CHANGES IN GENE EXPRESSION DURING DRYING AND IMBIBITION OF DESICCATION SENSITIVE *Magnolia ovata* (A. St.-Hil.) Spreng. SEEDS[1]

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ABSTRACT - Seeds of *Magnolia ovata* were dried to different water contents to assess the viability and transcript abundance of genes related to seed development, cell cycle, cytoskeleton and desiccation tolerance.The expression of development, cell cycle and cytoskeleton relative genes (*ABI3, CDC2-like* and *ACT2*) alone could not explain the germination behaviour of *M. ovata* seeds in relation to drying damage. Irrespective of their initial water content, the seeds performed in the same way during the initial period of germination and the deleterious effects of desiccation only occurred in later stages. Expression of *PKABA1, sHSP17.5* and *2-Cys-PRX* did not show a relationship with desiccation. However, the expression patterns of *PKABA1* and *sHSP17.5* suggested the participation of these genes in protective mechanisms during the imbibition of *M. ovata* seeds..

Index terms: desiccation tolerance, drying, gene expression, *Magnolia ovata.*

ALTERAÇÕES NA EXPRESSÃO GÊNICA DURANTE A SECAGEM E EMBEBIÇÃO DE SEMENTES SENSÍVEIS À DESSECAÇÃO DE *Magnolia ovata* A. ST.-HIL.

RESUMO – Sementes de *Magnolia ovata*, foram secas a diferentes conteúdos de água para avaliação da viabilidade e da expressão de genes relacionados ao desenvolvimento, ciclo celular, citoesqueleto e tolerância à dessecação. A expressão de transcritos dos genes relacionados ao desenvolvimento, ciclo celular e citoesqueleto (*ABI3, CDC2-like* e *ACT2*) não explicou o comportamento germinativo de sementes de *M. ovata* em relação aos danos ocasionados pela secagem. Aparentemente as sementes comportam-se da mesma maneira nos primeiros dias de embebição, independentemente do conteúdo de água inicial. A expressão dos transcritos dos genes *PKABA1, sHSP17.5* e *2-Cys-PRX* não mostraram relação com a dessecação, entretanto, o padrão de expressão de *PKABA1* e *sHSP17.5* sugere a participação destes genes em mecanismos de proteção durante a embebição de sementes de *Magnolia ovata*.

Termos para indexação: expressão gênica, *Magnolia ovata*, secagem, tolerância à dessecação.

INTRODUCTION

Desiccation tolerance of seeds and pollen is a widespread phenomenon among higher plants. Orthodox seeds exhibit the ability to withstand removal of water from their tissues to permit storage at low temperatures and be kept in seed banks for periods of time varying from decades to thousands of years. However, not all species tolerate drying to such low water contents. Recalcitrant seeds have high water content at shedding and do not withstand desiccation down to 0.10 g $H_2O \cdot g^{-1}$ dw (Hong et al., 1996).

Although all seeds experience some reduction in water content after maturation, the limit tolerated by recalcitrant species is narrow, with considerable variation among species (Farrant et al., 1996). In some recalcitrant seeds like *Camellia sinensis* (Berjak et al., 1993), *Quercus robur* (Finch-Savage, 1992), *Landolphia kirkii* (Vertucci et al., 1995) an increase in desiccation tolerance is observed during development,. A few recalcitrant species can also exhibit some degree of dormancy at shedding (Liu et al., 2005).

Studies of gene expression, associated with desiccation tolerance and sensitivity, have been used in order to understand the mechanisms leading to deterioration of recalcitrant seeds after drying (Faria et al., 2006). With the initiation of drying there is a shift in gene expression compelling seeds to a different physiological status, where genes coding for protective molecules have an important role in desiccation tolerant material (Ramanjulu and Bartels, 2002). Genes encoding for proteins associated with tolerance, especially those involved in metabolic changes (Avelange-Macherel et al., 2006), protection against oxidation (Pukacka and Ratajczak, 2005), and other putative protective molecules show a specific expression pattern during desiccation.

The phytohormone abscisic acid (ABA) is involved in the regulation of many complex plant physiological processes including seed development, seed dormancy, transpiration, adaptation to environmental stresses (Finkelstein, 2006) and plays an important role in seed desiccation tolerance. ABA INSENSTIVE 3/VIVIPAROUS 1 (ABI3/VP1) is a transcription factor that mediates ABA responses in seeds from a range of species (Parcy et al., 1994; Zeng et al., 2003). ABA acts through ABI3/VP1 during the later stages of seed development, which promotes seed maturation processes including storage reserve deposition, the acquisition of desiccation tolerance and dormancy (Li and Foley, 1997). Seeds of severe *abi3* mutants in Arabidopsis accumulate less storage proteins, are desiccation-intolerant and germinate precociously. Studies with transgenic plants overexpressing *ABI3* indicated that this gene is able to direct the expression of seed-specific genes in response to ABA in tissues other than seeds (Parcy et al., 1994).

