has remained somewhat enigmatic (Huang *et al*. 2007). At the transcriptional level ABA and polyethylene glycol (PEG) treatments are reported either induce or repress inhibitor transcripts (Rausch and Greiner 2004; Koh *et al*. 2008) and reversible protein phosphorylation serves as an intermediary in ABA-mediated regulation of invertase activity (Pan *et al*. 2005; Huang *et al*. 2007). More detailed localization and chromatography studies with a kernel-specific maize invertase inhibitor protein (INVINH1) revealed inhibitor transcripts were transiently localized to a defined region around the embryo. The authors proposed that transient and contained INVINH1 expression immediately following fertilization could coordinate hexose supply and contrasting developmental programs of the embryo and endosperm (Bate *et al*. 2004). An INVINH1-homolog identified in tomato was found to physically interact *in vivo* with LIN5, a well-characterized cell wall invertase (Jin *et al*. 2009). Silencing INVINH1 increased LIN5 invertase activity via release of post-translational inhibition, with increases in seed weight and size reported. In contrast, overexpressing INVINH1 or a tobacco cell wall invertase inhibitor dramatically reduced seed sets or resulted in complete infertility (Jin *et al*. 2009). *In situ* mRNA localization studies revealed LIN5 and INVINH1 transcripts co-localize to the placental vasculature. In addition to post-translational regulation, earlier studies revealed the LIN5 promoter is responsive to multiple hormones (GA, ABA, auxin) with LIN5 transcripts localizing to male and female gametophytic tissues (Godt and Roitsch 1997; Proels *et al*. 2003). RNAi silencing of LIN5 also induces aberrant floral and fruit phenotypes that are concomitant with altered hormone biosynthesis and signaling (Zanor *et al*. 2009).

ZYGOTIC EMBRYOGENESIS AND SEED DEVELOPMENT

Zygotic embryogenesis is an inherently complex developmental process due in large part to the enclosure and physical interaction of three genetically discrete tissues (zygotic embryo, triploid endosperm and maternal integuments) within the developing seed (Chaudhury *et al*. 2001; Berger *et al*. 2006). Studies of seed development are further complicated by the reciprocal interaction of individual seeds and whole fruits with maternal sporophytic tissues (Vivian-Smith and Koltunow 1999; Dorcey *et al*. 2009).

During early stages of seed development, maternal tissues hold a dominant position in determining the metabolic state and sugar composition of the embryo and endosperm. Throughout early and late stages of seed development carbohydrates such as glucose and sucrose serve as both nutrients and signal molecules (Wobus and Weber 1999; Borisjuk *et al*. 2004). By altering the temporal and spatial distribution of these particular metabolites cellular events and developmental programs including cell division and enlargement, transfer cell formation, embryo and endosperm differentiation, endoreduplication, photosynthetic activity and storage product accumulation, can be coordinated. As developing seeds progress from fertilization through to storage product accumulation a sequential transition from maternal to filial control occurs and can be correlated with defined transcriptional changes mediated in part by altered concentrations of metabolites and phytohormones (ABA or

GA) alongside modifications to the cellular energy status (ATP) (Weber *et al*. 2005).

As with pollen development certain HSPs are expressed during embryogenesis and seed maturation with distinct regulatory pathways triggering expression of individual family members in response to heat stress or developmental cues (Wehmeyer *et al*. 1996: Kotak *et al*. 2007). ABI3, a transcriptional activator involved in ABA signaling, has recently been implicated in a transcriptional cascade regulating HSP expression during seed development (Kotak *et al*. 2007). However, it is also intriguing that *abi3* mutants were isolated in a genetic screen to detect high temperature resistant germination mutants (Tamura *et al*. 2006). ABA and reactive oxygen species (ROS) have been further implicated embryogenesis and seed development by affecting plastid differentiation as well as seed after-ripening (Kim *et al*. 2009; Müller *et al*. 2009).

Studies of ABA-deficient mutants have clearly supported a role for maternal ABA in promoting seed development (Koornneef *et al*. 1989; Phillips *et al*. 1997; Frey *et al*. 2004). However, ample evidence also indicates that under environmental stress ABA produced in maternal sporophytic tissues (e.g. leaves) can be transported to reproductive tissues where it accumulates within developing seeds and subsequently impairs endosperm cell division and endoreduplication (Mambell and Setter 1998; Setter and Flannigan 2001). In severe cases ABA accumulation triggers seed abortion and reduces seed set (Ober and Setter 1990, 1992; Setter *et al*. 2001). How direct a role ABA plays in triggering ovule/ovary abortion has been questioned (Asch *et al*. 2001; Andersen *et al*. 2002). Nonetheless, under environmental stress sporophytic tissues such as the placenta which directly support endosperm and zygote development appear to demonstrate enhanced rates of ABA catabolism (Wang *et al*. 2002). These observations parallel those observed during pollen development under stress, with steady-state maintenance of endogenous ABA levels correlated with enhanced viability of developing seeds (Wang *et al*. 2002). Seed development, in turn, produces signals that stimulate fruit expansion, with endosperm-derived GA strongly implicated in this process (Hu *et al*. 2008; Dorcey *et al*. 2009). In the future, the dynamic interplay between of GA and ABA pathways warrants further investigation from both a developmental and agronomic standpoint. For example, whether environmental stress affects the flux of GA emanating from developing seeds and whether or not GA impinges upon ABA metabolism and signaling in surrounding maternal tissues.

Genetic approaches have further reinforced that an extensive reciprocal dialog exists amongst parental and zygotic components of seed development (Dilkes *et al*. 2002; Johnston *et al*. 2007). For example, in *cdc2a* mutants of *Arabidopsis* preferential delivery of a single sperm cell to the egg cell triggers endosperm proliferation, suggesting a positive signal emanates from the fertilized egg cell to trigger proliferation of the central cell (Nowack *et al*. 2006). Bayer *et al* (2009) also recently discovered that embryonic patterning in *Arabidopsis thaliana* is mediated by male gamete-specific transcripts of the SHORT SUSPENSOR (SSP) gene. SSP transcripts produced in a parent-of-origin manner are subsequently delivered to the egg cell or central cell at which point SSP transcripts are translated to initiate asymmetric cell divisions in the developing zygote (Bayer *et al*. 2009). Maternal parent-of-origin effects have also been described for several genes (e.g. MEDEA (MEA), FERTILIZATION INDEPENDENT ENDOSPERM (FIS), MULTI-COPY SUPPRESOR of IRA1 (MIS1)) in *Arabidopsis* which involve the differential or strict mono-allelic expression of maternal transcripts over paternal transcripts (reviewed in Chaudhury *et al*. 2001). For these particular genes parent-of-origin effects are derived through genomic imprinting which adds specific epigenetic marks to maternal (MEA^m) or paternal (MEA^p) alleles. In turn, these epigenetic marks result in repressive chromatin structure which selectively silences the maternal or paternal alleles. Thus far, PHERES (PHE1) has emerged as the only imprinted gene where higher levels of expression are observed from the paternal allele over that of the maternal allele. Several additional genes expressed predominantly in male or female gametophytic tissues (FERONIA, LORELEI, ANXUR1 and ANXUR2) of *Arabidopsis* interact to coordinate pollen tube targeting, rupture and delivery of sperm cells to the egg cell and synergid (Berger 2009).

While almost all of the genes listed above have been isolated in studies which sought to eludicate fundamental aspects of plant reproductive development, there is tremendous potential to explore these signaling pathways in the context of environmental stress and reproductive failure. The evolution of genomic imprinting is often hypothesized to arise from maternal and paternal conflicts over resource allocation when multiple offspring require nourishment (Köhler and Makarevich 2006). Differential dosage hypothesis also exist to explain the consequences of paternal or maternal excess in driving the growth rate and final size of developing seeds (Dilkes and Comai 2004). From an agronomic standpoint reciprocal crosses (control/stressed pollen × control/stressed pistil) represent an important starting point towards unravelling the molecular intricacies of maternal (megagametophyte) or paternal (microgametophytic) control of reproductive outcome during abiotic stress.

