Dehydrin-like proteins in castor bean seeds and seedlings are differentially produced in response to ABA and water-deficit-related stresses

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Abstract

The stress inducibility of dehydrin protein production in seedlings of castor bean was analysed by subjecting them to ABA and various water-deficit-related treatments including desiccation, water stress, high salt, high osmolarity, and low temperature. A further goal was to determine whether the immature seed (at stages prior to major dehydrin synthesis) would respond in a similar manner to these stresses. A number of dehydrin-like proteins increased in seedlings subjected to the various stress treatments. In the endosperm, these appear to be different from the dehydrin-related polypeptides that are induced during late seed development and which persist following germination/growth of mature seeds. In the endosperm of seedlings, ABA, water stress and desiccation induced the same dehydrin polypeptides, while high osmolarity, high salt and low temperature induced a different set. Stress-specific differences in dehydrin synthesis were also found in the cotyledons and radicle of castor bean seedlings; however, dehydrins inducible by exogenous ABA were consistently produced. Immature seeds treated with ABA or subjected to stress responded by producing dehydrin-like proteins associated with late development; however, the same proteins were induced following detachment of immature seeds from the parent plant and maintenance on water. When seedlings were exposed simultaneously to GA and either ABA, high salt, or low temperature, dehydrin production was suppressed. It is concluded that dehydrin production in castor bean is tissue-specific and is dependent upon the physiological stage of the seed. In the endosperm, the response to different stresses may rely upon more than one signal transduction pathway.

Key words: Dehydrin, castor bean, ABA, desiccation.

Introduction

Plants undergo a series of physiological, biochemical and molecular changes in response to adverse environmental conditions or stresses such as drought, low temperature or high salt. Following exposure of the plant to a water-related stress for a few days (or several hours depending on the plant or tissue), a group of proteins are induced that are presumed to play a protective role. This group of proteins, termed dehydrins (a subset of the proteins termed LEAs; Late Embryogenesis Abundant or RABs; Responsive to ABA) have some common features in their structure. In particular, they are characterized by a conserved 15-amino acid, lysine-rich sequence near the carboxyl terminus. This consensus polypeptide forms an amphiphilic α-helix which may serve as an ion trap in dehydrating cells, sequestering ions that become concentrated (Close et al., 1993a; Dure, 1993). The elevated levels of endogenous ABA, which often accompany stress imposition, are thought to induce dehydrin gene expression (reviewed in Bray, 1991; Chandler and Robertson, 1994; Kermode, 1995). However, the extent to which expression is regulated (directly or indirectly) by ABA alone, or whether other factors are important requires further investigation. ABA and water-deficit-related stresses may affect plant molecular processes through different pathways and induce different dehydrin genes. In Arabidopsis thaliana, there may be at least two independent signal transduction pathways between the environmental stress and the expression of dehydrin genes—one which is ABA-independent and the other ABA-responsive (Yamaguchi-Shinozaki and Shinozaki, 1994). Further, antagonizing factors, such as GA may also play
an important role in regulating the expression of dehydrin genes. For example, in barley aleurone protoplasts, GA suppresses the ABA-induced increase in transcription of a dehydrin gene (M Robertson, personal communication). It remains to be determined whether similar interactions occur between GA and ABA in other tissue types and if GA is capable of suppressing dehydrin synthesis elicited by water-deficit-related stresses.

Lea genes exhibit a flexible expression repertoire, being responsive to both developmental and environmental cues (reviewed in Thomas et al., 1991). In addition to being expressed under abnormal (stress) conditions, they exhibit temporal regulation during seed development where expression is most intense during mid-to late-development. Thus, the protective role of dehydrins in the survival of water loss is purported to be dual: during maturation drying of the developing seed and following germination/growth of the mature seed (i.e. in seedlings or plant vegetative tissues undergoing mild water stress).