Actin and tubulin are the major components of the plant cytoskeleton. Actin is required for correct cell-division, plate alignment and synthesis, cell shape determination, cell-polarity establishment, cytoplasmic streaming, organelle movement and tip growth (Meagher et al., 2005). The cell cycle is controlled by the molecular machinery that ensures the fidelity of DNA replication and responds to signals from both the external environment and intrinsic developmental programs. A central role in the regulation of the cell cycle is played by the cyclin-dependent kinases (CDKs) (De Veylder et al., 2001). CDKs govern, as in other eukaryotic organisms, the plant cell cycle (Inzé, 2005). Cell division is controlled by the activity of CDK complexes. In addition to their association with CYCs (Cyclins), the activity of CDKs is also regulated by other mechanisms, including activation of CDKs through phosphorylation. CDKs are also involved in the regulation of transcription and mRNA processing (Loyer et al., 2005).

Proteins kinases also play a critical role in the perception and transduction of cellular responses to environmental stresses (Holappa et al., 2005). PKABA1 is a serine/threonine protein kinase belonging to the SnRK2 group. Its transcription is induced by ABA and occurs in embryos and seedlings. *PKABA1* is transcriptionally up-regulated in response to environmental stress via changes in ABA concentration in vegetative tissues and developing embryos of wheat (Holappa and Walker-Simmons, 1995). *PKABA1* mRNA levels increase as ABA content increases in developing embryos and reach high levels at seed maturity. Levels remain high in isolated embryos treated with ABA but decline in germinating seeds. *PKABA1* is induced rapidly in seedlings when ABA levels increase in response to environmental stress like cold temperature and osmotic stress (Holappa and Walker-Simmons, 1995). *PKABA1-like* genes have been identified in many plant genomes including *Triticum aestivum* (*TaPK3*), soybean (*SPK3*), Arabidopsis (*SNRK2E*) and *Medicago truncatula* (Holappa and Walker-Simmons, 1997) and may be associated with the acquisition of desiccation tolerance (Faria et al., 2006).

Reactive oxygen species (ROS) play a significant role in causing damage to living cells under severe stress conditions. To deal with oxidative stress, complex protective mechanisms have been evolved by plants to mitigate and repair the damage initiated by free radicals (Price et al., 1994). The expression of the *2-CYS PRX* gene is up-regulated in Arabidopsis

plants in response to salt stress and in response to pathogen infection (Rouhier et al., 2004; Charlton et al., 2005). These antioxidants can also remove damaging reactive oxygen species produced by products of desiccation and respiration during late embryogenesis, imbibition of dormant seeds and germination (Stacy et al., 1996).

Another group of plant proteins with synthesis associated with stress is formed by the heat shock proteins (HSPs). HSPs are divided into low molecular mass proteins of approximately 15–28 kDa (sHSPs) and high molecular mass proteins of more than 30 kDa (HMM HSPs). sHSPs were first discovered as gene products whose expression is induced by heat and other forms of abiotic stresses. However, now their importance as molecular chaperones is generally accepted (Lee and Vierling, 2000).

This work aimed to study the expression of genes, at the mRNA level, related to seed development, cell cycle, cytoskeleton and desiccation tolerance during drying and subsequent imbibition of the desiccation sensitive seeds of *Magnolia ovata* (Magnoliaceae), a tree from the Brazilian Atlantic Forest, in order to better understand the mechanisms, leading to deterioration of recalcitrant seeds by desiccation and subsequent imbibition.

MATERIALS AND METHODS

Plant material and desiccation conditions

Magnolia ovata fruits were collected from 12 trees along the Rio Grande river near Lavras (State of Minas Gerais, Brazil), in September 2004. After collection, fruits were left at room temperature to allow the completion of dehiscence, which occurred between 3 to 4 days after collection. The red aril that covers the seed was removed by gentle rubbing on a mesh and rinsing with tap water. After cleaning, seeds were blotted dry with a paper towel to remove excess of water, and stored at 5ºC in closed plastic bags. Seeds were deployed for experimental work within 2 months of harvest.

Desiccation was carried out over a 90% (w/v) lithium chloride solution, providing a relative humidity of 11% at 20ºC. Water content was monitored every hour by the difference between initial weight of fresh and dried seeds. When seeds reached the estimated target water content, three samples were taken: one sample of 20 seeds for water content determination, one sample of 100 seeds for germination and one sample of 50 seeds for RNA extraction (Table 1). The latter seeds were frozen in liquid nitrogen and stored at -70ºC until processing.

