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Differential accumulation of abscisic acid and its catabolites in drought-sensitive and drought-tolerant sunflower seeds

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Abstract

Eleven sunflower (*Helianthus annuus* L.) inbred lines were evaluated in field and laboratory studies under drought and irrigation. In the field, lines B59, R419 and B67 had reduced seed and oil yield under drought, while no reduction was observed for R432, HAR4 and B71. Lines HA89, R415, R049, RHA274 and R423 presented intermediate responses. In laboratory tests, seeds of line B59 had reduced germination percentages at 200 and 400 mM mannitol, while germination of seeds of lines R432, B71, HAR4, RHA274 and HA89 was reduced only at 400 mM mannitol. Drought-sensitive B59 and drought-tolerant B71 grown under irrigation and drought conditions in the field were selected for hormone assays. Abscisic acid (ABA) and its catabolites in pericarp, embryonic axis and cotyledons of dry and germinated seeds of B59 and B71 were determined. ABA was the major component of the pericarp of dry seeds from B71 and B59 plants grown under drought. The embryonic axis of B71 dry seeds from drought-grown plants also showed high ABA content. The major findings from this study are: (1) the drought-sensitive and -tolerant lines exhibited different ABA and catabolite profiles; (2) water environment during maternal plant growth affected ABA content and the composition of catabolites in mature and germinated seeds; (3) ABA content did not affect germination performance in our conditions; and (4) the dry and germinated seed parts showed different ABA and catabolite profiles.

Keywords: abscisic acid, abscisic acid catabolites, sunflower inbred lines

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Introduction

Sunflower (*Helianthus annuus* L.) is one of the most widely cultivated oil crops in the world. Sunflower production area has increased steadily in recent years because of the crop's moderate cultivation requirements and high oil yield. Sunflower has the ability to tolerate periods of water deficit and therefore has the potential to become an important crop in semi-arid environments. However, to expand the sunflower planting area it is necessary to obtain genotypes tolerant to environmental stresses such as drought, salinity and low temperature.

A major obstacle to high yield in sunflower production is the lack of synchronized crop establishment due to undesirable weather and poor soil conditions. Water stress results in irregular seed germination, poor and unsynchronized seedling establishment (Mwale *et al.*, 2003), decreased leaf expansion (Connor and Sadras, 1992; Casadebaig *et al.*, 2008) and reduced seed oil content (Muriel and Downes, 1974; Hall *et al.*, 1985). Water stress also affects physical seed characteristics such as seed size (Baldini and Vannozzi, 1999), weight (Unger, 1982) and hull content (Connor and Hall, 1997). Thus, drought is a chronic limiting factor for sunflower yield, causing an annual loss of $\sim$1500 kg grain ha^{-1} in Argentina (Mercau *et al.*, 2001).

Drought tolerance in plants is genetically determined, but expression of this trait is affected by interaction with environmental factors. Through evolution, plants have developed a variety of biochemical and physiological mechanisms to respond and adapt to drought. Among physiological responses, abscisic acid (ABA) plays a central role in stress responses, affecting many developmental processes, e.g. seed maturation and inhibition of precocious germination (Raz *et al.*, 2001). ABA level in a particular tissue is determined by relative degrees of biosynthesis versus catabolism (Nambara and Marion-Poll, 2005),

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but may also involve release of ABA from its inactive glucose conjugate by a β-glucosidase (Lee *et al.*, 2006).

ABA in higher plants is synthesized through oxidative cleavage of a C_{40} carotenoid precursor (Nambara and Marion-Poll, 2005; Marion-Poll and Leung, 2006). The first step of the ABA biosynthetic pathway is epoxidation of zeaxanthin and antheraxanthin to violaxanthin, catalysed by zeaxanthin epoxidase (ZEP). Violaxanthin is converted to 9-*cis*-epoxycarotenoid. Oxidative cleavage of the major epoxycarotenoid 9-*cis*-neoxanthin by 9-*cis*-epoxycarotenoid dioxygenase (NCED) yields a C_{15} intermediate, xanthoxin, which is converted to ABA through a two-step reaction *via* ABA-aldehyde.

ABA is deactivated through oxidation pathways involving oxidation at different positions and a conjugation pathway (Kushiro *et al.*, 2004; Nambara and Marion-Poll, 2005). Three different ABA hydroxylation pathways have been shown to oxidize the methyl groups (C-7′, C-8′ and C-9′) of the ring structure. Hydroxylation at C-8′, considered the more common in plant catabolism, produces 8′-hydroxy-ABA (8′-HOABA), which isomerizes to phaseic acid (PA). PA is subsequently reduced to dihydrophaseic acid (DPA) and/or its analogue, *epi*-dihydrophaseic acid (*epi*-DPA). In addition, ABA and hydroxyl-ABA can be conjugated as glucose esters (ABA-GE) or glycosides, ABA-GE being the predominant form (Cutler and Krochko, 1999). Also, other conjugates with the hydroxyl groups of ABA and its hydroxylated catabolites have been reported (Nambara and Marion-Poll, 2005).

Changes in the environment during seed maturation influence development of the progeny. Seeds from sunflower plants grown under drought showed different jasmonate profiles from those in seeds grown under irrigation. Jasmonate content varies during sunflower germination, and environmental conditions that the mother plant encounters affect hormonal content of the seed progeny (Vigliocco *et al.*, 2007). In fact, among crop plants, sunflower often experiences drought stress. Therefore, it is necessary to elucidate the physiological mechanisms by which sunflower plants perceive and transduce the stress signals and initiate adaptive responses under these conditions. This will provide additional information about the resistance to drought of this agricultural crop which has not been studied as extensively as in other species.