In the present study, the stress inducibility of dehydrin-like protein production in seedlings of castor bean was examined by subjecting them to ABA and various water-deficit-related treatments including desiccation, water stress, high salt, high osmolarity, and low temperature. In several species, dehydrins are induced during the post-germinative stage when seedlings encounter water-deficit-related stresses. A further goal was to examine the response of the immature seed to the same water-deficit-related stresses when the stress is imposed at stages prior to the onset of major dehydrin synthesis associated with later stages of seed development. For these studies, a dehydrin antibody was used (Close et al., 1993a), directed against a synthetic polypeptide corresponding to a conserved sequence found in many dehydrins. The assumption was made that castor bean dehydrins contain the conserved sequence. However, this is not a certainty since no dehydrin genes from castor bean have been sequenced to date. Stress-specific differences in dehydrin synthesis were found in different organs/tissues of castor bean seedlings. In the endosperm of seedlings, ABA, water stress and desiccation induced the same dehydrin-like polypeptides, while high osmolarity, high salt and low temperature induced a different set. Dehydrin-like proteins inducible by exogenous ABA were consistently produced in the cotyledons and radicle of stressed seedlings. Immature seeds at 30 d after pollination (DAP) produced dehydrins characteristic of late seed development within the endosperm following the ABA or water-deficit-related treatments. However, the induction of this new pattern of synthesis was likely a consequence of seed detachment from the parent plant. The effects of GA on dehydrin induction in mature seedlings exposed to ABA and the water-deficit-related treatments were also studied. When seedlings were exposed simultaneously to GA$_3$ and either ABA, high salt, or low temperature, production of dehydrin-like polypeptides was suppressed.

Materials and methods

Plant material

Ricinus communis L. plants cv. Hale were grown in the field from seed at Simon Fraser University on Burnaby Mountain, BC. Seeds were collected at 30 days after pollination (DAP) and at maturity (60 DAP). The staging of seed was according to Greenwood and Bewley (1982). Endosperms were obtained from 30 DAP seeds subjected to the various treatments outlined below. Endosperms, cotyledons and radicles were obtained from 5-d-old seedlings (obtained by germinating mature seed) subjected to the same treatments.

Seed germination

Mature seeds were surface-sterilized in a solution of 1% hypochlorite, 0.01% SDS for 10 min, and then rinsed three times in sterilized water. The seed coats were then removed under aseptic conditions in a laminar flow hood. Seeds were placed in Petri dishes containing sterile filter paper moistened with 10 ml of sterile water and allowed to germinate at 26°C in the dark. After 5 d, seedlings were used for the various treatments.

ABA treatments

Five-day-old seedlings were incubated for 7 d in different concentrations of ABA ($10^{-3}$ M, $10^{-4}$ M, $10^{-5}$ M, and $10^{-6}$ M). For these treatments, the seedlings were placed in Petri dishes containing sterile filter paper and 10 ml of the different ABA solutions (sterilized by passing through a 0.22 µm filter). The 7 d treatment was carried out under ambient laboratory conditions.

Water stress treatment

Developing seeds and 5-d-old seedlings were subjected to water stress by equilibrating them in a desiccator over 25% glycerol for 7 d. The osmotic potential of the glycerol solution was determined to be -12.6 MPa, which produces a RH value in the chamber of approximately 87%.

Desiccation treatment

Developing seeds and seedlings were dried slowly to known percentage water contents by placing them in a desiccator over stirred saturated salt solutions. The drying regime used for 30 DAP seeds and for 5-d-old seedlings was as described in Kermode and Bewley (1985). This involved placing seeds or seedlings for 1 d sequentially in desiccators containing stirred saturated salt solutions maintaining RH values of 92, 86 (2 d), 74, 65, 44, and 22%. For both immature seeds and seedlings, the desiccation treatment resulted in a much faster rate of water loss than the water stress treatment and the extent of water loss at the end of the 7 d period was greater. For example, seedlings underwent the greatest loss of FW (approximately 40%) over the first 4 d of the desiccation treatment; the water-stress treatment resulted in very gradual water loss over 7 d in which 25% of the original FW was lost.