STRESS DURING *IN VITRO* MORPHOGENESIS

Cellular totipotency, proposed by Haberlandt (1902) is the inherent ability of plant cells to form organs or embryos in culture. This concept was first demonstrated by Levine (1947) who was able to obtain embryo-like structures from carrot cells. These observations were further elaborated by Steward *et al.* (1958) and Reinert (1958) who are accredited with an accurate description of the process. Today *in vitro* plant propagation represents an effective way to clone desirable species and capture genetic gain over a relatively short period of time. Over the past few years methods of propagation have prospered thanks to the optimization of culture conditions and the refinement of improved media and addenda. These efforts have been pivotal in increasing the number of species able to propagate in culture (see Thorpe and Stasolla 2001).

In vitro embryogenesis can be achieved through two distinct processes. The first is somatic embryogenesis, which is the capacity of somatic or non-sexual plant cells to form embryos by a process very similar to that occurring for zygotic (or seed) embryogenesis. This process leads to the formation of bipolar structures with a defined root-shoot axis and a closed vascular system. The morphology of somatic embryos is close to that of their zygotic counterparts and stages equivalent to globular, heart, and torpedo can be observed in culture. Embryos can also be generated *in vitro* through gametophytic embryogenesis, the formation of haploid embryos from cells of the male or female gametophyte. This process is very suitable for the capture of endogenous genetic variation through recovery of diploid homozygous plants following artificial chromosome doubling with applications of colchicine (Yao *et al*. 1997). As a general rule the most efficient and routinely used system for gametophytic embryogenesis is androgenesis in which immature pollen grains, referred to as microspores, can be used as starting material for the production of microspore-derived embryos (MDEs) (**Fig. 1**). Initial work was conducted using anther cultures but in recent years isolated microspore cultures have emerged as a more suitable alternative as they offer the advantage that the sporophytic anther wall does not interfere with the development of the embryos. Androgenesis has been demonstrated in a large variety of species and several protocols have been optimized over the years. In recent years this process has been fully integrated in many breeding programs aimed at recovering and propagating desirable lines.

The imposition of stress in the form of osmoticum, heat, or oxidative stress has often been used in both somatic and

microspore-derived embryogenesis during either the induction phase or to stimulate embryonic development. In this review two species will be used as model systems to describe the role of stress during morphogenesis: *Brassica napus* (canola) for androgenesis and *Picea glauca* (white spruce) for somatic embryogenesis. Both species have profound ecological and economical significance to Canada. According to the Canola Council of Canada (2010) canola-related industry in Canada can be estimated as $13.8 billion. Canola is not the main source of oil for foreign exchange by the export but also has diverse domestic usage for food, animal feed, chemical feedstocks and fuel. Like canola, white spruce holds a key position in Canada where it is used in the forestry sector serving as the most economically important tree for paper and pulp production (NRCAN 2007).

STRESS DURING *BRASSICA* MICROSPORE-DERIVED EMBRYOGENESIS

Embryogenesis from immature pollen requires a re-direction of the gametophytic pathway into the sporophytic pathway. Early worked show that several forms of stress imposed to donor plants, such as short days, nitrogen starvation, as well as cold treatments, and/or chemical treatments of the excised inflorescences, increased the number of microspores initiating the embryogenic pathways (see Sunderland 1982). As reviewed by Touraev *et al.* (2001) a better understanding on the role played by stress during the initiation of microspore-derived embryogenesis was only reached with the realization that spores isolated from non stressed donor plans, were also responsive to stress treatments. In the absence of plant growth regulators, a heat shock of 8 h at 32°C is sufficient to induce isolated *Brassica napus* microspores to initiate the embryogenic process and the resulting embryos are able to regenerate into viable plants (Custers 1994). The duration of the heat stress was further increased to 2 d (Ferrie and Keller 1995) to increase the number of *Brassica* microspore-derived embryos produced. The embryos produced with these stress treatments are generally similar to that of their zygotic counterparts although they do not display the same initial cell division and differentiation patterns observed in vivo (Tykarska 1976, 1979), as well as lacking a well developed suspensor (Yeung *et al*. 1996). Zygotic-like microspore-derived embryos were obtained by Joosen *et al*. (2007) who used a milder heat stress (25°C), together with a narrower range of microspore developmental stages. Such embryos display an elongated suspensor and the embryo proper originates from the distal cell of the suspensor and undergoes the same ordered cell divisions occurring during early zygotic embryogeny. The ability to "reproduce" embryos with zygotic-like features in culture has made this system a model for molecular studies which besides elucidating the genetic network responsible for the induction process (Joosen *et al.* 2007) have shed light on the role of the suspensor during embryogenesis (Supena *et al*. 2008). These advantages have prompted many labs to use *in vitro* embryogenesis as an alternative system for studying zygotic embryogenesis.

The role of heat shock for the redirection of the gametophytic pathway into the sporophytic pathway is not restricted to *Brassica* but also documented in other species, including tobacco where it triggers embryogenesis in isolated unicellular microspore (Touraev *et al*. 1996), and in wheat when combined with temporary starvation (Kyo and Harada 1986).

Characteristic stress-induced morphological changes have been observed during microspore-derived embryogenesis in a variety of species including *Brassica*, rice, wheat, and tobacco. Following the heat treatments the microspores swell and their nucleus moves in a central position. This is followed by the formation of cytoplasmic strands connecting the perinuclear cytoplasm with the subcortical cytoplasm through the vacuole (reviewed by Touraev *et al*. 2007). These structural cellular alterations, which have been associated to a state of highly mitotic activity, are needed for the reactivation of the cell cycle and the initiation of the embryogenic process. During normal pollen development microspores undergo an asymmetric cell division. The generative cell becomes arrested in the G2 phase of the cell cycle after a round of DNA replication, whereas the vegetative cell arrests in G1 phase (Zarsky *et al*. 1992). This is in contrast to "stressed" microspores in which the vegetative nucleus proceeds from the G1 to the S phase and this is followed by a reduction in the levels of protein, starch and RNA and a cessation of ribosome production. According to Zarsky *et al*. (1992) such events are needed to commit the cell to replicate its DNA once the stress is removed and to responds to the inductive signals required for the embryogenic commitment.

Changes in gene expression as a result of stress have also been documented during the initiation of the embryogenic process. Independent studies have shown that in both *Brassica* and tobacco the inductive starvation of microspores promotes the transcription of several mRNAs (Garrido *et al*. 1993; Cordewener *et al*. 1994). In some instances some of these transcripts remain transcriptionally inactive, as also observed in other developmental systems (i.e. animal oocytes), in which cells remain in a quiescent state prior fertilization, whereas in other they are translated into proteins. As suggested by Touraev *et al*. (1997) these differences might be attributed to the fact that in some species, including *Brassica*, cell arrest is not a requirement for embryogenic induction. As a result, several genes induced at the onset of *Brassica* microspore-derived embryogenesis belong to different classes of heat shock proteins (Cordewener *et al*. 1994), making it difficult, if not impossible, to discriminate between stress-induced and embryogenic-induced transcription. These problems have recently been overcome by the use of refined isolation methods coupled with advanced molecular and proteomic techniques. Joosen *et al* (2007) identified 135 robust markers for the transition of *Brassica* microspore-derived embryogenesis and several of these markers were co-regulated at the gene and protein expression levels. These findings are exciting as they open new avenues for unravelling the genetic network induced by stress and involved in embryo initiation.