TABLE 1. Treatments applied to *Magnolia ovata* seeds in order to study changes in gene expression during desiccation and after imbibition.

Sample	Treatment
F0	Fresh seeds (control, 0.28 g $H_2O \cdot g^{-1}$ dw)
PD0	Partially dried seeds (0.25 g $H_2O \cdot g^{-1}$ dw)
D0	Dried seeds (0.10 g $H_2O \cdot g^{-1}$ dw)
F2	Fresh seeds after 2 days of imbibition
PD2	Partially dried seeds after 2 days of
D2	imbibition
F10	Dried seeds after 2 days of imbibition
PD10	Fresh seeds after 10 days of imbibition
D10	Partially dried seeds after 10 days of imbibition
	Dried seeds after 10 days of imbibition

Water content assessment

Water content of whole seeds was determined gravimetrically of four replications of five seeds by oven drying at 103ºC/17hours (ISTA, 2005). Water contents were expressed on a dry weight basis (g $H_2O \cdot g$ dry weight^{-1}, or g·g^{-1}).

Seed germination and viability assessment

Before germination seeds were pre-humidified by incubation on moist filter paper (Ø 85 mm, Schleicher and Schuell 595, Dassel, Germany) in 9 cm Petri dishes for 24 hours with 5 ml distilled water. Subsequently, seeds were surface sterilised in 1% sodium hypochlorite for 10 minutes, rinsed with distilled water and imbibed in 5ml of distilled water. Germination experiments were executed with four replicates of 25 seeds. Seeds were placed in 9 cm Petri dishes on two sheets of filter paper (Schleicher and Schuell 595). Seeds were incubated at alternating temperature 20ºC/10ºC (day/night), with 8 daily hours of light.

A seed was regarded as germinated when the radicle protruded 2mm through the seed coat. After 0, 2 and 10 days of imbibition, a sample of 50 seeds was taken for RNA extraction. These seeds were frozen in liquid nitrogen and stored at -70ºC. Germination experiments were carried out for 80 days. Non germinated seeds after this period were evaluated by a cut test in order to assess viability of seeds. Seeds were regarded as dead if mushy, liquid or rotten endosperm was present. On the other hand, if a firm and white endosperm and embryo were present, seeds were scored as viable seeds (Goslin, 2002; ISTA, 2005) and reported as "dormant" seeds.

Total RNA isolation and reverse transcriptase PCR

Total RNA was extracted from whole seeds (without the seed coat). It was not possible to isolate the embryo from the seed without severely damaging it. For each treatment, 50 seeds were frozen in liquid nitrogen and stored at -70°C. Seeds were ground with a mortar and pestle in liquid nitrogen and added to a tube containing 1 ml of phenol:chloroform (5:1) plus TLE grinding buffer (0.18 M Tris, 0.09 M LiCl, 4.5 mM EDTA, 1% SDS, adjusted to pH 8.2) and 5μL of β-mercaptoethanol that was added to the extraction buffer. After centrifugation, the upper phase was transferred to a new tube containing 1ml of phenol-chloroform (1:1), centrifuged again and the supernatant was washed with chloroform to remove any residual phenol. RNA was precipitated overnight at -20°C in 0.1 volume 3 M sodium acetate and 2.5 volume of 100% ethanol, and then resuspended in 20 µL DEPC water. RNA quality was analyzed in 1.0% agarose gel electrophoresis, stained with ethidium bromide and quantified with a spectrophotometer (Eppendorf BioPhotometer, Eppendorf, Hamburg, Germany).

cDNA synthesis was performed using the iScript cDNA synthesis kit (Bio Rad, Hercules, CA, USA), according to the manufacturer's protocol. Reactions were prepared by mixing the total RNA with iScript reaction mix and iScript reverse transcriptase and performed in a thermal cycler (iCycler, Bio Rad, Hercules, CA, USA), as follows: 5 min at 25°C; 30 min at 42°C and 5 min at 85°C. A negative control for cDNA synthesis was also included, by omitting reverse transcriptase to the reaction.

Primer design and quantitative real-time PCR

Degenerate primers (Table 2) were designed based on gene sequences available in databases using the software Jellyfish version 3.2 (LabVelocity, Inc., Los Angeles, USA). Sequences amplified by PCR were cloned using pGemT vector (Promega, USA) following manufacturer's instructions, and sequenced. Sequences were annotated and deposited in GenBank at National Center of Biotechnology Information server. Specific primers were designed with the software Vector NTI10 (Invitrogen Coorporation, USA), based on gene sequences obtained after cloning and sequencing of partial cDNA products.

TABLE 2. Specific primers used in the real-time PCR reactions.