In the present study, we used different sunflower inbred lines to characterize their responses to water stress under field and laboratory conditions. The B71 (drought-tolerant) and B59 (drought-sensitive) lines were selected to evaluate ABA and its catabolite content in the pericarp, embryonic axis and cotyledons of dry and germinated seeds from plants grown under drought or irrigation. Relationships among genotypes, drought tolerance, growth conditions, hormone catabolites and seed germination were analysed.

Materials and methods

Inbred-line characterization

Eight sunflower (*Helianthus annuus* L.) lines produced by Experimental Station INTA-Manfredi, Argentina (B59, B67, B71, R415, R049, R419, R423 and R432) and three USA public lines (HA89, RHA274 and HAR4) were evaluated for drought tolerance. Two field experiments were conducted during 2003–2004 and 2004–2005 at the Experimental Station INTA-Manfredi, Argentina. Plants of the above lines were grown under irrigation and drought conditions, using a split-plot experimental design with complete randomization and two replications. Treatment was started when plants had the fourth pair of leaves (V4 stage). For drought, soil was covered with polypropylene sheets until harvest. For irrigation, plants were watered when soil moisture reached 60% of field capacity. The physiological growth parameters evaluated were: plant height, weight of 1000 seeds, number of seeds per head, oil yield ha^{-1} and seed yield ha^{-1}.

For laboratory studies, lines B59, B71, R432, HA89, RHA274 and HAR4 were used. For germination assays, 50 seeds were placed in pots containing sand and incubated in a Conviron E15 growth chamber (Conviron, Winnipeg, Manitoba, Canada) under 16 h light, 28°C, 60% relative humidity/8 h dark, 18°C and 70% relative humidity. Soil moisture at sowing time was 60% of field capacity. Stress treatments were performed at mannitol concentrations of 0 (control), 200 and 400 mM, corresponding approximately to osmotic potentials of 0, −0.5 and −1.0 MPa. At day 5 and every 3 d thereafter, seedlings were watered by capillary ascent with half-strength Hoagland solution for each mannitol concentration. Control seedlings were treated with half-strength Hoagland solution. Germination percentage and seedling dry and fresh weights were determined at day 11 after sowing. Experiments were performed in quintuplicate.

Relative germination was calculated as (number of germinated seeds under stress conditions/number of germinated seeds under control conditions) × 100. Relative plant height, 1000 seed weight, number of seeds per head, oil yield ha^{-1}, seed yield ha^{-1} and seedling dry and fresh weight were calculated in a similar fashion.

Seeds collected from homogeneous populations of lines B71 and B59 grown under irrigation and drought conditions in the field were used for hormone assays. After harvest seeds were stored a for 2 months at −80°C.

Hormone assays

Dry seeds of B71 and B59 were dissected into the pericarp, embryonic axis and cotyledons. Two hundred milligrams of each seed part were used

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for hormone assay. Quantification of ABA and its catabolites was performed in triplicate.

Fifty germinated seeds of B71 and B59 at 72 h were dissected into the pericarp, embryonic axis and cotyledons. Two hundred milligrams of each seed part were used for hormone assay. ABA and catabolites were quantified in triplicate.

Abscisic acid and catabolite extraction

ABA, PA, DPA and ABA-GE were extracted from the pericarp, embryonic axis and cotyledons of dry or germinated seeds using a modification of the protocol of Durgbanshi *et al.* (2005). Plant material was homogenized in liquid nitrogen with a mortar and a pestle and dissolved in 5 ml ultra-pure water. One hundred nanograms of [$^2H_6$]ABA (OlChemIm Ltd, Olomouc, Czech Republic), [$^2H_3$]PA, [$^2H_3$]DPA and [$^2H_5$]ABA-GE (NRC-Plant Biotechnology Institute, Saskatoon, Canada) were added as internal standards. Extracts were transferred to 50-ml tubes, centrifuged at 1500 *g* for 15 min. The supernatant was collected, adjusted to pH 2.8 with 15% (v/v) acetic acid and extracted twice with an equal volume of diethyl ether. The aqueous phase was discarded and the organic fraction was evaporated by vacuum. Dried extracts were dissolved in 1 ml methanol. Samples were filtered through a syringe filter tip on a vacuum manifold at flow rate less than 1 ml min^{-1}, and the eluate was evaporated at 35°C under vacuum in a SpeedVac SC110 (Savant Instruments, Inc., New York, USA).

Liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI–MS–MS)

Mass spectrometry analysis was performed on a quadruple tandem mass spectrometer (MS–MS, Quattro Ultima; Micromass, Manchester, UK) outfitted with an electrospray ion source (ESI). A mixture containing all unlabelled compounds and internal standards was separated by reversed-phase high-performance liquid chromatography (HPLC) and analysed by tandem mass spectrometry with single-ion recording (SIR) to determine retention times for all compounds. The spectrometer software MassLynx™ v. 4.1 (Micromass) was used. The response was calculated as product ion peak area × (IS concentration/IS product ion peak area), where IS concentration is the known amount of the internal standard added.

