Planta (1988) 175:485–492

Planta
© Springer-Verlag 1988

Onset of desiccation tolerance during development of the barley embryo

D. Bartels*, M. Singh, and F. Salamini

Abteilung Pflanzenzüchtung und Ertragsphysiologie, Max-Planck-Institut für Züchtungsforschung, Egelspfad, D-5000 Köln 30, Federal Republic of Germany

Abstract. We have investigated events which take place in the developing barley (*Hordeum vulgare* L.) embryo during its acquisition of desiccation tolerance. Excised embryos are capable of precocious germination as early as 8 d after pollination (DAP). At this age, however, they are not capable of resisting a desiccation treatment which induces a loss of 96–98% of their initial water content. At 16 DAP the embryos germinate despite the drastic drying treatment. The pattern of in-vivo and in-vitro proteins synthesized by the developing embryos from 12 DAP (desiccation-intolerant) and 16 DAP (desiccation-tolerant) were compared. A set of 25–30 proteins was identified which is de-novo synthesized or enhanced during the developmental period leading to desiccation tolerance. Abscisic acid (ABA; 100 μM) applied in vitro for 5 d to 12-DAP embryos induces desiccation tolerance and represses a subset of polypeptides preferentially associated with 16-DAP embryos. During in vitro culture of barley embryos ABA stimulates the appearance of a set of proteins and prevents the precocious germination allowing embryogenesis to continue in vitro. It also suppresses a set of germination-related proteins which appear 4 h after the incubation of the dissected embryo on a germination medium without ABA. Almost all mRNAs remain functional for translation when isolated embryos are dried at the desiccation-intolerant and tolerant stages of embryo development.

Key words: Abscisic acid and desiccation tolerance – *Hordeum* (embryo development) – Embryo (development, desiccation tolerance) – Desiccation tolerance – Protein synthesis

Introduction

In the majority of higher plants, tolerance to protoplastic dehydration is restricted to the seeds. Despite their low water content these organs are viable and, under proper environmental conditions, capable of regrowth. The embryo tolerates water potentials as low as 50% relative humidity (Gaff 1980), a property it acquires during seed maturation. After the seed germinates the growing plant loses the ability to survive desiccation. Therefore, the ability to tolerate dehydration is developmentally programmed and, with a few notable exceptions (Gaff 1971; Gaff and Latz 1978) restricted to the embryo.

Desiccation tolerance is a complex property. The phenomenon has been frequently described at morphological, physiological and biochemical levels, but still it is far away from being understood at the molecular level (reviewed by Bewley and Krochko 1982). Developing embryos can be used as an experimental system to study desiccation tolerance. They can be isolated from the endosperm tissue, desiccated and tested in vitro for their capacity to germinate precociously, that is, germination after the embryos are removed from premature seeds. Under constant environmental conditions the onset of desiccation tolerance, together with that of correlated metabolic events, can be precisely monitored. Moreover, the in-vitro cultivation of isolated embryos offers the possibility of testing the influence of exogeneously supplied hormones, such as (GA) gibberellic acid or abscisic acid (ABA), which are thought to regulate important events during embryogenesis (for reviews see King 1982; Ross 1984; Walbot 1978).

The objective of the work reported in this paper was to investigate molecular events which take place in the developing barley embryo during the acquisition of desiccation tolerance. Two-dimen-

* To whom correspondence should be addressed

Abbreviations: ABA = abscisic acid; DAP = days after pollination; GM = germination medium; poly(A)RNA = polyadenylated RNA; SDS = sodium dodecyl sulfate.

sional polyacrylamide gel electrophoresis of in-vitro- and in-vivo-synthesized proteins has allowed us to associate qualitative and quantitative changes with specific stages of embryo development. Furthermore, the effect of desiccation on the expression of embryo mRNAs was studied. Also it was possible to manipulate desiccation tolerance in vitro by exogenous applications of ABA.

Material and methods

Plant material. Barley (*Hordeum vulgare* L. cv. Aura) plants were grown in a Conviron growth chamber (16 h light, 18° C, 8 h dark, 14° C, 75–80% relative humidity) in 8-cm-diameter pots. Ears were labelled at anthesis.

Isolation and culture of embryos. Seeds from the middle part of the ear were collected, the outer glumes were removed, and the grains were surface sterilized with 0.5% NaOCl (15 min) and 70% ethanol (7 min) and washed thoroughly in sterile water. Embryos were aseptically isolated under a dissecting microscope and immediately frozen in liquid N_2 or treated as described below. The weight of 30 seeds was always determined and the sizes of the embryos were measured to ensure that all experiments were performed with embryos of uniform age.