Gene	Forward (sequences written 5' to 3')	Reverse (sequences written 5' to 3')
ABI3	GCTTCTTTCTTTGGCAACACAATCC	AACCGCCATGCAAACACAACAGC
ACT2	TGCTGTGATCTCCTTGNCTCATACG	GAAGCTGCTGGAATCCATGAGACC
CDC2-like	AGATGAGGGTGTTCCAAGCACAGC	CGTGGATTCTTGGCAAAATCTGG
PKABA1	GAGATTTAGCGAGGATGAGGCAAGG	GATTTTCAAGCGCGGAGCTGG
2-CYS- PRX	CCTACGCCGAAGAGTTCGAGAAGC	CCTTTTCGGGTCTGCTATTATCGG
sHSP17.5	AGGGCTGAAGAAAGAGGAAGTCAGG	TTTTCCATCGCTGCCTTCACG
18S	TGACGGAGAATTAGGGTTCG	CCTCCAATGGATCCTCGTTA

Reactions were performed using an iCycler iQ instrument (Bio Rad, Hercules, CA, USA) with gene specific primers, cDNA and iQ SYBR green supermix (Bio Rad, Hercules, CA, USA). The amplification protocol consisted of 3 min at 95°C; then 40 cycles of 15s at 95°C followed by 1 min at 60°C. A primer for 18S gene was used as internal control to normalize the other products for analysis purposes. A negative control (negative cDNA) and a water control were used in every PCR plate. Real-time PCR evaluations were replicated four times for each treatment, derived from 2 biological replicates repeated twice. Values of fold change in gene expression (mRNA) in relation to the control (fresh seeds) were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), and plotted in graphs for comparison. Data were compared by Scott and Knott test ($p \leq 0.05$) (Scott and Knott, 1974).

RESULTS AND DISCUSSION

The *Magnolia ovata* seed

The true *M. ovata* seed consists of a woody seed coat (testa), a soft oily endosperm and a small but fully differentiated embryo (Figure 1). The average seed size was 0.97 x 0.62 x 0.42 cm (length x width x thickness) whereas the average embryo length was 0.9 mm and represented approximately 1/40 of the total seed volume.
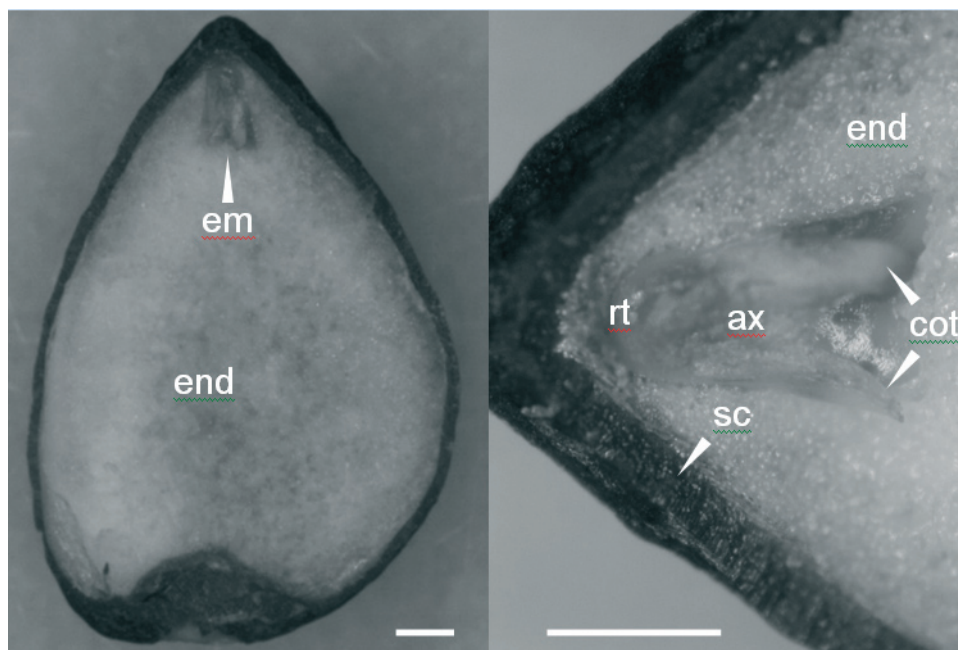
FIGURE 1. Transversal section of a *M. ovata* **seed (A) and enlarged section with the embryo (B). em, embryo; end, endosperm; rt, radicle tip; ax, axis; cot, cotyledon; sc, seed coat. Bars represent 1 mm.**

Viability of *Magnolia ovata* seeds after desiccation and Expression of genes related to seed development, cell cycle and cytoskeleton during drying and imbibition of Magnolia ovata seeds.