HPLC was used to separate ABA and its catabolites extracted from seeds. An Alliance 2695 separation module (Waters, Milford, Massachusetts, USA) equipped with a 100 mm × 2.1 mm, 3-μm RESTEK C_{18} column was used to maintain performance of the analytical column. Fractions were separated using a gradient of increasing methanol concentrations, constant glacial acetic acid concentration 0.2% (v/v) in water and initial flow rate 0.2 ml min^{-1}. The gradient was increased linearly from 40% (v/v) methanol–60% (v/v) water–acetic acid at 25 min to 80% (v/v) methanol–20% (v/v) water–acetic acid. After 1 min, initial conditions were restored and the system was allowed to equilibrate for 7 min. The Monitoring Reaction Multiple mode was used for determination of ABA, ABA-GE, PA and DPA. These compounds were monitored at m/z transitions of 263/153, 425/263, 279/139 and 281/171 with retention times of 9.6, 3.75, 4.5 and 2.8 min, respectively. The collision energies used were 15 eV (electron volts) for ABA and 45 eV for ABA-GE, PA and DPA. The cone voltage was 35 V.

Seed germination assay

A simple random design was used to record time-courses of germination. For each experiment in quadruplicate, twenty-five B71 and B59 seeds were germinated between wet filter paper towels in a Conviron E15 germination chamber (Conviron) under controlled conditions (16 h light, 130 μE m^{-2} s^{-1}, 28°C, 60% relative humidity/8 h dark, 18°C, 70% relative humidity). Numbers of germinated seeds were recorded at 24, 48 and 72 h. Germination was defined as the emergence of a clearly visible radicle (Ni and Bradford, 1993).

Statistical analysis

Results of hormone analyses and germination percentages were analysed using Statgraphics Plus, version 3 (Manugistics, Rockville, Maryland, USA). Germination data as percentage of total number of seeds were arc-sine transformed. A one-way analysis of variance test (ANOVA) was used to determine the statistical significance of differences. Normality was confirmed by the Shapiro–Wilk test. Homogeneity of variance was confirmed by the Bartlett test. P values ≤ 0.05 were considered statistically significant.

Results

Evaluation of drought tolerance in sunflower inbred lines

In the field, differences among sunflower lines were observed only for seed yield ha^{-1} and oil yield ha^{-1} in plants grown under drought or irrigation. These parameters were therefore used to differentiate responses of lines to water stress. Under drought, lines B59, R419 and B67 had reduced relative seed yield ha^{-1}; lines R432, HAR4 and B71 showed no change in this parameter; and HA89, R415, R049,

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RHA274 and R423 showed an intermediate reduction (Fig. 1A). For oil yield ha^{-1}, lines B59, R419 and B67 showed a reduction under drought and line B71 was less affected (Fig. 1B). No significant differences among lines were found for plant height, 1000 seed weight or number of seeds per head (data not shown).

In laboratory tests, relative germination percentage was the only parameter differentiating responses of sunflower lines to water stress. A substantial reduction of germination percentage at 200 and 400 mM mannitol was observed for line B59. For R432, B71, HAR4, RHA274 and HA89, germination percentage was reduced at 400 mM mannitol but not at 200 mM (Fig. 1C). No significant differences among lines were found for dry or fresh seedling weight (data not shown).

Based on these results, B59 was characterized as drought-sensitive and R432, HAR4, and B71 as drought-tolerant lines under field and laboratory conditions.

ABA and catabolite ratios in dry and germinated seeds

ABA, PA, DPA and ABA-GE were detected in dry and germinated seeds of B71 (drought-tolerant) and B59 (drought-sensitive) lines. ABA catabolite content was much higher than ABA content in B71 dry seeds from plants grown under irrigation (Fig. 2A) and in B59 dry seeds from plants grown under both soil moisture conditions (Figs 2B and 3B). In contrast, ABA was abundant (6236 ± 289 pmol g^{-1}) in the B71 dry seeds from drought-grown plants (Fig. 3A).

ABA content decreased after germination in both B71 and B59 lines and in both soil moisture conditions (Figs 2 and 3), although the decrease in ABA content in B71 seeds from the irrigated plants was only moderate (Fig. 2A, C). Germination (or imbibition) triggered a substantial increase in ABA-GE content in seeds in all treatments (Figs 2 and 3).

PA content in dry seeds from the drought-grown B71 plants was approximately twofold lower compared to those in dry seeds from irrigated plants. DPA content was not modified by soil moisture conditions (Figs 2A and 3A). Approximately, a fourfold increase in DPA and threefold decrease in PA were found in seeds from the drought-grown B59 plants compared to those in dry seeds from irrigated plants (Figs 3B and 2B).

ABA and ABA catabolite contents in parts of dry and germinated seeds

ABA and its catabolites in the pericarp, embryonic axis and cotyledons of dry or germinated seeds were analysed in drought-sensitive B59 and drought-tolerant B71 lines. In B71, the pericarp and