Embryos were cultured according to Triplett and Quatrano (1982) in Petri dishes on filter papers soaked either with germination medium (GM; Murashige and Skoog salts as supplied by Flow Laboratories (Meckenheim, FRG), 2% (w/v) sucrose and 150 μM glutamine), or GM supplemented with 100 μM ABA (Sigma, München, FRG); this medium is indicated as GM+ABA. The dishes were sealed with parafilm and kept at 27° C with a 16-h photoperiod. Embryo growth or germination was assessed daily and recorded after 5 d. Germination was defined as the emergence and elongation of radicles and coleoptiles of minimum length of 0.2–0.3 cm. For an evaluation of the germinated plantlets the lengths of the radicles and coleoptiles were measured 5 d after incubation.

For the drying treatment the embryos were placed on two layers of filter paper in the constant air stream of a ventilating hood for 18–20 h at 20° C.

Determination of the water content. To determine the water content of fresh embryos their weights were compared before and after a 24 h incubation at 100° C.

In-vivo labelling. Ten isolated embryos were incubated in a sterile Petri dish in 200 μl GM or GM+ABA medium containing $1.9 \cdot 10^6$ Bq [^{35}S]methionine (Amersham, Braunschweig, FRG; $3.02 \cdot 10^{13}$ Bq $\cdot$ mmol^{-1}). The incorporated radioactivity was determined after precipitation of an aliquot with trichloroacetic acid (TCA).

Protein extraction. Proteins were extracted from 10 embryos as described by Damerval et al. (1986) Frozen embryos were ground to a fine powder under liquid N_2. Proteins were precipitated by adding 1.5 ml 10% (w/v) TCA in acetone containing 0.07% (v/v) β-mercaptoethanol. The pellet was washed twice with ice-cold acetone containing 0.07% (v/v) β-mercaptoethanol, dried under vacuum and dissolved in UKS-solution (9.5 M urea, 5 mM K_2CO_2, 1.25% sodium dodecyl sulfate (SDS), 0.5% dithiothreitol (DTT), 2% LKB (Gräfeling, FRG) ampholines pH 3.5 to 10, 6% NP-40). Non-soluble material was separated by centrifugation.

Two-dimensional electrophoresis. For the first dimension the proteins were separated on 18-cm-long isoelectrofocusing (IEF) rod gels (1.5 mm diameter). The gel mixture was 4.2% acrylamide, 0.2% N,N′-methylenbis-acrylamide, 9 M urea, 3% NP-40, 6% carrier ampholytes (LKB) consisting of a mixture of one-fifth Ampholine pH 5–10, two-fifths Ampholine pH 4–6, one-fifth Ampholine pH 5–8, one-fifth Ampholine pH 7–9. The IEF was performed for 14000 V·h. The rod gels were equilibrated in 10% (w/v) glycerol, 2.3% (w/v) SDS, 5% DTT and 0.625 M 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HCl pH 6.8. In the second dimension the focused proteins were either separated on a 12.5% or on a gradient (7.5–15%) polyacrylamide gel overlaid with a 4% polyacrylamide stacking gel (Laemmli 1970). After the tracking dye (bromophenol blue) had run to the bottom the gels were fixed in 6% (w/v) TCA, 5% (v/v) ethanol and then prepared for fluorography according to Bonner and Laskey (1974). Methylated ^{14}C-proteins (Amersham) were used as molecular-weight markers for SDS gel electrophoresis.

Extraction and in-vitro translation of polyadenylated RNA (poly(A)RNA). Extraction of poly(A)RNA was done as described in Bartels and Thompson (1983). The poly(A)RNAs were translated in rabbit reticulocyte lysate, which was prepared and used according to Jackson and Hunt (1983). For 20-μl translation assays, saturating amounts of poly(A)RNA (0.5–1 μg) were incubated for 2 h at 30° C, using $3.77 \cdot 10^5$ Bq [^{35}S]methionine ($3.02 \cdot 10^{13}$ Bq $\cdot$ mmol^{-1}, Amersham). For IEF separation, urea (11 mg $\cdot$ 10 μl^{-1}) was dissolved in the in-vitro translation assay and an equal volume of sample buffer (9.5 M urea, 2% Ampholines – same composition as for IEF gels –, 5% β-mercaptoethanol and 2% NP-40) was added. The sample was immediately loaded onto a rod gel.