Oxidative stress, can be defined as the "imbalance in the pro- versus anti-oxidant ratio in the cells" (Cassells and Curry 2001) resulting in high levels of ROS such as hydrogen peroxide, superoxide dismutase, and peroxyl and alkoxyl radicals. These molecules are harmful for the cellular environment as they cause several aberrations including DNA mutations, hyper- and hypomethylation, changes in chromosome numbers, and structural damages to membranes and other cellular components. Oxidative stress is inevitable in culture as tissue manipulation, sterilization, and the subsequent subculturing cause wounding, which are a known cause of oxidative burst (Yahraus *et al*. 1995). Chemicals used in sterilization solutions are also elicitors of oxidative stress, as well as sub-optimal light and temperature requirements (reviewed by Cassells and Curry 2001). Both plant and animal cells respond to oxidative stress through changes in metabolism of glutathione, a small peptide which is synthesized enzymatically and not as a result of mRNA translation (Alsher 1989). This molecule can exist in a reduced form (GSH) or as an oxidized form (GSSG), with a disulfide bridge linking two molecules (reviewed by Yeung *et al.* 2005). Since glutathione acts as a major redox buffer within plant cells it is not surprising that cells maintain a balance between GSH and GSSG through several feedback mechanisms regulating the production of both forms (May *et al*. 1998). Besides functioning as an antioxidant the glutathione redox pair has been implicated in several developmental processes. Sanchez-Fernandez *et al.* (1997) documented an increase in root growth in mature Arabidopsis plants after GSH treatments. Highest levels of this metabolite were found in the epidermal and cortical initials showing increased cell division. Additional information comes from the study by Vernoux *et al*. (2000) who documented a requirement of GSH for cell division initi-

ation and maintenance of the *Arabidopsis* root meristem. The *ROOT MERISTEMLESS 1* (RML1) gene is needed for cell proliferation within the root tip (Cheng *et al*. 1995) and encodes γ-glutamylcysteine synthetase, which is a precursor of the de-novo synthesis of GSH. In the same study it was found that plants homozygous for the *rml1* mutation show a reduction in root growth and have lower levels of cellular GSH. It is interesting to note that the shoot apical meristem of RML1 mutant plants remained functional, producing vegetative structures. Additional evidence on the participation of glutathione on root development comes from maize seedlings (Jiang *et al*. 2003). Quiescent center integrity of the root meristem is proposed to be regulated by the redox state of the ascorbate and glutathione pool (Jiang *et al*. 2003).

Cell proliferation and elongation are two key components of plant growth that govern morphogenesis. It appears that there is a strong correlation between glutathione levels and mitotic activity. Elevated concentrations of GSH, which result in a high GSH/GSSG ratio, are often associated with rapidly growing tissues including meristematic regions and newly expanding leaves of pea (Bielawski and Joy 1986). However, at present, it is not clear as to how glutathione regulates mitotic activity and cell cycle events (Potters *et al*. 2002).

The realization that alteration in the glutathione redox state (ratio between GSH and GSSG) could affect morphogenesis *in vitro* was first ascribed to Marre and Arrigoni (1957). Using a variety of systems they concluded that the auxin-induced changes is growth rate were the results of endogenous fluctuations of the gluthatione redox state, with an oxidized environment (low GSH/GSSG ratio) inhibiting growth and a reduced environment (high GSH/GSSG ratio) promoting cell proliferation. The concept that oxidative stress, mediated by the glutathione redox state, could be use to manipulate *in vitro* embryogenesis was proved in several species including *Brassica* microspore-derived embryogenesis. The imposition of a glutathione oxidized environment (low GSH/GSSG ratio) through applications of buthionine sulfoximine (BSO), a specific GSH biosynthetic inhibitor) enhanced significantly the quality of *Brassica* MDEs and their ability to convert into viable and vigorous plants (Belmonte *et al*. 2006). The authors showed that applications of 0.1 mM BSO increased the percentage of 25 day old MDE able to convert, i.e. form a fully developed shoot and root system at germination, from 26% to more than 60%. Histological analyses revealed that compared to their control counterparts BSO-treated embryos form well organized meristems and accumulate storage products in a similar pattern to that observed in seed embryos. The imposed oxidized stress was beneficial especially for the differentiation of shoot apical meristems which displayed a zygotic-like appearance having a dome-shaped architecture and a well defined zonation pattern, i.e. visible tunica-corpus boundaries. This was in contrast to control MDEs which had meritems disrupted by the presence of intercellular spaces and exhibited signed of cellular differentiation (Belmonte *et al*. 2006). The BSO beneficial effects were also ascribed to increased levels of abscisic acid (ABA), a plant growth regulator induced by stress in plant tissue. Besides increasing the endogenous ABA level, applications of BSO induced structural changes that were reproducible by exogenous ABA treatments (Belmonte *et al*. 2006). These findings are very promising as they can be applied to improve propagation of recalcitrant Brassica lines produced by breeding programs.

In order to investigate the molecular basis of the glutathione oxidized environment during micorpore-derived embryogenesis, Stasolla *et al*. (2008) monitored the profile of 15k transcripts between control MDEs and MDEs treated with BSO. BSO applications induced major changes in transcript accumulation patterns, especially during the late phases of embryogenesis. Several key enzymes involved in ascorbate metabolism, which resulted in major fluctuations in cellular ascorbate levels were affected by BSO. These changes were related to morphological characteristics of the embryos and their post-embryonic performance. BSO applications also activated many genes controlling meristem formation and function, including *ZWILLE*, *SHOOT-MERISTEMLESS*, and *ARGONAUTE 1*. The authors speculated that increased expression of these genes may contribute to the improved structural quality of the shoot poles observed in the presence of BSO (Stasolla *et al*. 2008). Changes in transcript profiles were more abundant especially during the second half of development. Compared to their control counterparts, middle and late-stage BSO-treated embryos also showed increased accumulation of transcripts associated with the maturation phase of zygotic embryo development, including genes encoding ABA-responsive proteins and storage- and late-abundant (LEA) proteins. From these studies it was concluded that the BSO-induced oxidized glutathione redox state allows cultured embryos to reach both morphological and physiological maturity, which in turn guarantees successful regeneration and enhanced post-embryonic growth (Stasolla *et al*. 2008).

Based on the above, it is evident that stress, in the form of heat shock or oxidative stress contributes to the proper development of *Brassica* MDEs. The heat shock treatment appears to be critical during the induction phase for redirecting the gametophitic pathway towards the embryogenic fate, whereas the oxidative stress might be required for the subsequent stages of development characterized by histodifferentiation and tissue patterning.

Overall the understanding of how stress regulates morphogenesis, and in particular embryogenesis, is key for the Canadian breeding industry, where haploid plants derived from microspores are often used to propagate elite genotypes to be introduced in agronomic programs.

STRESS DURING WHITE SPRUCE SOMATIC EMBRYOGENESIS

White spruce somatic embryogenesis is a multi-step process which includes (1) induction and maintenance of the embryogenic tissue, and (2) embryo development and maturation. As reviewed by Stasolla *et al*. (2003) embryogenic tissue is initiated from immature zygotic embryos and maintained either on solid or liquid medium in the presence of levels of auxin and cytokinins which need to be optimized for each cell line. During both processes, i.e. induction and maintenance, tissue is constantly under stress, due to the excision of the zygotic embryos and the sub-culturing of the embryogenic tissue on a regular basis. However, the role played by stress during these phases remains elusive. It is during the developmental phase, in which filamentous embryos increase in size that the role of stress, especially water and oxidative stresses, has been investigated in details. Embryo development is achieved through the removal of auxin and cytokinin, addition of abscisic acid (ABA), and applications of increased osmolarity in the medium. When all these conditions are applied fully developed embryos are produced (see Stasolla *et al*. 2003). The role of ABA during somatic embryogenesis is mainly related to promotion of storage product accumulation and the prevention of precocious germination. The requirement of ABA during embryo development has also been described in vivo. Studies conducted by Kong *et al*. (1997) revealed that ABA levels in developing white spruce seeds are low during the initial phases of development, reaching a maximum point during the middle phase of maturation, prior to declining again at later stages when seeds dry.

Production of endogenous ABA is tightly linked to the "stress level" of the tissue as synthesis of this plant growth regulator generally increases under sub-optimal conditions.