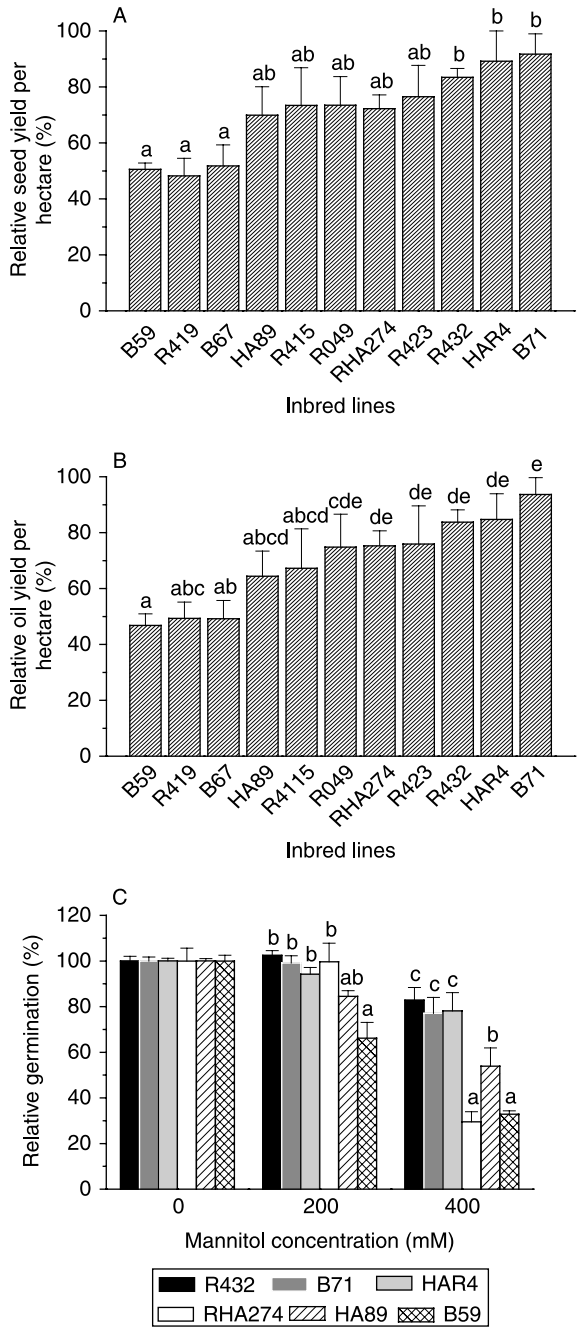

Figure 1. (A) Relative seed yield for inbred lines grown under drought versus irrigation in field studies during 2003–2004 and 2004–2005. (B) Relative oil yield for inbred lines grown under drought versus irrigation in field studies during 2003–2004 and 2004–2005. (C) Relative germination percentage of seeds from drought-grown inbred lines in mannitol. Data are means of five replicates. Values with the same letter are not significantly different ($P > 0.05$).

embryonic axis of dry seeds from irrigated plants contained very low ABA, with DPA being the major component (Fig. 4A, C). Germination (or possibly imbibition) caused a significant decrease of DPA in both tissues (Fig. 4A, C). In contrast, ABA was

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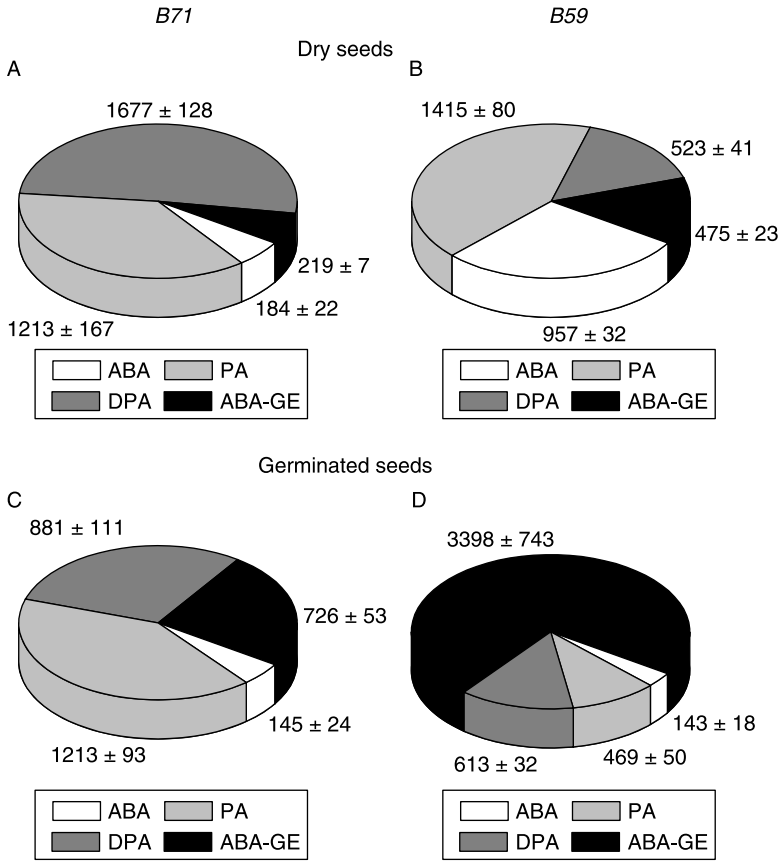

Figure 2. Ratio of ABA and its catabolites in dry and germinated seeds from the drought-tolerant B71 and drought-sensitive B59 lines grown under irrigation [values are expressed in pmol (g dry weight)$^{-1}$]. ABA, abscisic acid; ABA-GE, ABA glucose esters; DPA, dihydrophaseic acid; PA, phaseic acid.

predominant in the pericarp and embryonic axis of dry seeds from the drought grown B71 plants. ABA content in the embryonic axis was higher than that in the pericarp of drought-grown B71 dry seeds. ABA content in both tissues decreased dramatically after germination (Fig. 5A, C). DPA content was sevenfold higher in the embryonic axis compared to the pericarp (Fig. 5A, C). PA was the major component in cotyledons of dry seeds from both soil moisture conditions and ABA was almost undetectable. In cotyledons of germinated seeds from drought-grown plants, ABA, DPA and ABA-GE contents were higher than those in germinated seeds from plants grown under irrigation; however, PA remained at the same level (Figs 4E and 5E).