Results

Germination capacity of isolated embryos. To obtain embryos typical for the various developmental stages, in addition to dissection from the seed at a precise day after pollination (DAP), grain weight and embryo length were also controlled and taken as indicators of embryo growth (Fig. 1a). Excised embryos were capable of precocious germination as early as 8 DAP (Fig. 1b). Their desiccation tolerance was tested by subjecting them to a drying treatment as specified in *Material and methods.* During desiccation the embryos lost between 50 and 60% of their fresh weight and around 96–98% of the initial water content. After the drying treatment the embryos were incubated on filter paper soaked with GM to test their germination capacity. More than 95% of the embryos excised at 15 DAP germinated on GM despite the drying treatment. Younger embryos did not have this capacity, although they germinated precociously if not dried. The conclusion is that during embryo development the ability to germinate after drying is acquired at a later stage than the ability to germinate precociously without a drying treatment.

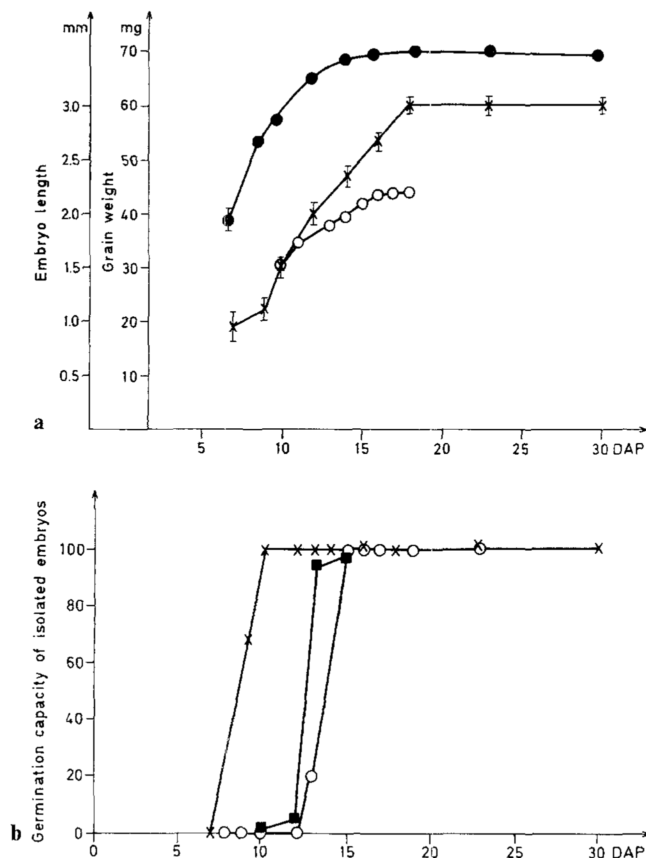

Fig. 1 a, b. Monitoring embryogenesis of *Hordeum vulgare*. **a** The grain weight (●——●) was determined and the embryo length (×——×) was measured at the indicated days after pollination (DAP; removed from the ear). Each measurement represents the average of 30 embryos. ○——○, Length of embryos during in-vitro culture on ABA with 10- and 12-DAP embryos on ABA (100 μM). **b** Embryos were isolated from seeds at different DAP; the capacity of embryos to germinate precociously was tested for embryos in the fresh state (×——×) or for dried (○——○) embryos. ■——■, Germination capacity of embryos which were excised at 10 DAP and cultured in vitro on 100 μM ABA. Embryos were removed every day, dried and then tested for germination. Each time point and treatment represents the average of 30 embryos

Influence of exogenous ABA on the acquisition of desiccation tolerance. In the presence of ABA, precocious germination of immature isolated embryos grown in vitro could be arrested. Concentrations of ABA between 10^2 and 10^{-3} μM were tested; no germination was observed with concentrations above 1 μM; 10^2 μM ABA was chosen for subsequent experiments. During in-vitro culture in the presence of ABA the size of the embryos increased, although not as much as in vivo (Fig. 1 a).

When isolated embryos were cultured in the presence of ABA, they acquired the capacity to germinate precociously after drying for a shorter time interval in comparison with the time course for embryos isolated each day from an ear. Every day a sample was removed and the germination capacity tested after the drying treatment. Three days after culturing on ABA (corresponding to 13 DAP in vivo), more than 95% germination capacity could be reached, a value higher than that of 20% shown by embryos of 13 DAP directly dissected from the plant (Fig. 1b). When embryos of 7–10 DAP were subjected to the same treatment, desiccation tolerance could not be induced by ABA. It is concluded that ABA induces the tolerance response, but only in embryos which have reached a particular developmental stage.