High salt and high osmolarity treatments

Developing seeds and 5-d-old seedlings were placed in Petri dishes containing sterile filter paper moistened with either 10 ml of 0.2 M NaCl (high salt treatment) or 10 ml of 0.3 M mannitol.
(high osmolarity treatment). Both solutions were previously sterilized by passing through a 0.22 μm filter. The treatment was carried out for 7 d under ambient laboratory conditions.

Cold treatment

Developing seeds and 5-d-old seedlings were placed in Petri dishes containing sterile filter paper moistened with 10 ml of sterile water, and then maintained at 4°C for 7 d.

GA$_3$ treatment

To determine the effect of exogenous GA$_3$ on the response to cold treatment, 5-d-old seedlings were placed in Petri dishes containing sterile filter paper moistened with 10 ml of the following solutions: ABA (10$^{-3}$ M), GA$_3$ (10$^{-4}$ M); NaCl (0.2 M) + GA$_3$ (10$^{-4}$ M); mannitol (0.3 M) + GA$_3$ (10$^{-4}$ M). All solutions were sterilized by passing through a 0.22 μm filter. The treatments were carried out for 7 d under ambient laboratory conditions.

Extraction of heat-soluble proteins and Western blot analysis

Heat-soluble proteins were extracted from endosperms isolated from immature seeds and seedlings, and from cotyledons and radicles isolated from seedlings as outlined in Close et al. (1993a). Briefly, total soluble proteins were extracted by grinding tissues in 30 mM TES buffer pH 8.0, containing 20 mM NaCl and 1 mM PMSF using a ground glass homogenizer. This was followed by centrifugation at top speed in a microcentrifuge for 15 min at 4°C. The supernatants were boiled at 100°C for 10 min, kept on ice and then centrifuged as before. Protein concentrations in the supernatants were determined by the Bio-Rad protein assay (Bradford, 1976) using bovine serum albumin as a standard. Aliquots containing equal amounts of heat-soluble proteins were precipitated with 4 vols of acetone and then resuspended in SDS-sample buffer (0.125 M TRIS-HCl, pH 6.7, 2% SDS, 10% glycerol, 0.003% bromophenol blue, and 5% β-mercaptoethanol). The mixture was heated for 10 min at 95°C, cooled and then fractionated by SDS-PAGE on 12% gels using a Hoefer Model SE 280 apparatus, according to the method of Laemmli (1970). After electro blotting on to nitrocellulose (using a BioRad Trans-Blot Semi-Dry Transfer Cell), dehydrin proteins were detected with rabbit anti-dehydrin serum (diluted 1/6000), followed by goat anti-rabbit IgG (Promega, Madison, W1) conjugated with alkaline phosphatase. The anti-dehydrin serum was produced from a synthetic polypeptide containing a highly conserved sequence (KIKEKLPG) (kindly provided by TJ Close) (Close et al., 1993a, b). The pre-immune serum was utilized as a control.

Results

Dehydrin-like proteins induced in developing seeds and in seedlings of castor bean by ABA and different water-deficit-related treatments

The stress inducibility of dehydrin protein synthesis in immature seeds of castor bean was analysed by submitting them to ABA and various water-deficit-related treatments including desiccation, water stress, high osmolarity, high salt, and low temperature. Further examination was carried out to determine whether the dehydrin-like proteins induced in immature seeds were similar to those induced following the transition to germination and growth (i.e. in seedlings subjected to stress or ABA treatment). Figure 1A shows dehydrin-related proteins produced in the endosperm of developing fresh seeds at 30 DAP and those produced in the mature dry (60 DAP) seed. The Western blots are of heat-soluble proteins (rather than total soluble proteins) since many heat-soluble proteins have been demonstrated to be LEAs (Jacobsen and Shaw, 1989). At 30 DAP, prominent bands on the Western blot had $M_r$ of 25, 32, 46, and 60 kDa. Of these, only the 25 kDa products are dehydrin-related polypeptides; the others were detected on control Western blots using the pre-immune serum (Fig. 1C). This profile of dehydrin production persists within the developing endosperm until at least 40 DAP (Han et al., 1995). In the mature dry seed at 60 DAP, the nature of the dehydrin-related polypeptides accumulated in the endosperm changed dramatically. In particular, new polypeptides were detected with $M_r$ of 28–30, 33 and 41 kDa (Fig. 1A). Figure 1B shows the effect of exogenous ABA and the various stress treatments on dehydrin accumulation in developing 30 DAP castor bean seeds. Most of the dehydrin-like proteins produced in response to ABA or the various stress treatments in the endosperm (that do not appear on control blots, using the pre-immune serum; Fig. 1C) were similar to those induced during normal late development, and are prominent in the mature dry (60 DAP) seed (Fig. 1A). However, the induction of this late developmental pattern may not be due to the stress imposed, but rather the period of detachment from the parent plant required to effect the treatment. For example, a similar profile of dehydrin-like proteins was induced in 30 DAP seeds detached and hydrated on water for the same period required to effect the stress treatments (Fig. 1B; H$_2$O).