In B59, the pericarp of germinated seeds from irrigated plants showed a lower ABA content compared to that in dry seeds, whereas ABA-GE content was slightly higher in germinated seeds than that in dry seeds. No significant change was observed in PA content (Fig. 4B). In the pericarp of dry seeds from drought-grown B59 plants, ABA was predominant, which was drastically decreased after germination (Fig. 5B). This pattern was similar to that observed in dry seeds from the drought-grown B71 plants (Fig. 5A, B). ABA-GE was the main catabolite in embryonic axes in germinated seeds from irrigated plants (Fig. 4D). However, DPA was the major catabolite in the embryonic axes of dry seeds from drought-grown plants (Fig. 5D). In cotyledons of dry seeds from irrigated plants, PA was the most abundant catabolite (Fig. 4F), whereas ABA and DPA showed almost similar content in cotyledons of dry seeds from drought-grown plants (Fig. 5F). In cotyledons of germinated seeds from irrigated plants, ABA-GE content was threefold higher compared to that in the cotyledons of germinated seeds from drought-grown plants (Figs 4F and 5F). Germinated seeds from irrigated plants accumulated mainly ABA-GE in the embryonic axis and cotyledons (Fig. 4D, F).

Germination of B59 and B71 seeds

The germination time-courses during early hours of imbibition were quite different between B71 and B59 lines (Fig. 6). At 24 h, more than 70% of B71 seeds from both drought-grown and irrigated plants germinated. On the contrary, germination of B59 seeds from the plants grown under both soil conditions was very low

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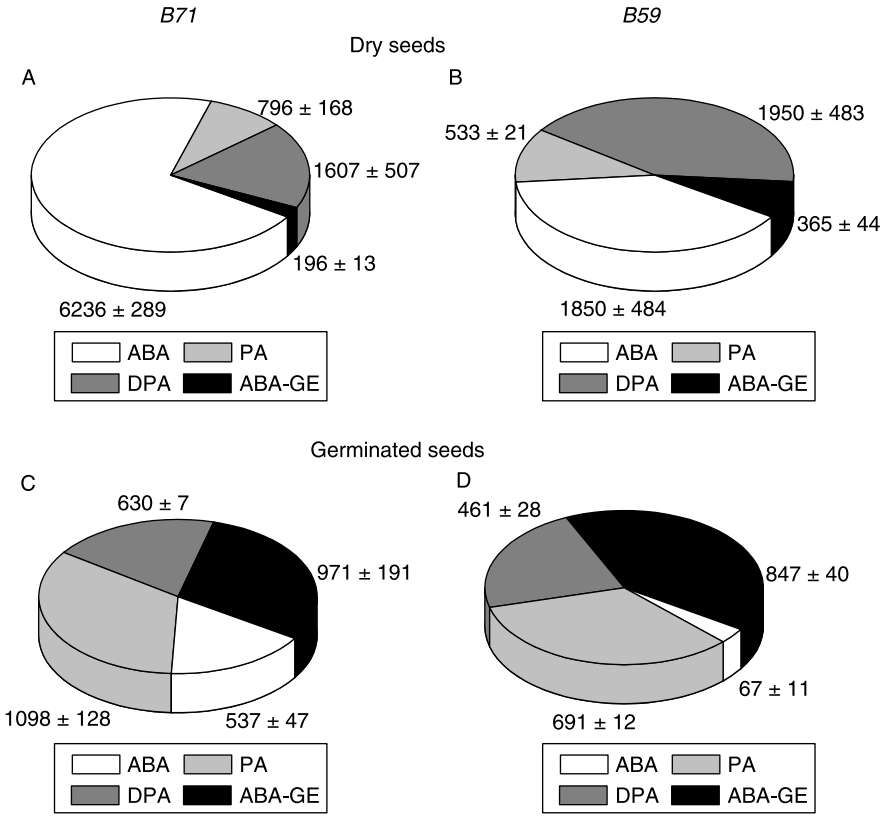

Figure 3. Ratio of ABA and its catabolites in dry and germinated seeds from the drought-tolerant B71 and drought-sensitive B59 lines grown under drought [values are expressed in pmol (g dry weight)$^{-1}$]. ABA, abscisic acid; ABA-GE, ABA glucose esters; DPA, dihydrophaseic acid; PA, phaseic acid.

at 24 h of imbibition. After 48 h, all groups of seeds reached a high germination percentage, although the germination percentage of B59 seeds from irrigated plants was slightly lower than that of other seeds.

Discussion

Sunflower production is expanding to the arid regions of the world, where it faces the challenges of low rainfall and the use of salt-contaminated water for irrigation. Tolerance of crop plants to drought is genetically determined and involves a wide range of processes. Among them, differences in stress-related hormone levels have been suggested (Pedranzani *et al.*, 2003, 2007; Mahajan and Tuteja, 2005; Perales *et al.*, 2005; Andrade *et al.*, 2008).

The great genetic diversity of sunflowers can be exploited for selection of stress-tolerant lines based on specific phenotypic markers. Sunflower genotypes were selected for drought tolerance indirectly by high achene yield on the basis of head diameter and 100 achene weight (Tahir *et al.*, 2002). In the present study, it was demonstrated that physiological efficiency of various sunflower lines, in terms of the sensitivity to drought, can be differentiated by monitoring relative oil yield, seed yield and germination percentages under stress conditions. These parameters were able to distinguish the drought-tolerant B71 and drought-sensitive B59 lines.

In a previous study we reported the highest accumulation of jasmonates in the pericarp, followed by the embryonic axis and cotyledons of sunflower B71 and B59 dry seeds. Likewise, we found that jasmonate profiles varied during seed germination and that the environmental conditions experienced by the mother plant modified the hormonal content of the seed progeny (Vigliocco *et al.*, 2007).