Several studies have shown that ABA increases both *in vivo* and *in vitro* as a result of osmoticum. Yeung and Brown (1982) documented that the liquid endosperm of flowering plants has more negative osmotic values than those of the embryo. This negative osmotic potential is important for a slow development of the zygotic embryo,

necessary for regulating the pattern of histodifferentiation (Yeung 1995). Restriction of water uptake in vitro can be achieved through the use of either permeating osmoticum agents, such as mannitol, sucrose, and inorganic acids, or by non-permeating osmotica, such as polyethyleneglycol (PEG) and dextran (reviewed by Stasolla *et al*. 2003). Utilization of PEG as an osmotic agent has been extensively reported during white spruce somatic embryogenesis. Imposition of water stress through applications of PEG (5-10%) resulted in a three-fold increase in the maturation frequency of white spruce somatic embryos and produced somatic embryos with superior appearance to those matured with ABA alone. These embryos also exhibited an increased tolerance to drying, a nine fold increase in the amount of storage lipid triacylglycerol with a fatty acid composition resembling that of zygotic embryos (Attree and Fowke 1993), and a three-fold higher protein content than somatic embryos matured in the absence of PEG (Misra *et al*. 1993). The physiological "maturity" observed in PEG-treated white spruce somatic embryos was also confirmed by the appearance of some of the major matrix and crystalloid polypeptides which were absent from somatic embryos matured in ABA and low osmoticum, but present in mature seed embryos (Misra *et al*. 1993). In the same study it was observed that the effects of ABA and osmoticum in the regulation of somatic embryo development, especially protein synthesis, appear to be additive. Crystalloid protein synthesis is first initiated by ABA alone, but sequentially regulated by PEG at a translational or post-translational level (Misra *et al*. 1993).

Spruce somatic embryos which are morphologically mature cannot successfully germinate and convert into viable plantlets unless they undergo a desiccation period, either through applications of non-plasmolysing osmoticum or by a partial drying treatment, which lowers water content of about 20% (reviewed by Stasolla *et al*. 2003). To date there are only very few studies dealing with the physiological and biochemical events occurring during the desiccation period. Changes in hormone level are most likely to occur as the embryos dry. Compared to mature embryos, partially dried white spruce somatic embryos had a lower endogenous level of ABA, as well as a reduced sensitivity to ABA (Kong and Yeung 1995). Kong (1994) suggested that these alterations in ABA metabolism are beneficial for both germination and conversion processes. The poor post-embryonic growth of white spruce somatic embryos following applications of ABA in the germination medium supports this notion. Similarly to ABA, ethylene production was significantly reduced in partially dried embryos (Kong 1994).

Besides altering hormone level, the partial drying treatment may be required for increasing the ability of white spruce somatic embryos to generate purine and pyrimidine nucleotides in preparation for the resumption of growth at germination. Both uridine and adenine salvage enzymes, uridine kinase (UK) and adenine phosphoribosyltransferase (APRT) were found to increase in partially dried embryos (Stasolla *et al*. 2001a). High activities of these enzymes will be required for the extensive salvage of both purine and pyrimidine precursors occurring at the inception of germination, before the restoration of the *de novo* nucleotide biosynthesis (Stasolla *et al*. 2001b, 2001c). The critical role played by the salvage pathway at germination is also demonstrated by the observations that inclusions of ascorbic acid in the germination medium increased the conversion frequency of white spruce somatic embryos (Stasolla and Yeung 1999), as well as the ability of the embryos to salvage purine nucleosides for nucleotide production (Stasolla *et al*. 2001b).

As observed during microspore-derived embryogenesis, the imposition of an oxidative stress, effected by applications of oxidized glutathione GSSG promotes proper histodifferentiation and tissue patterning in developing somatic embryos. Several studies (Belmonte *et al*. 2005; Belmonte and Stasolla 2007) revealed that white spruce somatic embryos cultured in a low GSH/GSSG ratio have better organized shoot apical meristems, which are able to reactivate promptly at the onset of germination. The apical pole of these embryos also exhibit a larger expression pattern of HBK1, a gene preferentially expressed in the meristematic cells and involved in proper meristem formation (Belmonte *et al*. 2005).

These studies are relevant to the Canadian biotechnology/forestry sector dealing with propagation of coniferous and tree species. Over the past few years heavy deforestation practices in the Canadian boreal forest are putting pressure on the private sector to provide suitable genotypes to be integrated in reforestation practices. This holds true especially for spruce, which is the preferred species utilized for paper and pulp production. Therefore the understanding on how stress can be applied in culture to enhance embryonic yield and quality is crucial for accelerating the propagation of elite genotypes.

CONCLUSIONS AND PERSPECTIVES

In the following review we have discussed molecular and physiological aspects of plant reproductive development, highlighting the dual role which stress plays in reducing reproductive output but also serving as a tool to reprogram plant growth and development. For the Canadian agriculture sector maintaining or improving the productivity of cropping systems is of paramount importance and is most frequently assessed according to the quantity and quality of seed derived from reproductive processes. Abiotic stress and variable climactic conditions are both hallmarks of Canadian cropping systems: past, present and future. However, in response to forecasted climate changes and rising input costs, an increased focus has been placed upon yield stability or safety in modern breeding programs.

In an evolutionary context, reproductive processes are associated with continuation of the genetic line and may actually be at odds with agronomic goals. The maternal sporophyte exerts a dominant influence in allocating resources to reproductive processes and developing progeny. Abiotic stress or resource limitation might also trigger the maternal sporophyte to undergo developmental arrest or invest in metabolic processes which improve stress tolerance, thus delaying reproductive processes or reducing reproductive output.

While the following review has focused on molecular and cellular aspects of stress in specific reproductive structures it is important to emphasize that yield is determined by a hierarchy of yield components which operate at the tissue, organ, whole plant and population level. For example, a specific communication system termed global proliferative arrest (GPA) operates between developing seeds/fruits and proliferating meristems, with putative systemic signals (e.g. nutrients, hormones) emanating from seeds or fruits to coordinate meristem arrest (Hensel *et al*. 1994). At present the molecular mode of action underlying GPA is poorly understood but the implications of this process towards the reproductive success of crop species is readily apparent. Competing theories exist to explain seed and fruit abortion in plants (Ganeshaiah and Shaanker 1994) but it is agreed that intrinsic mechanisms are under genetic and environmental control and function to regulate whole plant reproductive output (fecundity) in a non-random manner (Lee and Bazzaz 1986; Cox and Swain). In the context of modern agriculture and forestry it is also imperative to consider the extent to which the maternal genotype and maternal environmental interact to influence the phenotype of the progeny. For instance the maternal environment (e.g. photoperiod, temperature) in which seeds are produced can subsequently affect seed quality, seed germination, biomass accumulation and stress tolerance of progenies (Blönder *et al*. 2007; Boyd *et al*. 2007; Donahue *et al*. 2009). While the following review has focused largely on molecular studies of reproductive tissues and associated physiological or biochemical processes, it also critical to emphasize the caveats of extrapolating laboratory and growth chamber studies to

enhanced crop productivity or yield stability in extreme environments. Crop growth, development and yield are affected by environmental stress in both linear and non-linear ways depending upon severity of the imposed stress, developmental stage of the crop, and defined thresholds of growth assigned to individual species and cultivars (Porter and Semenov 2005). Moreover, in the field crop plants can be exposed to a combination of stress factors, which reinforces the importance of studying genotype × environment interactions across different management practices.