The dehydrin-related polypeptides that are prominent within mature dry seeds of castor bean persist during germination/growth for at least 48–72 h after the start of imbibition (Han et al., 1995). To determine whether these polypeptides increase in seedlings subjected to stress or to exogenous ABA, 5-d-old castor bean seedlings were treated with different concentrations of ABA (10$^{-3}$ to 10$^{-5}$ M). As shown in Fig. 2, dehydrin-related polypeptides of 48 kDa were induced in the endosperm by ABA and were different in size from those produced during late seed development. In all three tissues of the treated seedlings (endosperm, cotyledons and radicle; Fig. 2), ABA applied at concentrations less than 10$^{-5}$ M was ineffective in eliciting dehydrin production. As in the endosperm, Western blot analysis detected polypeptides of similar molecular weights (48 kDa) in the cotyledons.
Fig. 1. Changes in dehydrin-related proteins in the endosperm of immature (30 days after pollination, DAP) and mature (60 DAP) castor bean seeds (A) and in immature seeds subjected to ABA and water-deficit-related stresses (B). (A) and (B) are Western blots of heat-soluble proteins (64 µg in 32 µl sample buffer containing 5% (v/v) β-mercaptoethanol) using a rabbit antibody to detect dehydrin-related polypeptides. Numbers on the right indicate the approximate molecular weights of the dehydrin-related polypeptides. Polypeptides found on control Western blots using pre-immune serum are shown in (C). Refer to Materials and methods for details on the stress treatments imposed. (D, desiccation; WS, water stress; ABA, abscisic acid at 10^{-5} M; Man, mannitol.)

of seedlings subjected to ABA treatment; in contrast, those produced in radicles were 51 and 32 kDa.

Different dehydrin genes may be induced by different stresses and these responses may also be tissue-specific. In barley seedlings, three lea mRNAs respond differently to salt, cold, mannitol, and ABA (Espelund et al., 1992). These mRNAs also show different expression patterns during seed development. To determine differences between the responses of immature seeds versus seedlings, the stress-inducibility of dehydrin production in seedlings was examined and also whether the various stress treatments would induce a similar set of dehydrin-related polypeptides as the exogenous ABA treatment. Two distinct patterns of dehydrin production were elicited in the endosperms of castor bean seedlings subjected to the different stresses for 7 d, in which there were both quantitative as well as qualitative differences (Fig. 3, Endosperm). Western blot analysis showed the induction of one set of dehydrins (48 kDa) in response to ABA, desiccation and water stress; another set, in which proteins of 43 kDa were particularly prominent, was elicited by the high osmoticum (mannitol), high salt, and low temperature treatments. Production of some of the 43 kDa proteins also occurred following water stress and desiccation, albeit in a lower amount; in ABA-treated seedlings these proteins were barely detectable. Similarly, other dehydrin-related proteins (36 kDa) were common to most of the water-related stresses examined, although they were produced in much higher amounts in response to high salt and low temperature. Consistent with this, the combination of ABA and high osmoticum (ABA and Man, Fig. 3) resulted in the production of the 36, 43 and 48 kDa proteins in the endosperm.