ABA is known to play a key role in adaptation of plants to drought (Mahajan and Tuteja, 2005; Zhang *et al.*, 2006). Comparisons between stress-tolerant and stress-sensitive cultivars within the same species have shown higher ABA levels in tolerant cultivars. The degree of stress tolerance of a given line appears to be related to the different rate of ABA synthesis under stress (Perales *et al.*, 2005).

In the present study, the ABA accumulation observed in dry seeds of B71 and B59 grown under drought or irrigation conditions confirms that ABA metabolism is responsive to drought. The differences observed in the ABA catabolite profiles between B71

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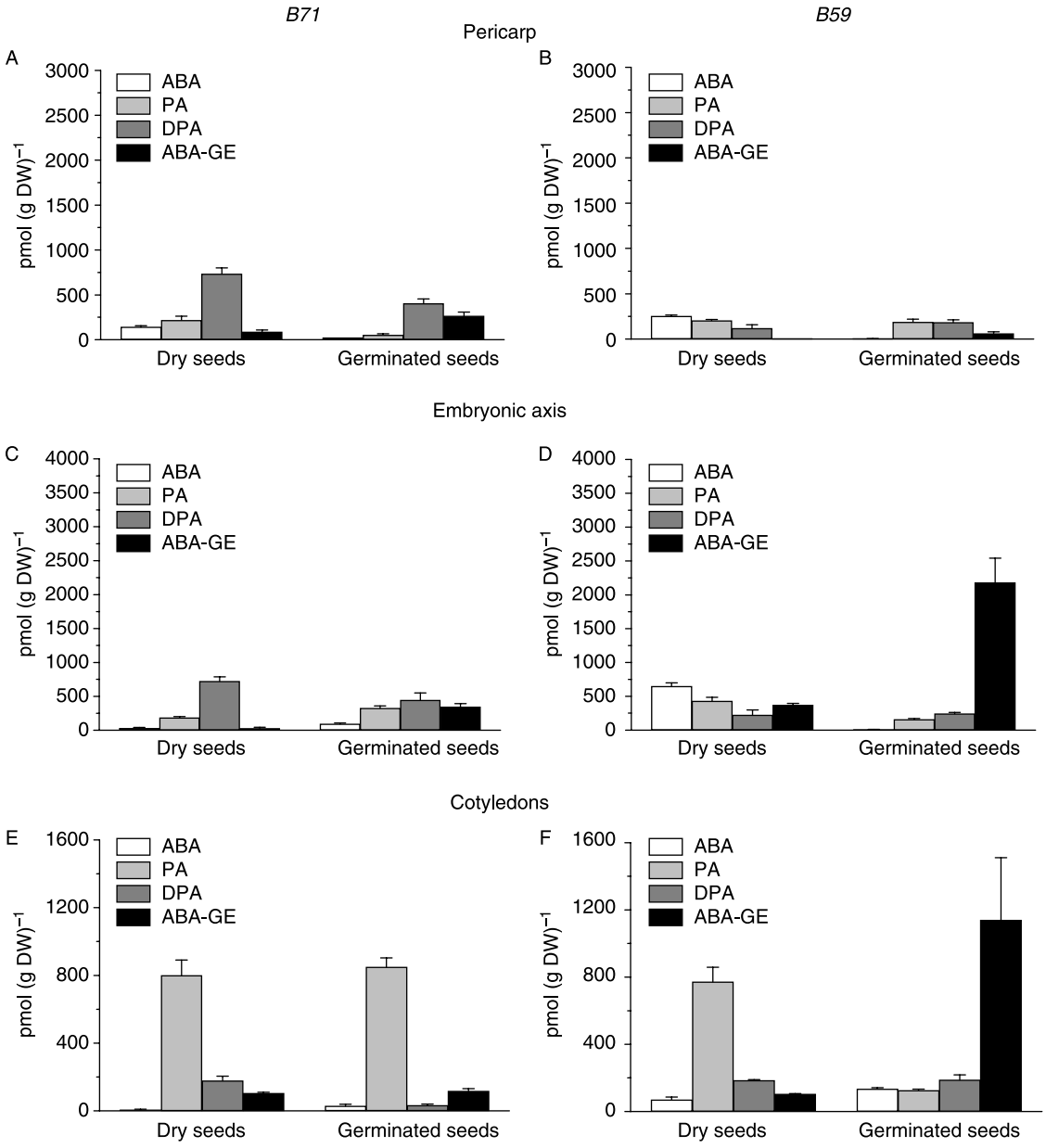

Figure 4. ABA catabolite content in pericarp, embryonic axis and cotyledons of dry and germinated seeds of drought-tolerant B71 and drought-sensitive B59 lines grown under irrigation. Data are means of three replicates with SEs, $P \leq 0.05$. ABA, abscisic acid; ABA-GE, ABA glucose esters; DPA, dihydrophaseic acid; PA, phaseic acid.

and B59 dry seeds from irrigated plants suggest that stress tolerance of a given line grown under irrigated conditions is related not only to endogenous ABA content but also to different rates of ABA synthesis and catabolism. This is consistent with previous reports comparing stress-sensitive and stress-tolerant cultivars in different species (Zheng and Li, 2000; Chen *et al.*, 2002; Perales *et al.*, 2005). In addition, in our work a slight difference in germination speed was observed between B71 and B59 seeds. The high percentage of B71 line germination in the early hours of imbibition could contribute to better seedling emergence and establishment. ABA content in sunflower seeds did not necessarily correlate with germination performance, which is not consistent with results from other species. Studies using *Arabidopsis* (Ali-Rachedi *et al.*, 2004), lettuce (Toyomasu *et al.*, 1994), barley (Millar *et al.*, 2006) and tomato (Andrade *et al.*, 2008) demonstrated a correlation between ABA levels in imbibed seeds and seed germinability.