REFERENCES

Adie BAT, Perez-Perez J, Perez-Perez MM, Godoy M, Sanchez-Serrano JJ, Schmelz EA, Solano R (2007) ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defenses in *Arabidopsis*. *Plant Cell* **19**, 1665-1681

Alscher RG (1989) Biosynthesis and antioxidant function of glutathione in plants. *Physiologia Plantarum* **77**, 457-464

Anderson MN, Asch F, Wu Y, Jensen CR, Naested H, Mogensen VO, Koch KE (2002) Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. *Plant Physiology* **130**, 591-604

Angadi SV, Cutforth HW, Miller PR, McConkey BG, Entz MH, Brandt SA, Volkmar KM (2000) Response of three *Brassica* species to high temperature stress during reproductive growth. *Canadian Journal of Plant Sciences* **80**, 693-701

Asch F, Andersen MN, Jensen CR, Mogensen VO (2001) Ovary abscisic acid concentration does not induce kernel abortion in field-grown maize subjected to drought. *European Journal of Agronomy* **15**, 119-129

Attree SM, Fowke LC (1993) Embryogeny of gymnosperms: Advances in synthetic seed technology of conifers. *Plant Cell, Tissue and Organ Culture* **35**, 1-35

Barnabás B, Jäger K, Fehér A (2007) The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell and Environment* **31**, 11-38

Bate NJ, Niu X, Wang Y, Reimann KS, Helentjaris TG (2004) An invertase inhibitor from maize localizes to the embryo surrounding region during early kernel development. *Plant Physiology* **134**, 246-254

Bayer M, Nawy T, Giglione C, Galli M, Meinnel T, Lukowitz W (2009) Paternal control of embryonic patterning in *Arabidopsis thaliana. Science* **323**, 1485-1488

Belmonte M, Donald J, Reid MD, Yeung EC, Stasolla C (2005) Alterations of glutathione metabolism improve meristem integrity and embryo quality of white spruce somatic embryos. *Journal of Experimental Botany* **56**, 2355-2364

Belmonte M, Ambrose SJ, Ross ARS, Abrams SR, Stasolla C (2006) Improved development of microspore derived embryo cultures of *Brassica napus* cv Topaz following changes in glutathione metabolism. *Physiologia Plantarum* **127**, 690-700

Belmonte M, Stasolla C (2007) Applications of DL-buthionine-[*S*,*R*]-sulfoximine deplete cellular glutathione and improve white spruce (*Picea glauca*) somatic embryo development. *Plant Cell Reports* **26**, 517-523

Berger F, Grini PE, Schnittger A (2006) Endosperm: an integrator of seed growth and development. *Current Opinion in Plant Biology* **9**, 664-670

Berger F, Hamamura Y, Ingouff M, Higashiyami T (2008) Double fertilization – caught in the act. *Trends in Plant Science* **13**, 437-443

Berger F (2009) Reproductive biology: receptor-like kinases orchestrate love songs in plants. *Current Biology* **19**, R647-649

Bielawski W, Joy KW (1986) Reduced and oxidised glutathione and glutathione-reductase activity in tissues of *Pisum sativum. Planta* **169**, 267-272

Blönder C, Goebel C, Feussner I, Gatz C, Polle A (2007) Warm and cold parental reproductive environments affect seed properties, fitness, and cold responsiveness in *Arabidopsis thaliana* progenies. *Plant, Cell and Environment* **30**, 165-175

Borisjuk L, Rolletschek R, Radchuk R, Weschke W, Wobus U, Weber H (2004) Seed development and differentiation: A role for metabolic regulation. *Plant Biology* **6**, 375-386

Boyd EW, Dorn LA, Weinig C, Schmitt J (2007) Maternal effects and germination timing mediate the expression of winter and spring annual life histories in *Arabidopsis thaliana*. *International Journal of Plant Sciences* **168**, 205-214

Boyer JS, McLaughlin JE (2007) Functional reversion to identify controlling genes in multigenic responses: Analysis of floral abortion. *Journal of Experimental Botany* **l58**, 267-277

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H_2O_2. *Plant Journal* **45**, 113-122

Brocard IM, Lynch TJ, Finkelstein RR (2002) Regulation and role of the *Arabidopsis abscisic acid-insensitive 5* gene in abscisic acid, sugar, and stress response. *Plant Physiology* **129**, 1533-1543

Canola Council of Canada (2010) Available online: http://www.canolacouncil.org

Cassells AC, Curry RF (2001) Oxidative stress and physiological, epigenetic variability in plant tissue culture: Implications for micropropagators and genetic engineers. *Plant Cell, Tissue and Organ Culture* **64**, 145-157

Chaudhury AM, Koltunow A, Payne T, Luo M, Tucker MR, Dennis ES, Peacock WJ (2001) Control of early seed development. *Annual Review of Cell Developmental Biology* **17**, 677-699

Cheng JC, Seeley KA, Sung ZR (1995) RML1 and RML2, *Arabidopsis* genes required for cell proliferation at the root tip. *Plant Physiology* **107**, 365-376

Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiba T, Sheen J (2002) A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. *Plant Cell* **14**, 2723-2743

Chibi F, Matilla AJ, Angosto T, Garrido D (1994) Changes in polyamine synthesis during anther development and pollen germination in tobacco (*Nicotiana tabacum*). *Physiologia Plantarum* **92**, 61-68

Cipollini D (2007) Consequences of the overproduction of methyl jasmonate on seed production, tolerance to defoliation and competitive effect and response of *Arabidopsis thaliana. Physiologia Plantarum* **173**, 146-153

Corderwener JHG, Hause G, Corgen E, Busink R, Hause B, Dons HJM, van Lammeren AAM, van Lookeren Campagne MM, Pechan P (1994) Changes in synthesis and localization of members of the 70-kDa class of heat-shock proteins accompany the induction of embryogenesis in *Brassica napus* L. microspores. *Planta* **195**, 50-56

Cox CM, Swain SM (2006) Localised and non-localised promotion of fruit development by seeds in *Arabidopsis*. *Functional Plant Biology* **33**, 1-8

Crawford BC, Yanofsky MF (2008) The formation and function of the female reproductive tract in flowering plants. *Current Biology* **18**, R972-R97

Cross RH, McKay AB, McHughen AG, Bonham-Smith PC (2003) Heat-stress effects on reproduction and seed set in *Linum usitatissimum* L. (flax). *Plant Cell Environment* **26**, 1013-1020

Custers JBM (1994) Temperature controls both gametophytic and sporophytic development in microspore cultures of *Brassica napus*. *Plant Cell Reports* **13**, 267-271

Cutforth HW, McGinn SM, McPhee KE, Miller PR (2007) Adaptation of pulse crops to the changing climate of the northern great plains. *Agronomy Journal* **99**, 1684-1699

Dilkes BP, Dante RA, Coelho C, Larkins BA (2002) Genetic analysis of endoreduplication in *Zea mays* endosperm: Evidence of sporophytic and zygotic maternal control. *Genetics* **160**, 1163-1177

Dilkes BP, Comai L (2004) A differential dosage hypothesis for parental effects in seed development. *Plant Cell* **16**, 3174-3180

Donahue KD (2009) Completing the cycle: Maternal effects as the missing link in plant life histories. *The Philosophical Transactions of the Royal Society B* **364**, 1059-1074

Dorcey E, Urbez C, Bláquez MA, Carbonell J, Perez-Amador MA (2009) Fertilization-dependent auxin response in ovules triggers fruit development through the modulation of gibberellin metabolism in *Arabidopsis*. *Plant Journal* **58**, 318-332

Dorion S, Lalonde S, Saini HS (1996) Induction of male sterility in wheat by meiotic-stage water deficit is preceded by a decline in invertase activity and changes in carbohydrate metabolism in anthers. *Plant Physiology* **111**, 137-145

Fan J, Hill L, Crooks C, Doerner P, Lamb C (2009) Abscisic acid has a key role in modulating diverse plant-pathogen interactions. *Plant Physiology* **150**, 1750-1761

Ferrie AMR, Keller WA (1995) Microspore culture for haploid plant production. In: Gambors OL, Phillips GC (Eds) *Plant Cell, Tissue and Organ Culture: Fundamental Methods*, Springer, Berlin, Germany, pp 155-164

Frank G, Pressman, Ophir R, Althan L, Shaked R, Freedman M, Shen S, Firon N (2009) Transcriptional profiling of maturing (*Solanum lycopersicon* L.) microspores reveals the involvement of heat shock proteins, ROS scavengers, hormones, and sugars in the heat stress response. *Journal of Experimental Botany* **60**, 3891-3908

Frey A, Godin B, Bonnet M, Sotta B, Marion-Poll A (2004) Maternal synthesis of abscisic acid controls seed development and yield in *Nicotiana plumbaginifolia*. *Planta* **218**, 958-964

Ganeshaiah KN, Shaanker RU (1994) Seed and fruit abortion as a process of self organization among developing sinks. *Physiologia Plantarum* **91**, 81-89