Gene expression investigated by in-vivo labelling of intact embryos. Embryos of different ages were incubated in the presence of [^{35}S]methionine and the extracted proteins characterized by gel electrophoresis. The results obtained from labelling 10- and 16-DAP embryos for 2 h are reported in Fig. 2. The two stages of development allow a comparison of the protein pattern of intolerant embryos with that of a desiccation-tolerant stage. A set of proteins was identified which is present at a high level in 16-DAP embryos and absent or present only at a low level in 10-DAP embryos (as indicated by arrows in Fig. 2b). The appearance of some of these proteins belonging to the group identified here may specifically correlate with the onset of a biochemical pathway leading to desiccation tolerance.

The effect of the ABA treatment on growth and properties of isolated embryos was studied by analysing the time course of label incorporation into proteins. Embryos of 16 DAP, a stage where most proteins of a fully developed embryo are already synthesized, were labelled on GM+ABA medium for 0.5, 1, 2, 4, 8 and 24 h and the results compared with those of embryos incubated on GM only. Incorporation of [^{35}S]methionine into embryos was detected as early as 0.5 h after incubation. On GM, embryos started to germinate, giving rise to a group of proteins not present in vivo at 16 or 18 DAP. These proteins started to appear after an incubation period of 4 h and they were designated 'germination-related' (GM) proteins. Proteins from all timepoints studied were visualized by two-dimensional gels. In Fig. 3, sectors of two-dimensional gels are presented which show the most important differences between the protein patterns from embryos incubated for 24 h on GM and GM+ABA. Under the experimental conditions described, ABA interferes with protein synthesis by suppressing the appearance of germination-related (GM) proteins which probably lead to

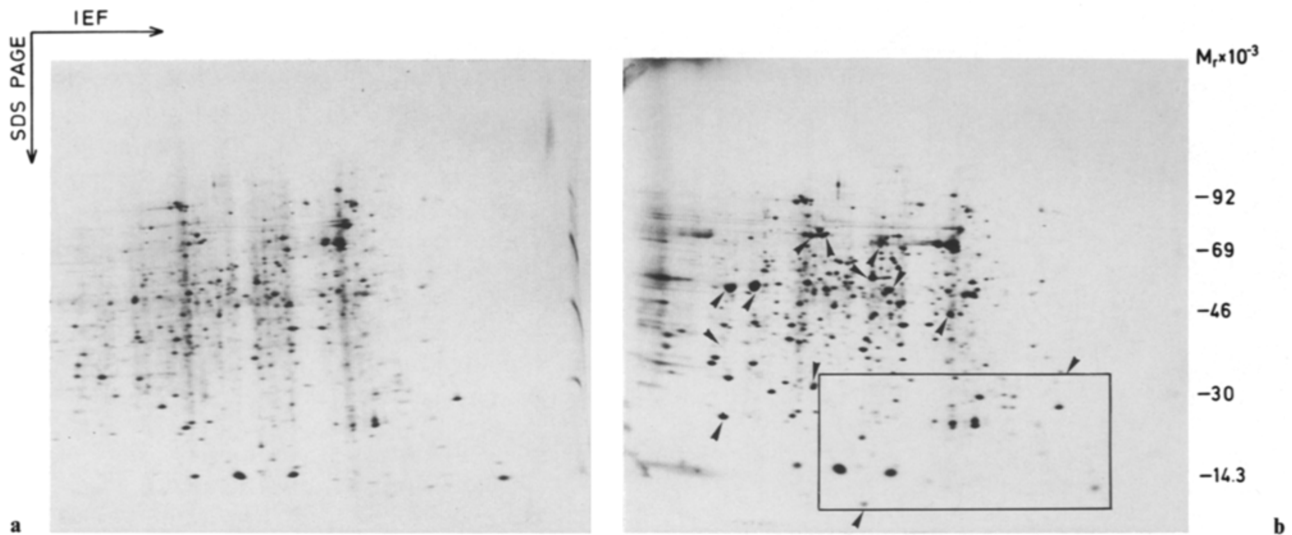

Fig. 2a, b. Isolated 10-DAP (**a**) and 16-DAP (**b**) barley embryos were labelled for 2 h with [^{35}S]methionine, and the proteins were compared by two-dimensional electrophoresis. In the second dimension (SDS-PAGE) the proteins were separated in a 7.5–15% gradient gel. A fluorograph of the labelled proteins is presented. Proteins of 16-DAP embryos which change in comparison with those at 10 DAP are indicated by *arrows*. The area of the protein pattern which is considered in **Fig. 3** is indicated