The viability of both fresh and partially-dried *Magnolia ovata* seeds was about 80%. However, the proportion of dormant seeds was reduced after a partial drying treatment to 0.25 g·g^{-1} (Table 3). Drying seeds to a water content below 0.10 g·g^{-1} led to an almost complete loss of viability, although still some dormant seeds were detected at the end of the germination experiment.

TABLE 3. Effect of drying on germination and number of dormant seeds of *Magnolia ovata*. Final germination was scored after 80 days of incubation at 20/10ºC. Data are average of four replicates (± standard deviation). Lower case letters in the same column indicate significance of difference based on Scott-Knott test (p ≤ 0.05).

Treatment	Drying time (hours)	Water content (g H$_2$O·g^{-1} dw)	Germination (%)	Dormant (%)
Fresh	0	0.28	51 ± 10.8 b	27 ± 2.9 a
Partially dried	2	0.25	72 ± 8.0 a	7 ± 2.0 b
Dried	20	0.10	18 ± 7.7 c	13 ± 2.5 b

In general, differences in transcript abundance were not as pronounced as could be expected from comparable studies (Faria, 2006; Faria et al., 2007). Since the selected genes are expected to be expressed exclusively in the embryo, the use of whole seeds for RNA extraction may have resulted in a considerable dilution of embryonic RNA. The embryo volume is an estimated 1/40 of the whole seed.

ABI3 and *ACT2* showed a similar expression pattern after desiccation (Figure 1A, B). Transcript abundance of both did not change significantly after drying the seeds (PD0 and D0 treatments) compared to the control (fresh seeds, F0). After imbibition, there was an increasing trend of the relative mRNA levels for both genes. *ABI3* expression was higher in fresh seeds imbibed for 2 days (F2) than in fresh seeds imbibed for 10 days (F10), whilst the relative levels of *ACT2* did not differ in dried seeds imbibed for 2 days

(D2) and dried seeds imbibed for 10 days (D10). The relative abundance of *CDC2-like* in F0 and PD0 did not differ (Figure 2 C). It was significantly down-regulated when seeds were

dried to 0.10g·g⁻¹ and up-regulated after imbibition for 2 and 10 days in all treatments.
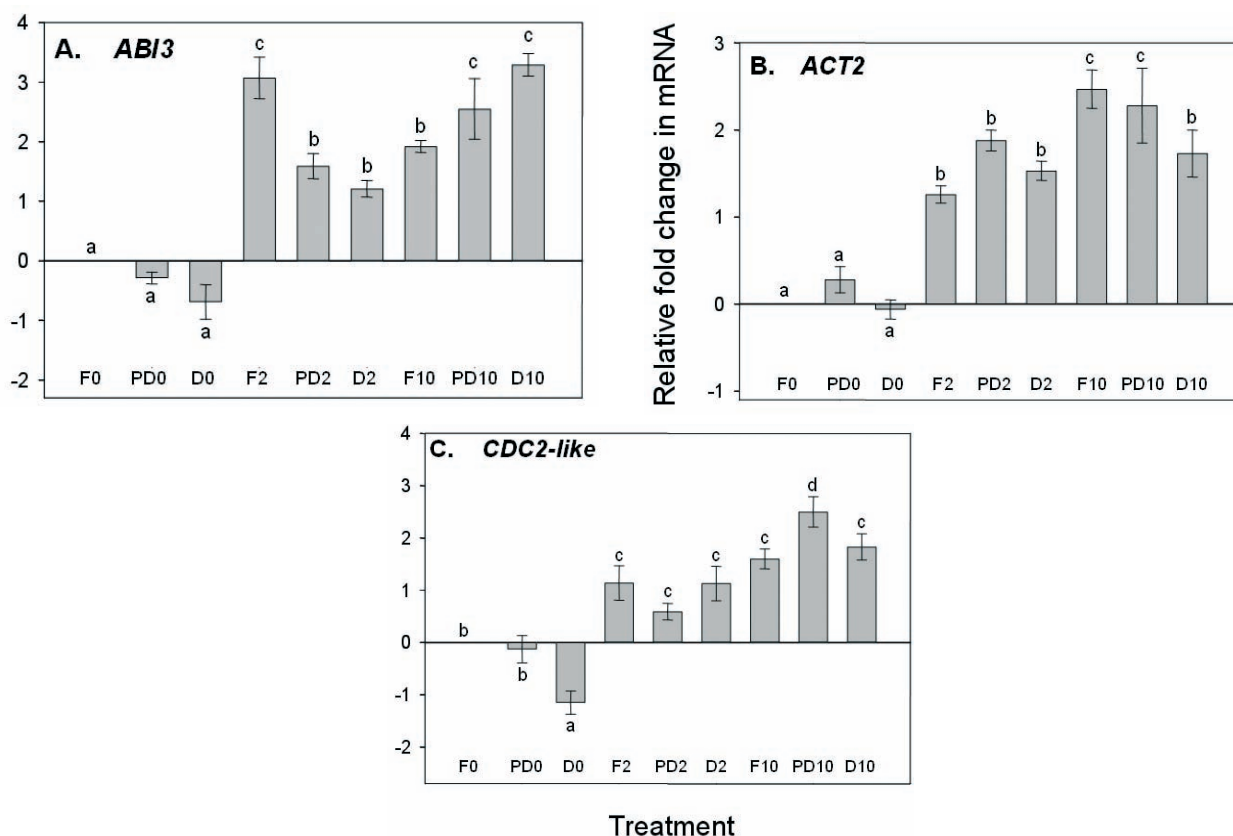