Haberlandt G (1902) Culturvesuche mit isolierten Pflanzenzellen. *Sitzungsberg. Kais. Aked. Wiss. Wien, Mat-Natuirwiss. Kl., Abt.* **111**, 69-92

Hauser BA, Sun K, Oppenheimer DG, Sage TL (2006) Changes in mitochondrial membrane potential and accumulation of reactive oxygen species precede ultrastructural changes during ovule abortion. *Planta* **223**, 492-499

Hedhly A, Hormaza JI, Herrero M (2009) Global warming and sexual plant reproduction. *Trends in Plant Science* **14**, 30-36

Hensel LL, Nelson MA, Richmond TA, Bleecker AB (1994) The fate of inflorescence meristems is controlled by developing fruits in *Arabidopsis*. *Plant Physiology* **106**, 863-876

Hu J, Mitchum MG, Barnaby N, Ayele BT, Ogawa M, Nam E, Lai W-C, Hanada A, Alonso JM, Ecker JR, Swain SM, Yamaguchi S, Kamiya Y, Sun T (2008) Potential sites of bioactive gibberellin production during reproductive growth in *Arabidopsis*. *Plant Cell* **20**, 320-336

Huang L-F, Bocock PN, Davis JM, Koch KE (2007) Regulation of invertase: a 'suite' of transcriptional and post-transcriptional mechanisms. *Functional Plant Biology* **34**, 499-507

Jin Y, Ni D-A, Ruan Y-L (2009) Posttranslational elevation of cell wall inver-

tase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. *Plant Cell* **21**, 2072-2089

Jiang K, Meng YL, Feldman L (2003) Quiescent center formation in maize roots is associated with an auxin-regulated oxidizing environment. *Development* **130**, 1429-1438

Johnston AJ, Meier P, Gheyselinck J, Wuest SEJ, Federer M, Schlagenhauf E, Becker JD, Grössniklaus U (2007) Genetic subtraction profiling identifies genes essential for *Arabidopsis* reproduction and reveals interaction between the female gametophyte and the maternal sporophyte. *Genome Biology* **8**, R204

Joosen R, Cordewener J, Supena EDJ, Vorst O, Lammers M, Maliepaard C, Zeilmaker T, Miki B, America T, Custers J, Boutilier K (2007) Combined transcriptome and proteome analysis identifies pathways and markers associated with the establishment of rapeseed microspore-derived embryo development. *Plant Physiology* **144**, 1-18

Kandasamy MK, Nasrallah JB, Nasrallah ME (1994) Pollen-pistil interactions and developmental regulation of pollen tube growth. *Development* **120**, 3405-3418

Kim EH, Kim YS, Park S-H, Koo YJ, Choi YD, Chung Y-Y, Lee I-J, Kim J-K (2009) Methyl jasmonate reduces grain yield by mediating stress signals to alter spike development in rice. *Plant Physiology* **149**, 1751-1760

Kim C, Lee KP, Baruah A, Nater M, Göbel C, Feussner I, Apel K (2009) $^1O_2$–mediated retrograde signalling during late embryogenesis predetermines plastid differentiation in seedlings by recruiting abscisic acid. *Proceedings of the National Academy of Sciences USA* **106**, 9920-9924

Koonjul PK, Minhas JS, Nunes C, Sheoran IS, Saini HS (2005) Selective transcriptional down-regulation of anther invertases precedes the failure of pollen development in water- stressed wheat. *Planta* **56**, 179-190

Kong L, Attree SM, Fowke LC (1997) Changes in endogenous hormone levels in developing seeds, zygotic embryos, and megagametophyte in *Picea glauca* Voss. *Physiologia Plantarum* **101**, 23-30

Koh E-J, Lee SJ, Hong S-W, Lee HS, Lee H (2008) The ABA effect on the accumulation of an invertase inhibitor transcript that is driven by the CAMV35S promoter in Arabidopsis. *Plant Cell* **20**, 1676-1687

Köhler C, Makarevich G (2006) Epigenetic mechanisms governing seed development in plants. *EMBO Reports* **7**, 1223-1227

Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karsssen (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. *Theoretical and Applied Genetics* **61**, 385-393

Kong L (1994) Factors affecting white spruce somatic embryogenesis and embryo conversion. PhD dissertation, University of Calgary, Calgary

Kong L, Yeung EC (1995) Effects of silver nitrate and polyethylene glycol on white spruce (*Picea glauca*) somatic embryo development: enhancing cotyledonary embryo formation and endogenous ABA content. *Physiologia Plantarum* **93**, 298-304

Kotak S, Vierling E, Bäumlein H, von Koskull-Döring P (2007) A novel transcriptional cascade regulating expression of heat stress proteins during seed development of *Arabidopsis*. *Plant Cell* **19**, 182-195

Kwak JM, Mori IC, Pei Z-M, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. *EMBO Journal* **22**, 2623-2633

Kyo M, Harada H (1986) Control of developmental pathway of tobacco pollen *in vitro*. *Planta* **168**, 427-432

Lan L, Li M, Lai Y, Xu W, Kong Z, Ying K, Han B, Xue Y (2005) Microarray analysis reveals similarities and variations in genetic programs controlling pollination/fertilization and stress responses in rice (*Oryza sativa* L.). *Plant Molecular Biology* **59**, 151-164

Lee TD, Bazzaz FA (1986) Maternal regulation of fecundity: non-random ovule abortion in *Cassia fasciculate* Michx. *Oecologia* **68**, 459-465

Levine M (1947) Differentiation of carrot root tissue grown in culture. *Bulletin Torrey of the Botany Club,* **74**, 321-323

Li M, Xu W, Yang W, Kong Z, Xue Y (2007) Genome-wide gene expression profiling reveals conserved and novel molecular functions of the stigma in rice. *Plant Physiology* **144**, 1797-1812

Liu F, Jensen CR, Andersen MN (2005) A review of drought adaptation in crop plants: Changes in vegetative and reproductive physiology induced by ABA-based chemical signals. *Australian Journal of Agricultural Research* **56**, 1245-1252

Liu Z, Yuan Y-L, Liu S-Q, Yu X-N, Rao L-Q (2006) Screening for high-temperature tolerant cotton cultivars by testing *in vitro* pollen germination, pollen tube growth and boll retention. *Journal of Integrative Plant Biology* **48**, 706-714

Ma H (2005) Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. *Annual Review in Plant Biology* **56**, 393-434

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **324**, 1064-1067

Mäkelä P, McLaughlin JE, Boyer JS (2005) Imaging and quantifying carbohydrate transport to the developing ovaries of maize. *Annals of Botany* **96**, 939-949

Mambell S, Setter TL (1998) Inhibition of maize endosperm cell division and endoreduplication by exogenously applied abscisic acid. *Physiologia Plantarum* **104**, 266-272

Marre E, Arrigoni O (1957) Metabolic reactions to auxin: The effects of auxin on glutathione and the effects of glutathione on growth of isolated plant parts. *Physiologia Plantarum* **10**, 289-301

May M, Vernoux T, Leaver C, Van Montagu M, Inzé D (1998) Glutathione homeostasis in plants: Implications for environmental sensing and plant development. *Journal of Experimental Botany* **49**, 649-667

McInnis SM, Desikan R, Hancock JT, Hiscock SJ (2006) Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signaling crosstalk? *New Phytologist* **172**, 221-228

Misra S, Attree SM, Leal I, Fowke LC (1993) Effects of abscisic acid, osmoricum and desiccation on synthesis of storage proteins during the development of white spruce somatic embryos. *Annals of Botany* **71**, 11-22

Morgan JM (1980) Possible role of abscisic acid in reducing seed set in water-stressed wheat plants. *Nature* **285**, 655-657

Motha RP, Baier W (2005) Impacts of present and future climate change and climate variability on agriculture in the temperate regions: North America. *Climate Change* **70**, 137-164

Müller K, Carstens AC, Linkies A, Torres MA, Leubner-Metzger G (2009) The NADPH-oxidase *AtrohB* plays a role in *Arabidopsis* seed after ripening. *New Phytologist* **184**, 885-897