It is possible that ABA, desiccation and water stress share one signal transduction pathway which is ABA-dependent. On the other hand, high salt, high osmoticum, and cold may induce other dehydrin genes via an additional signal transduction pathway that is ABA-independent. However, whether ABA accumulates in the endosperm during the latter treatments was not assessed.

Somewhat different results were found in relation to dehydrin production in the cotyledons and radicles of the stressed castor bean seedlings as compared to the ABA-treated seedlings. Here, most of the differences in dehydrin production appeared to be quantitative, rather than qualitative. In the cotyledons, all of the water-deficit-related stresses elicited the production of the 48-kDa polypeptides (similar to exogenous ABA treatment), and to a lesser extent, proteins of 36 kDa. Additional polypeptides of 30 kDa (induced in mannitol-treated seedlings) and 18 kDa (particularly evident in the seedlings subjected to desiccation, water stress and the combination of ABA and mannitol; Fig. 3) were also induced in response to ABA at 10^{-4} M (Fig. 2, Cotyledons). Proteins of 43 kDa appeared to be elicited exclusively in the cotyledons of seedlings treated with high salt or the combination of ABA and mannitol. Like exogenous ABA treatment, the various water-related stress treatments induced the
Fig. 2. Changes in dehydrin-related proteins in the endosperm, cotyledons and radicle of castor bean seedlings following treatment with different exogenous ABA concentrations. Western blots are of heat-soluble proteins extracted from the endosperm, cotyledons and radicle of seedlings subjected to different concentrations of ABA (10^{-3} to 10^{-6} M). Samples were loaded with equal protein as in Fig. 1. Numbers on the right indicate the approximate molecular weights of the dehydrin-related polypeptides detected by the rabbit antibody. Polypeptides detected on control Western blots using pre-immune serum are also shown (Control). Lane 1: Endosperm; Lane 2: Cotyledons; Lane 3: Radicle. (For the pre-immune controls, all samples are from seedlings subjected to ABA at 10^{-5} M.)

production of 51- and 32-kDa proteins in the radicle (Fig. 3, Radicle). Proteins of 25 kDa were particularly prominent in the radicles of seedlings subjected to desiccation and water stress, but these were also detected (faintly) on control Western blots using pre-immune serum (data not shown).

Effects of GA3 on dehydrin production in castor bean seedlings elicited by ABA and water-deficit-related stresses

In order to address the possible role of ABA in mediating plant responses to water-deficit-related stresses further, the effects of an antagonist of ABA action, i.e. gibberellic acid (GA3) were examined. Figure 4 shows the effects of GA3 on dehydrin induction when castor bean seedlings were exposed simultaneously to GA, and either ABA, high mannitol, high salt, or low temperature. In the endosperm, the production of the 48-kDa dehydrins elicited following exposure of seedlings to exogenous ABA, water stress, and desiccation (Fig. 3), was suppressed when seedlings were exposed simultaneously to ABA and GA3 (Fig. 4). Salt and low temperature induced dehydrin-related proteins of 36 and 43 kDa in relatively high amounts in the endosperm (Figs 3, 4); production of
Fig. 3. Changes in dehydrin-related proteins in the endosperm, cotyledons and radicle of castor bean seedlings following ABA treatment and different water-deficit-related treatments. As a control, seedlings were kept hydrated on water for the same length of time required to effect the ABA or stress treatment (H₂O). Samples were loaded with equal protein as in Fig. 1. Numbers on the right indicate the approximate molecular weights of the dehydrin-related polypeptides detected by the rabbit antibody; only those polypeptides not found on control Western blots using pre-immune serum are indicated. An exception is the 25 kDa protein in radicles that is barely detectable on control blots of water stressed/desiccated seedlings. (D, desiccation; WS, water stress; ABA, abscisic acid at 10⁻⁵ M; Man, mannitol.)