FIGURE 2. Relative fold change in mRNA abundance of (A) *ABI3* (accession number: FJ668600), (B) *CDC2-like* (accession number: FJ668602) and (C) *ACT2* (accession number: FJ668597) in *Magnolia ovata* seeds after drying to different water contents and subsequently imbibition, as compared to fresh seeds (F0). Data show the mean ± standard deviation of four replicates. Different lower case letters mean statistically significant difference based on Scott-Knott test (p ≤ 0.05).

In orthodox seeds, the maturation stage of development is characterized by desiccation. Concomitantly, seeds may enter a state of dormancy, which allows survival under unfavorable environmental conditions and ensures dispersal in place and time (Ingram and Bartels, 1996). Molecular studies of seed maturation in model species have revealed that it is driven mainly by three key genes: *ABAINSENSITIVE3* (*ABI3*), *FUSCA3* (*FUS3*), and *LEAFYCOTYLEDON1* (*LEC1*) (Wobus and Weber, 1999). The expression pattern of *ABI3* in *Arabidopsis thaliana* seeds is characterized by a continuous increase throughout development and transient expression during the first week of germination (Parcy et al.,

1994). On the other hand, Bassel et al. (2006), studying *ABI3* expression in tomato (*Solanum esculentum*) and Arabidopsis seeds, observed high transient expression of this gene during imbibition, prior to the completion of germination (*i.e.* radicle protrusion), but no expression in dry mature seeds. Our results show that the relative levels of *ABI3* transcripts remained approximately constant during drying (Figure 1A), whereas they increased in abundance during subsequent imbibition. However, the timing of increase of *ABI3* transcript abundance depended on the treatment. Rehydration from the driest state resulted in the slowest increase in *ABI3* transcript abundance. Although an increase in *ABI3* expression during germination

has been observed in species such as tomato and Arabidopsis (Bassel et al., 2006) or yellow cedar (Zeng et al., 2003), there is no clear evidence of a participation of *ABI3* in signaling leading to the completion of germination. Moreover, most of the *abi* mutants in Arabidopsis show normal germination (Koornneef et al., 1984). Thus, it is plausible that ABI3 is participating in stress signaling during imbibition. It is important to note, however, that even with high expression of *ABI3* in dried seeds after imbibition, the vast majority of the seeds did not germinate, mainly because they had lost viability. These results are in accordance with Faria et al. (2007) who compared changes in *ABI3* expression in radicle tips during the loss and re-establishment of desiccation tolerance in germinated seeds of *Medicago truncatula*. Upon drying, desiccation intolerant radicles showed 15-fold higher *ABI3* transcript levels compared with desiccation tolerant radicles.

ACT2 expression did not change during drying, and showed an increase during imbibition. Transcript abundance remained high but dried seeds imbibed for 2 and 10 days displayed lower levels than those of fresh and partially dried seeds at 10 days of imbibition, thus apparently reflecting the loss of viability in the dried seeds. These results are partially in accordance with the expression of *ACT7*, a gene from the same family, in Arabidopsis seeds, where no expression was detected in developing embryos but high expression at seed maturity after drying. This high expression persisted during imbibition (McDowell et al., 1996). Changes in expression of ACT2 during seed development have not been reported so far. In vegetative tissues, water deficit causes a decrease in mitotic activity, which is attributed to the regulation of *CDC2* genes and other cyclin-dependent kinase (*CDK*) proteins (Schuppler et al., 1998). These authors proposed that water-stress signaling acts on *CDC2* by increasing phosphorylation of tyrosine, causing a shift to the inhibited form and slowing cell production. In *M. ovata* there was a reduction in the levels of *CDC2* mRNAs when seeds were dried to low water contents (D0). However, when seeds were imbibed, the transcripts levels increased even in dried seeds, which have low viability.