Naylor R, Steinfeld H, Falcon W, Galloways J, Smil V, Bradford E, Alder J, Mooney H (2005) Losing the links between livestock and land. *Science* **310**, 1021-1024

Nguyen GN, Hailstones DL, Wilkes M, Sutton BG (2009) Drought-induced oxidative conditions in rice anthers leading to a programmed cell death and pollen abortion. *Journal of Agronomy and Crop Science* **195**, 157-164

Nowack MK, Grini PE, Jakoby MJ, Lafos M, Koncz C, Schnittger A (2006) A positive signal from the fertilization of the egg cell sets off endosperm proliferation in angiosperm embryogenesis. *Nature Genetics* **38**, 63-67

NRCAN (National Research Council of Canada) (2007) Available online: http://canadaforests.nrcan.gc.ca/rpt#forests

Ober ES, Setter TL, Madison JT, Thompson JF, Shapiro PS (1991) Influence of water deficit on maize endosperm development. Enzyme activities and RNA transcripts of starch and zein synthesis, abscisic acid, and cell division. *Plant Physiology* **97**, 154-164

Ober ES, Setter TL (1992) Water deficit induces abscisic acid accumulation in endosperm of maize viviparous mutants. *Plant Physiology* **98**, 353-356

Oliver SN, Van Dongen J, Alfred SC, Mamun EA, Zhao X, Saini HS, Fernandes SF, Blanchard CL, Sutton BG, Geigenberger P, Dennis ES, Dolferus R (2005) Cold-induced repression of the rice anther-specific cell wall invertase gene OSINV4 is correlated with sucrose accumulation and pollen sterility. *Plant, Cell and Environment* **28**, 1534-1551

Oliver SN, Dennis ES, Dolferus R (2007) ABA regulates apoplastic sugar transport and is a potential signal for cold-induced pollen sterility in rice. *Plant Cell Physiology* **48**, 1319-1330

Pan QH, Li MJ, Peng CC, Zhang N, Zou X, Zou KQ, Wang XL, Yu XC, Wang XF, Xhang DP (2005) Abscisic acid activates acid invertases in developing grape berry. *Physiologia Plantarum* **125**, 157-170

Patrick JW, Offler CE (2001) Compartmentation of transport and transfer events in development seeds. *Journal of Experimental Botany* **52**, 551-564

Peng Y-B, Cheng Z, Wang D-H, Gong H-Q, Xu Z-H, Bai S-N (2006) Preferential localization of abscisic acid in primordial and nursing cells of reproductive organs of Arabidopsis and cucumber. *New Phytologist* **170**, 459-466

Phillips J, Artsaenko O, Fiedler U, Horstmann C, Mock HP, Muntz K, Conrad U (1997) Seed-specific immunomodulation of abscisic acid activity induces a developmental switch. *EMBO Journal* **16**, 4489-4496

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L (2008) The gibberellic acid signaling repressor RGL2 inhibits *Arabidopsis* seed germination by stimulating abscisic acid synthesis and ABI5. *Plant Cell* **20**, 2729-2745

Porter JR, Semenov MA (2005) Crop responses to climactic variation. *The Philosophical Transactions of the Royal Society B* **360**, 2021-2035

Potocky M, Jones MA, Bezvoda R, Smirnoff N, Zarsky V (2007) Reactive oxygen species produced by NADPH oxidase are involved in pollen tube growth. *New Phytologist* **174**, 742-751

Potters G, De Gara L, Asard H, Horemans N 2002) Ascorbate and glutathione: Guardians of the cell cycle, partners in crime? *Plant Physiology and Biochemistry* **40**, 537-548

Prado AM, Colaco R, Moreno N, Silva AC, Feijó JA (2008) Targeting of pollen tubes to ovules is dependent on nitric oxide (NO) signaling. *Molecular Plant* **4**, 703-714

Proels RK, Hause B, Berger S, Roitsch T (2003) Novel mode of hormone induction of tandem tomato invertase genes in floral tissues. *Plant Molecular Biology* **52**, 191-201

Rausch T, Greiner S (2004) Plant protein inhibitors of invertases. *Biochimica et Biophysica Acta* **1696**, 253-261

Reinert J (1958) morphogenese und ihre kontrolle an gewebekulturen aus carotten. *Naturwissenschaften* **45**, 344-345

Reisner L, Fischer RL (1993) The ovule and the embryo sac. *Plant Cell* **5**, 1291-1301

Roitsch T, González M-C (2004) Function and regulation of plant invertases: Sweet sensations. *Trends in Plant Science* **9**, 606-613

Rothstein SJ (2007) Returning to our roots: Making plant biology research relevant to future challenges in agriculture. *Plant Cell* **19**, 2695-2699

Sabelli PA, Larkins BA (2009) The development of endosperm in grasses. *Plant Physiology* **149**, 14-26

Saini HS, Westgate ME (2000) Reproductive development in grain crops during drought. *Advances in Agronomy* **68**, 59-96

Salem MA, Kakani VG, Koti S, Reddy KR (2007) Pollen-based screening of soybean genotypes for high temperatures. *Crop Sciences* **47**, 219-231

Sanchez-Fernandez R, Fricker M, Corben LB, White NS, Sheard N, Leaver C, Van Montagu M, Inzé D, May M (1997) Cell proliferation and air tip growth in the *Arabidopsis* roots are under mechanistically different forms of redox control. *Proceeding of the National Academy of Sciences USA* **94**, 2745-2750

Schindler DW, Donahue WF (2006) An impending water crisis in Canada's western prairie provinces. *Proceedings of the National Academy of Sciences USA* **103**, 7210-7216

Scott RJ, Spielman M, Dickinson HG (2004) Stamen structure and function. *Plant Cell* **16**, S46-S60

Seo M, Hanada A, Kuwahara A, Endo A, Okamoto M, Yamauchi Y, North H, Marion-Poll A, Sun TP, Koshiba T, Kamiya Y, Yamaguchi S, Nambara E (2006) Regulation of hormone metabolism in *Arabidopsis* seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellins metabolism. *Plant Journal* **48**, 354-366

Setter TL, Flannigan BA (2001) Water deficit inhibits cell division and expression of transcripts involved in cell proliferation and endoreduplication in maize endosperm. *Journal of Experimental Botany* **52**, 1401-1408

Setter TL, Flannigan BA, Melkonian J (2001) Loss of kernel set due to water deficit and shade in maize: Carbohydrate supplies, abscisic acid, and cytokinins. *Crop Science* **41**, 1530-1540

Shivanna KR, Linskens HF, Cresti M (1991) Response of tobacco pollen to high humidity and heat stress: Viability and germinability *in vitro* and *in vivo*. *Sexual Plant Reproduction* **4**, 104-109

Shukla A, Sawhey VK (1994) Abscisic acid: One of the factors affecting male sterility in *Brassica napus*. *Physiologia Plantarum* **91**, 522-528

Singh S, Sawhey VK (1992) Endogenous hormones in seeds, germination behaviour and early seedling characteristics in a normal and ogura cytoplasmic sterile line of rapeseed (*Brassica napus*). *Journal of Experimental Botany* **44**, 1497-1505

Singh DP, Jermakow AM, Swain SM (2002) Gibberellins are required for seed development and pollen tube growth in *Arabidopsis*. *Plant Cell* **14**, 3133-3147

Singh SK, Kakani VG, Brand D, Baldwin B, Reddy KR (2008) Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameter. *Journal of Agronomy and Crop Science* **194**, 225-236

Song J, Nada K, Tachibana S (1999) Amerliorative effect of polyamines on the high temperature inhibition of *in vitro* pollen germination in tomato (*Lycopersicon esculentum* Mill.). *Scientia Horticulture* **80**, 203-212

Stadler R, Lauterbach C, Sauer N (2005) Cell-to-cell movement of green fluorescent protein reveals post-phloem transport in the outer integument and identifies symplastic domains in *Arabidopsis* seeds and embryos. *Plant Physiology* **139**, 701-712

Stasolla C, Loukanina N, Ashihara H, Yeung EC, Thorpe TA (2001c) Pyrimidine nucleotide biosynthesis in germinating white spruce somatic embryos. *In Vitro Cellular and Developmental Biology – Plant* **37**, 286-293