The ABA distribution among different parts of sunflower dry seeds showed that ABA accumulates in the pericarp and embryonic axis, as does jasmonates (Vigliocco *et al.*, 2007). High ABA content in sugar beet pericarp was also reported by Hermann *et al.* (2007). Pericarp composition is determined by genetic factors,

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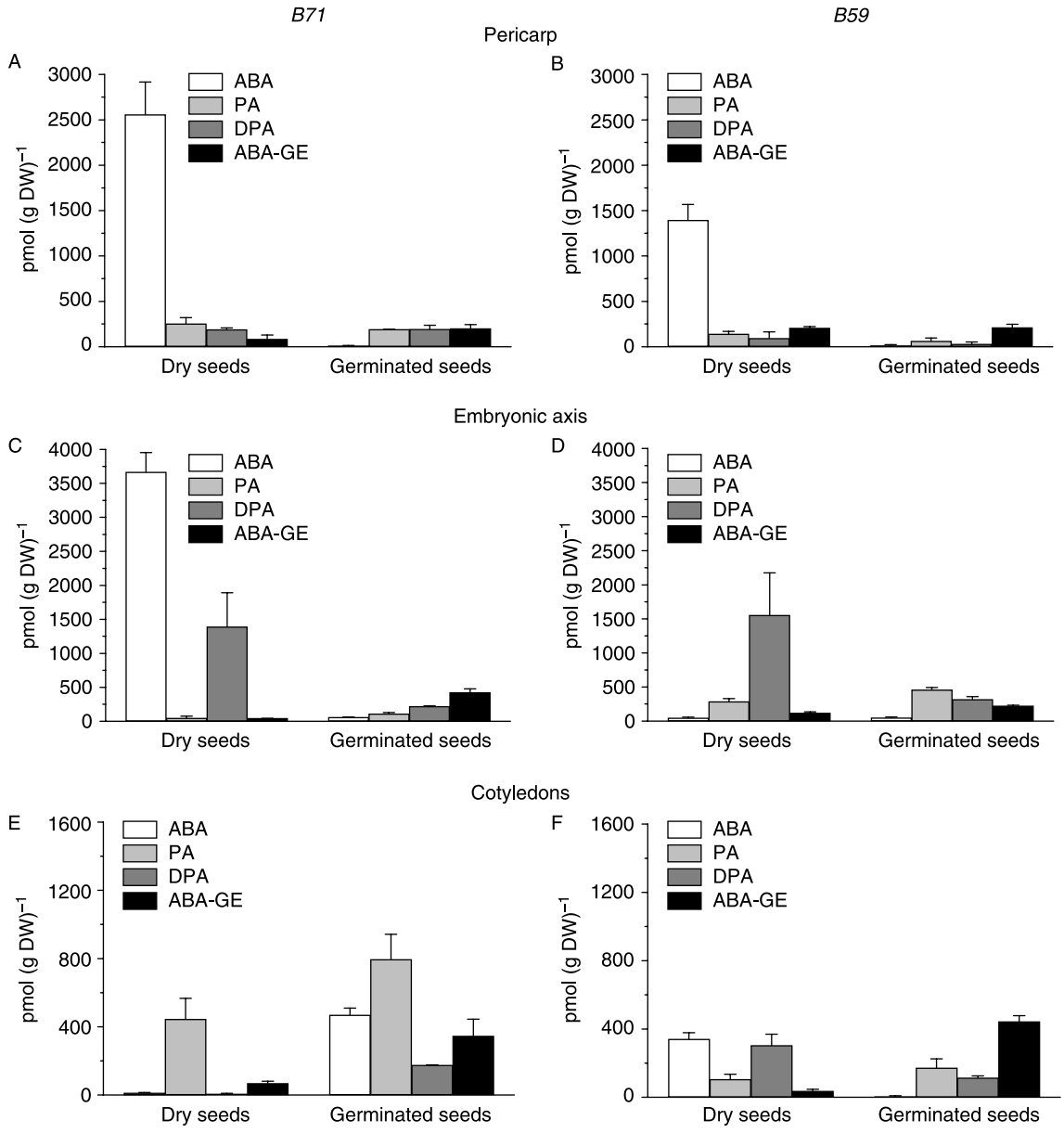

Figure 5. ABA catabolite content in pericarp, embryonic axis and cotyledons of dry and germinated seeds of drought-tolerant B71 and drought-sensitive B59 lines grown under drought. Note that different scales are used in the panels. Data are means of three replicates with SEs, $P \leq 0.05$. ABA, abscisic acid; ABA-GE, ABA glucose esters; DPA, dihydrophaseic acid; PA, phaseic acid.

although its structural properties are also affected by environmental growth conditions (Dennis, 2004; Rondanini *et al.*, 2006) or crop management (Baldini and Vannozzi, 1996). We found higher ABA content in the pericarp of dry seeds from drought-grown sunflower than in the pericarp of seeds from irrigated plants, which suggests that the stress condition affected ABA accumulation in the pericarp. In contrast, when the seeds were germinated, there was no difference in ABA content that was relevant to germination in seed parts from drought-grown plants. Thus, the ABA accumulation observed in dry seeds from drought-grown plants may not be important for germination capacity and could be simply interpreted as a consequence of the stress imposed on mother plant.