The expression of *ABI3*, *ACT2* and *CDC2* alone do not explain the germination behaviour of *M. ovata* seeds. It seems that the seeds, irrespective of their initial water content, perform in the same way during the initial period of germination whereas the deleterious effects of desiccation occur only later, since the germination of *M. ovata* seeds is very slow, with first radicle protrusion starting at 40 days after imbibition. These results suggest that seeds of this species may not be sensitive to desiccation to a water content of 0.10g·g^{-1}. Instead, seeds may become sensitive to imbibitional stress, leading to loss of viability, which was also observed in neem seeds (*Azadirachta indica*) (Sacandé, 2000).

Expression of genes related to desiccation tolerance

The expression pattern of *PKABA1* and *sHSP17.5* showed the same behaviour after drying to different water contents. There was a trend in reduction in the expression after desiccation, but it did not differ statistically from fresh seeds (Figure. 2A, C). The expression pattern of *PKABA1* was similar to that of *ABI3* (Figure 3A), showing no differences after drying and an increase in transcript abundance after imbibition. In the same way, relative expression in fresh seeds imbibed for 2 days (F2) was higher than in those imbibed for 10 days (F10). The relative levels of *sHSP17.5* increased with the time of imbibition, with no significant differences between the drying treatments. Drying fresh seeds to 0.25g·g^{-1} (PD0) did not change the relative amounts of *2-Cys-PRX* mRNA (Figure 3B) but a significant reduction occurred after drying to 0.10g·g^{-1} (D0). There was no difference in transcript level before and after imbibition of fresh and partially dried seeds (F2 and PD2), but a clear reduction was observed after 10 days of imbibition (F10 and PD10). The *2-Cys-PRX* transcript levels in dried seeds remained the same after imbibition for 2 days (D2) but increased after imbibition for 10 days (D10) to the same value as observed in fresh seeds.

PKABA1 is involved in a signaling pathway by which ABA suppresses the induction of gibberellin responsive genes in cereal aleurone layers (Gomez-Cadenas et al., 2001). Its expression is up-regulated by ABA, dehydration, cold, and osmotic stress in other plant tissues (Holappa and Walker-Simmons, 1995). During wheat seed development the levels of *PKABA1* mRNA increased, but decreased during imbibition of nondormant seeds and transiently increased during imbibition of dormant seeds (Johnson et al., 2002). *PKABA1* was not up-regulated during drying of *M. ovata* seeds, but its expression was induced after imbibition, even in fresh seeds. It suggests the participation of ABA-signaling during the imbibition of *M. ovata* seeds. Seeds of some temperate recalcitrant species such as *Aesculus hippocastanum* (Tompsett and Pritchard, 1993) and *Aesculus pseudoplatanus* (Hong and Ellis, 1990) have been reported to have dormancy at shedding. Fresh seeds of *M. ovata* showed a high number of dormant seeds after 80 days of imbibition (Table. 3), which is in accordance with high expression of *PKABA1* at 2 and 10 days of imbibition.
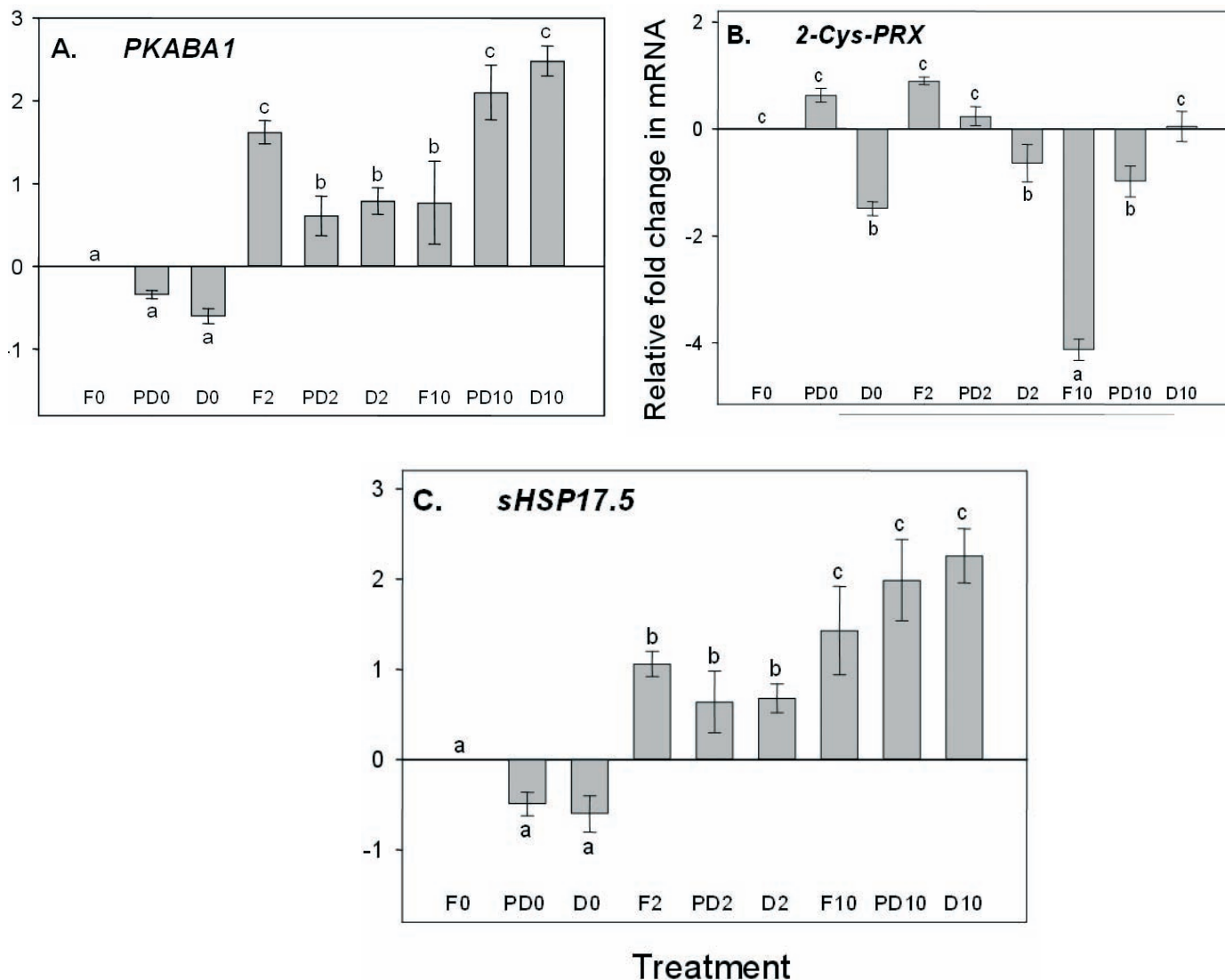
FIGURE 3. Relative fold change in mRNA abundance of (A) *PKABA1* (accession number: FJ668599), (B) *2-Cys-PRX* (accession number: FJ668601) and (C) *sHSP17.5* (accession number: FJ668598) in *Magnolia ovata* seeds after drying to different water contents and subsequently imbibition, as compared to fresh seeds (F0). Data show the mean ± standard deviation of four replicates. Different lower case letters mean statistically significant difference based on Scott-Knott test (p≤ 0.05).