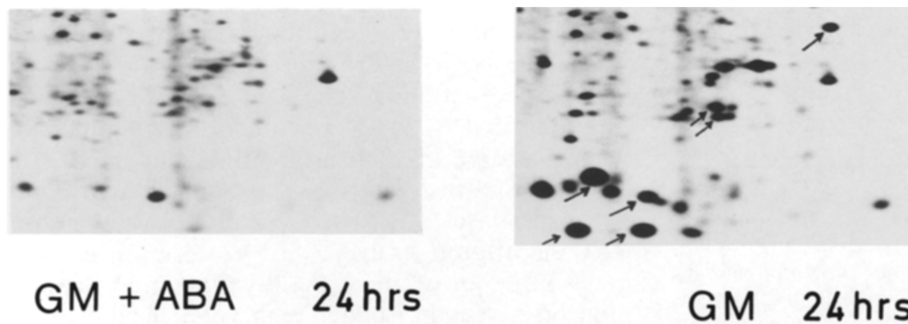

Fig. 3. Sections of a two-dimensional gel separation are shown as indicated in **Fig. 2b**. Barley embryos (16 DAP) were labelled with [^{35}S]methionine for 24 h on GM or GM+ABA. *Arrows* point to the GM-related proteins

the germination pathway. The effect of ABA will be further considered in experiments dealing with in-vitro translation of mRNA (see later).

Expression of poly(A)RNA during embryogenesis. Polyadenylated RNA was isolated from 10-, 12-, 14-, 16-, 18-, 22-DAP and mature embryos and translated in vitro. The proteins synthesized were separated by two-dimensional electrophoresis. Figure 4a shows the in-vitro-synthesized proteins derived from poly(A)RNA of 16- to 18-DAP desiccation-tolerant embryos. This pattern is schematically presented in Fig. 4b, to which the other patterns (data not shown) were related. Major changes were observed between 12 (desiccation-intolerant) and 16 days (desiccation-tolerant), and the prominent polypeptides which change during this time interval are indicated and numbered. All the changes observed are summarized in Table 1. Several proteins are not present, or are present at a much lower level, in products resulting from RNA translation of 10- to 12-DAP embryos. Several polypeptides appear first at around 16 DAP, a stage that can be considered a switch point in the synthetic activity of the barley embryo. Only spot No. 28 was identified to be present at 10 and 12 DAP but missing in the translation products from embryos of 14–16 DAP onwards. Between 16 and 22 DAP the mRNA population available for in-vitro translation does not seem to change. The pattern of in-vitro-synthesized proteins derived from poly(A)RNA of mature embryos shows only a few quantitative changes when compared with the 16-DAP pattern.

Expression of mRNA from dried embryos. As described, 16–18 DAP embryos have the ability to germinate after desiccation. The influence of the drying treatment on the expression of poly(A) RNAs available for in-vitro translation was investigated. Twelve-DAP and 16- to 18-DAP embryos were isolated, dried and poly(A)RNA was extract-