These results suggest that the soil moisture conditions under which the mother plants were grown affected the hormonal content of seeds, as reported previously (Benech Arnold *et al.*, 1991; Amzallag *et al.*, 1998; Vigliocco *et al.*, 2007). Chono *et al.* (2006) found that environmental conditions surrounding the mother plant during seed development, such as rainfall, humidity and temperature, affected the expression of *HvNCED1*, an ABA biosynthesis gene and *HvCYP707A1*, an ABA deactivation gene in barley grains, resulting in different ABA content.

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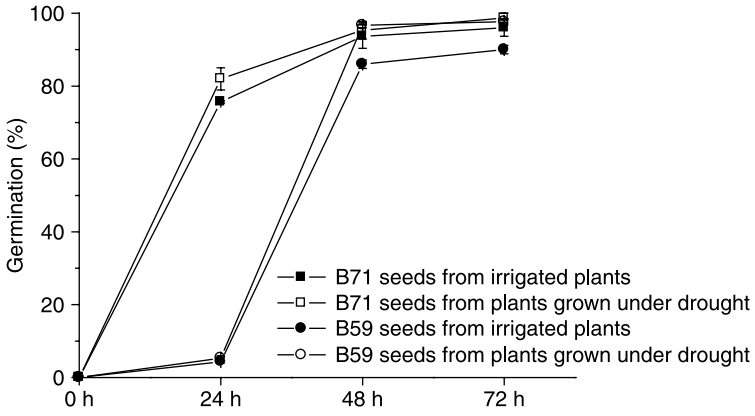

Figure 6. Germination time course of drought-tolerant B71 and drought-sensitive B59 sunflower seeds under controlled conditions (16 h light, 130 μE m^2 s^{-1}; 28°C; 60% relative humidity/8 h dark; 18°C; 70% relative humidity). Data are means of three replicates with SEs, $P \leq 0.05$.

Previous work indicated that ABA accumulation depends not only on accelerated ABA biosynthesis but also on ABA deactivation, including catabolism and conjugation (Ren *et al.*, 2007). The differences in the content of ABA and its catabolites found in sunflower dry seeds from the two soil moisture conditions, and the dramatic changes in the content of these compounds in *Arabidopsis* plants under drought stress (Huang *et al.*, 2008), confirm the close association between drought stress and ABA metabolism.

The accumulation of ABA catabolites (PA and DPA) in sunflower dry seed embryonic axis and cotyledons suggests that ABA catabolism is an active pathway in these parts during seed development. ABA catabolism to PA and DPA is also active in plant tissues of other species, including apple seeds, sweet cherry fruit and seeds (Setha *et al.*, 2005) and suspension-cultured maize cells (Balsevich *et al.*, 1994). The accumulation pattern of ABA catabolites is also dependent on the half-life of each catabolite. In particular, DPA is also converted into the conjugate form, which is not quantified in the present study. The low ABA-GE contents found in the pericarp, embryonic axes and cotyledons of B59 and B71 dry seeds from both drought-grown and irrigated plants suggest that ABA conjugation during seed development is not a major ABA deactivation pathway. The general decrease in ABA content following sunflower seed germination suggests activation of catabolic pathways during imbibition, in agreement with previous reports (Jacobsen *et al.*, 2002; Kushiro *et al.*, 2004; Zhou *et al.*, 2004; Nambara and Marion-Poll, 2005).

In the present study, seed germination (most likely imbibition) decreased ABA and increased ABA-GE. Therefore, glucosylation may be an active ABA deactivation pathway in imbibing sunflower seeds. ABA-GE was the main ABA catabolite in germinating lettuce seeds (Chiwocha *et al.*, 2003) and in germinating wild-type *Arabidopsis* seeds (Chiwocha *et al.*, 2005). ABA-GE has been considered to be a reversible conjugate of ABA (Dietz *et al.*, 2000) and to contribute to ABA homeostasis (Sauter *et al.*, 2002). If this is the case, germination (and normal growth) may require ABA to be distributed in growing tissues in a physiologically inactive form and accumulate in vacuoles, from which it can be released rapidly under adverse environmental conditions (Lee *et al.*, 2006). ABA-GE is the best candidate for a hormonal stress signal over long distance (Jiang and Hartung, 2007).

Under normal conditions, ABA accumulation is restricted by active degradation; changes in ABA content do not always vary in parallel to the ABA conjugate content (Nambara and Marion-Poll, 2005). Consistent with this, our results suggest that the decrease in endogenous ABA during imbibition could be related, at least in part, to its conjugation to ABA-GE. Besides conjugated ABA the changes in PA and DPA content confirm that activation of the 8′-hydroxylation pathway occurs in sunflower germinated (or germinating) seeds. In addition, the decrease in ABA during seed imbibition of barley and *Arabidopsis* is associated with increased PA (Jacobsen *et al.*, 2002; Kushiro *et al.*, 2004). On the other hand, PA was a minor component in germinated seeds of tomato cv. Moneymaker. This may be explained by its rapid reduction to DPA (Andrade *et al.*, 2008), as was observed for germinating seeds of *Brassica napus* (Zhou *et al.*, 2003). Thus, ABA catabolic profiles will help define the relative importance of competing pathways, conversion efficiencies within pathways and the relationship between ABA metabolism, environmental conditions and development (Zhou *et al.*, 2003).

In summary, we conclude that the environmental conditions experienced by the mother plant modified the contents of ABA and its catabolites in seed progeny, although they affected seed germination performance *per se* only slightly under our conditions.

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Such hormone profiles also differed between sunflower drought-tolerant and drought-sensitive lines.

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