Fig. 4. Effect of GA₃ on the production of dehydrin-related proteins in the endosperm of castor bean seedlings in response to ABA treatment and water-deficit-related treatments. Western blots are of heat-soluble proteins extracted from the endosperms of seedlings exposed to ABA or the stress treatment alone or from the endosperms of seedlings simultaneously exposed to GA₃ and either ABA or the water-deficit-related stress (cold, high salt or mannitol). Samples were loaded with equal protein as in Fig. 1. Numbers on the right indicate the approximate molecular weights of the dehydrin-related polypeptides detected by the rabbit antibody; only those polypeptides not found on control Western blots using pre-immune serum are indicated. (ABA, abscisic acid at 10⁻⁵ M; Man, mannitol; GA, GA₃ at 10⁻⁴ M.)

both these proteins was effectively suppressed when GA₃ was applied simultaneously with the stress treatment (Fig. 4). An exception occurred in seedlings treated with mannitol, in which GA₃ was relatively ineffective in preventing the production of the 36 and 43 kDa proteins. A similar GA-suppression of dehydrin production occurred in the cotyledons and radicle of stressed seedlings (data not shown).

Discussion

In the present study, the stress inducibility of dehydrin protein production was examined in immature seeds and seedlings of castor bean by subjecting them to ABA and various water-deficit-related treatments including desiccation, water stress, high salt, high osmolarity, and low temperature. In castor bean, production of dehydrin-related polypeptides was tissue-specific, dependent upon the physiological stage of the seed and exhibited some qualitative and quantitative differences in response to different water-deficit-related stresses. In several other species, dehydrin production exhibits tissue/organ-specificity. For example, in wheat plants, dehydration leads to the production of LEA proteins exclusively within the shoot (Reid and Walker-Simmons, 1993), with no synthesis occurring in the roots. However, in other plants or seeds, dehydrin/LEA proteins are present in all tissue types (e.g. in wheat seedlings and in embryos of maize and cotton) (Danyluk et al., 1994; Asghar et al., 1994; Roberts et al., 1993). Several researchers have restricted their analyses of dehydrin gene expression to specific tissue types and to certain stress conditions and it is not yet clear whether dehydrin genes which are expressed in cells of different tissues respond equally to ABA and water-deficit-related stresses.

Stress-specific differences in dehydrin synthesis were found in different organs/tissues of castor bean seedlings; however, dehydrins inducible by exogenous ABA were
consistently produced in the cotyledons and radicle of stressed seedlings. On the other hand, two distinct patterns of dehydrin production were elicited in the endosperms of castor bean seedlings subjected to the different stresses—one in response to ABA, desiccation and water stress; another was elicited by the high osmoticum (mannitol), high salinity, and low temperature treatments. Although no conclusions can be made about the differential induction of specific dehydrin genes in castor bean, others have found that ABA and water-deficit-related stresses affect plant molecular processes through different pathways. In Arabidopsis thaliana, two dehydration responsive genes (rd29A and rd29B) respond differently to ABA, drought, cold, and salt (Yamaguchi-Shinozaki and Shinozaki, 1994). The authors propose the existence of at least two independent signal transduction pathways (one which is ABA-independent and the other ABA-responsive) between the environmental stress and the expression of the two genes. Both pathways are involved in the expression of one dehydration responsive gene (rd29A); the other (rd29B) requires only the ABA responsive pathway for induction. For example, the rd29A gene has at least two cis-acting elements. One appears to be involved in an ABA-associated slow response to dehydration (ABRE), and the other may function in ABA-independent rapid induction (DRE). The stresses also function differently; salt and drought may affect gene expression by the ABA-dependent pathway, while cold and drought stimulate gene expression by the ABA-independent pathway. Further support for two pathways in A. thaliana comes from studies of ABA-deficient and ABA-insensitive mutants in relation to freezing tolerance (Mäntylä et al., 1995).

More work is needed to clarify whether there are dehydrin genes in castor bean that respond differently to specific stress inducers or whether different inducers stimulate dehydrin synthesis by different pathways. Dehydrin synthesis is often tissue-specific and the signal transduction pathways may also be tissue-specific.

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References