PKABA1 and *sHSP17.5* are genes related to desiccation tolerance, but in the present study they did not show any relationship with drying. Transcript abundance of these genes remained constant during drying, but was up-regulated after imbibition. This suggests the participation of these genes in protective mechanisms during imbibition. The *ABI3* gene product is thought to activate expression of genes coding for specific small heat shock proteins during seed development (Rojas et al., 1999). Comparing the expression pattern

of *ABI3* (Figure 3A) and *sHSP17.5* (Figure 3C) a similar trend can be observed, with increasing expression during imbibition, which partly confirms the preposition of ABA signaling control of *PKABA1* and *sHSP17.5* expression.

Peroxiredoxins may protect barley embryos and aleurone cells against desiccation-induced free radical damage during late seed development and early imbibition (Stacy et al., 1996). The peroxiredoxin gene has previously been shown to be up-regulated during seed maturation

and down-regulated during germination in Arabidopsis (Haslekås et al., 1998) and in *Brassica oleracea* (Soeda et al., 2005), and its expression is mediated by *ABI3* (Haslekås et al., 2003). In *B. oleracea*, expression of this gene differed between fast and slow-dried osmoprimed seeds. Our results show no differences in gene expression between fresh (F0) and partially-dried seeds (PD0). However, its expression was down-regulated when seeds were dried to 0.10 $g \cdot g^{-1}$ water content (Figure 3B). This gene appears to have some role in fresh and partially dried seeds (F2 and PD2) only during the first days of germination, since its expression was up-regulated in F2 and PD2 treatments, while in dried seeds (D10) an increase in abundance of *2-Cys-PRX* mRNA occurred only after 10 days of imbibition.

CONCLUSION

Desiccation sensitive seeds of *Magnolia ovata* generally displays similar patterns of abundance of *ABI3, ACT2, CDC2, PKABA1* and *sHSP17.5* transcripts during imbibition with or without previous drying. Since the differences between dried and fresh seeds were so minor during 10d of imbibition, we conclude that desiccation damage in this species is expressed at later stages of the germination process, e.g. at the time of radicle protrusion after 40d. The observed normal stress responses to drying suggest that the seeds may have become increasingly sensitive to another type of stress induced by lower moisture contents, e.g. imbibitional stress. Only expression of *2-Cys-PRX* was a clear discriminator of the drying conditions (Figure 3B). The lowest water content induced the highest level of expression as compared to the non-dried seeds.

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