Stasolla C, **Loukanina N, Ashihara H, Yeung EC, Thorpe TA** (2001b) Ascorbic acid changes the pattern of purine metabolism during germination of white spruce somatic embryos. *Tree Physiology* **21**, 359-367

Stasolla, C, Loukanina N, Ashihara H, Yeung EC, Thorpe TA (2001a) Purine and pyrimidine metabolism during the partial drying treatment of white spruce (*Picea glauca*) somatic embryos. *Physiologia Plantarum* **111**, 93-101

Stasolla C, Yeung EC, Thorpe TA (2003) Somatic embryogenesis in white spruce: Physiology and biochemistry. In: Bhojawani SS (Ed) *Agibiotechnology and Plant Tissue Culture*, Science Publishers, Inc. Enfield NH, pp 10-27

Steward F, Mapes M, Mears K (1958) Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. *American Journal of Botany* **45**, 705-708

Suain SM, Muller AJ, Singh DP (2004) The *gar2* and *rga* alleles increase the growth of gibberellin-deficient pollen tubes in Arabidopsis. *Plant Physiology* **134**, 694-705

Sun K, Cui Y, Hauser BA (2005) Environmental stress alters gene expression and induces ovule abortion: reactive oxygen species appear as ovules commit to abort. *Planta* **222**, 632-642

Sun K, Hunt K, Hauser BA (2004) Ovule abortion in *Arabidopsis* triggered by stress. *Plant Physiology* **135**, 2358-2367

Sunderlandt N (1982) Induction of growth in the culture pollen. In: Yeoman MM, Truman DES (Eds) *Differentiation in Vitro*, Cambridge University Press, Cambridge, pp 1-24

Supena EDJ, Winarto B, Riksen T, Dubas E, van Lammeren A, Offringa R, Boutilier K, Custers J (2008) Regeneration of zygotic-like microspore-derived embryos suggests an important role for the suspensor in early embryo patterning. *Journal of Experimental Botany* **59**, 803-810

Thakur P, Kumar S, Malik JA, Berger JD, Nayyar H (2010) Cold stress effects on reproductive development in grain crops: An overview. *Environmental and Experimental Botany* **67**, 429-443

Tamura N, Yoshida T, Tanaka A, Sasaki R, Bando A, Toh S, Lepiniec L, Kawakami N (2006) Isolation and characterization of high temperature-resistant germination mutants of *Arabidopsis thaliana*. *Plant and Cell Physiology* **47**, 1081-1094

Tan BC, Joseph LM, Deng WT, Liu LJ, Li QB, Cline K, McCarty DR (2003) Molecular characterization of the *Arabidopsis* 9-*cis* epoxycarotenoid dioxygenase gene family. *Plant Journal* **35**, 44-56

Thorpe TA, Stasolla C (2001) Somatic embryogenesis. In: Bhojwani SS, Soh WY (Eds) *Current Trends in the Embryology of Angiosperms*, Kluwer Academic Publisher, Dordrecht, pp 279-336

Toh S, Imamura A, Watanabe A, Nakabayashi K, Okamoto M, Jikumaru Y, Hanada A, Aso Y, Ishiyama K, Tamura N, Iuchi S, Kobayashi M, Yamaguchi S, Kamiya Y, Nambara E, Kawakami N (2008) High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellins action in *Arabidopsis* seeds. *Plant Physiology* **146**, 1368-1385

Touraev A, Ilham A, Vicente O, Heberle-Bors E (1996) Stress induced microspore embrogenesis from tobacco microspores: An optimized system for molecular studies. *Plant Cell Reports* **15**, 561-565

Touraev A, Vicente O, Heberle-Bors E (2001) Initiation of microspore embryogenesis by stress. *Trends in Plant Science* **2**, 297-301

Tung C-W, Dwyer KG, Nasrallah ME, Nasrallah JB (2005) Genome-wide Identification of genes expressed in *Arabidopsis* pistils specifically along the path of pollen tube growth. *Plant Physiology* **138**, 977-989

Tykarska T (1976) Rape embryogenesis: The proembryo development. *Acta Societatis Botanicorum Poloniae* **45**, 3-15

Vernoux T, Wilson RC, Seeley KA, Reichheld P-P, Muroy S, Brown S, Maughan SC, Cobbett CS, Van Montagu M, Inzé D, May MJ, Sung ZR (2000) The *ROOT MERISTEMLESS/CADMIUM SENSITIVE2* gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell* **12**, 97-109

Vivian-Smith A, Koltunow AM (1999) Genetic analysis of growth-regulator induced parthenocarpy in *Arabidopsis*. *Plant Physiology* **121**, 437-451

Vizcay-Barrera G, Wilson ZA (2006) Altered tapetal PCD and pollen wall development in the *Arabidopsis ms1* mutant. *Journal of Experimental Botany* **57**, 2709-2717

Vriezen WH, Feron R, Maretto F, Keijman J, Mariani C (2008) Changes in tomato ovary transcriptome demonstrate complex hormonal regulation of fruit set. *New Phytologist* **177**, 60-76

Wang M, Hoekstra S, van Bergen S, Lamers GEM, Oppendijk BJ, van der Heijden MW, de Preister W, Schilperoort RA (1999) Apoptosis in the developing anthers and the role of ABA in this process during androgenesis in *Hordeum vulgare* L. *Plant Molecular Biology* **39**, 489-501

Wang Z, Mambelli S, Setter TL (2002) Abscisic acid catabolism in maize kernels in response to water deficit at early endosperm development. *Annals of Botany* **90**, 623-630

Warner RM, Erwin JE (2005) Naturally occurring variation in high temperature induced floral bud abortion across *Arabidopsis thaliana* accessions. *Plant Cell Environment* **28**, 1255-1266

Weber H, Borisjuk L, Wobus U (2005) Molecular physiology of legume seed development. *Annual Reviews in Plant Biology* **56**, 253-279

Wehmeyer N, Hernandez LD, Finkelstein RR, Vierling E (1996) Synthesis of small heat-shock proteins is part of the developmental program of late seed maturation. *Plant Physiology* **112**, 747-757

Wobus U, Weber H (1999) Sugars as signal molecules in plant seed development. *Biological Chemistry* **380**, 937-944

Yahraus T, Chandra S, Legendre L, Los PS (1995) Evidence for a mechanically-induced oxidative burst. *Journal of Radiation Research* **38**, 1-4

Yao QA, Simion E, William M, Krochko J, Kasha KJ (1997) Biolistic transformation of haploid isolated microspores of barley (*Hordeum vulgare* L.). *Genome* **40**, 570-581

Yeung EC, Rahman MH, Thorpe TA (1976) Comparative development of zygotic and microspore-derived embryos in *Brassica napus* L. cv Topas. *International Journal of Plant Sciences* **157**, 27-39

Yeung EC, Belmonte MF, Tu LTT, Stasolla C (2007) Glutathione modulation of *in vitro* development. *In Vitro Cellular and Developmental Biology – Plant* **41**, 584-590

Young LW, Wilen RW, Bonham-Smith PC (2004) High temperature stress of *Brassica napus* during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production. *Journal of Experimental Botany* **55**, 485-495

Yu L-X, Setter TL (2003) Comparative transcriptional profiling of placenta and endosperm in developing maize kernels in response to water deficit. *Plant Physiology* **131**, 568-582

Zanor MI, Osorio S, Nunes-Nesi A, Carrari F, Lohse M, Usadel B, Kühn C, Bleiss W, Giavalisco P, Willmitzer L, Sulpice R, Zhou Y-H, Fernie AR (2009) RNA Interference of LIN5 in tomato confirms its role in controlling Brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. *Plant Physiology* **150**, 1204-1218

Zarsky V, Garrido D, Rihova L, Tupy J, Vicente O, Heberle-Bors E (1992) Depression of the cell cycle by starvation is involved in the induction of tobacco pollen embryogenesis. *Sexual Plant Reproduction* **5**, 189-194