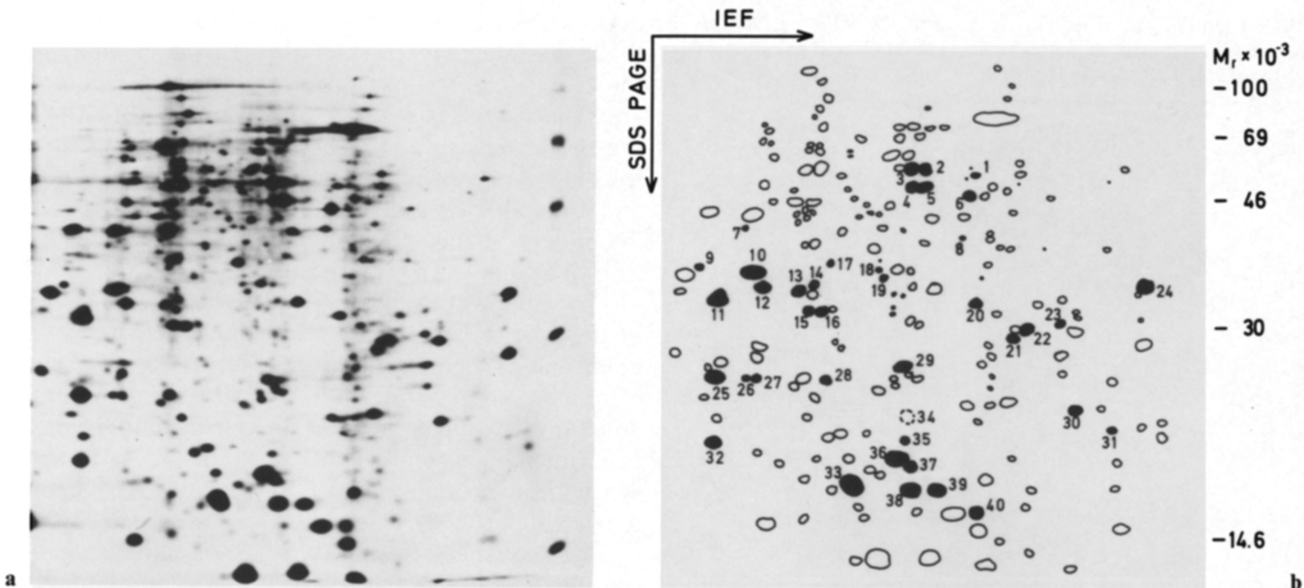

Fig. 4. **a** A fluorograph of in-vitro-synthesized proteins derived from poly(A)RNA of 16- to 18-DAP barley embryos. The proteins were first separated by isoelectric focusing and then in a 7.5–15% gradient polyacrylamide SDS gel. **b** A tracing of the protein pattern of **a** is presented; the proteins which change in comparison with other translation patterns (10- to 12-DAP embryos, mature embryos, ABA-treated embryos, dried embryos) are indicated and numbered (see also **Table 1** for a summary)

ed and translated in vitro. The protein pattern observed indicates that in our in-vitro system all the mRNAs present in fresh embryos remain translationally functional also in dried embryos. The expression of one polypeptide appears to be enhanced during this drying process (see Fig. 4, spot no. 31).

Effect of ABA on the expression of mRNA during embryogenesis. As mentioned earlier, when isolated embryos (10–12 DAP) are cultured in vitro in the presence of ABA, embryogenesis continues so that desiccation tolerance is acquired. Therefore the pattern of RNA expression during this in-vitro culture was studied. Young embryos (12 DAP) were cultured on ABA for 5 d, then the mRNA was extracted and translated in vitro. The translation products were separated in a two-dimensional gel and the protein pattern was compared with the in-vitro translation pattern obtained from mRNA of 16- to 18-DAP embryos. Out of the translation products enhanced or synthesized de novo when 16- to 18-DAP embryos were compared with 12-DAP embryos, a set of ten translation products increased during the in-vitro culture on ABA, the other translation products which were induced in vivo between 12 and 16 DAP were not induced during the in-vitro culture on ABA (see Table 1).

Discussion

Isolated embryos have been used as an experimental system to investigate morphogenetic and other developmental processes. Rogers and Quatrano (1983) distinguished five stages of embryo development in wheat. Their description applies also to barley: stage 1 corresponds to the undifferentiated embryo; stage 2 to rapid cell divisions; stage 3 to completion of tissue differentiation; stage 4 to increasing cell size and storage protein accumulation; and stage 5 to the onset of desiccation leading to developmental arrest. The events we describe take place during stages 2 and 3. Early in stage 2, barley embryos have the capacity to germinate precociously, a characteristic behaviour of the embryo of several plant species (for reviews see Raghavan 1976, pp. 163–203; Rappaport 1954). Between phases 2 and 3 the transition from desiccation intolerance to tolerance takes place. A similar observation has been reported for the developing maize embryo which germinates precociously at a developmental stage well separated temporally from the acquisition of desiccation tolerance (Bochiccio et al. 1988). It is now well established that young embryos of these Gramineae can remain viable after a drastic drying treatment which reduces their water content to around 5%. For other species, such as *Ricinus communis* (Kermode and Bewley 1985) and *Phaseolus vulgaris* (Long et al. 1981), the permissive level of drying is higher (23% and 15% respectively). It was not tested, whether the younger embryos might be desiccation tolerant if they were dried more slowly. Kermode and Bewley (1985) reported for *Ricinus communis* seeds that the younger the seeds were the more slowly the

Table 1. Survey of major in-vitro synthesized barley embryo polypeptides which change according to DAP and presence of ABA

No. of polypeptide (in Fig. 4b)	Comparative intensity at 18 DAP versus 12 DAP[1]	Modification by ABA[2]	Increase or decrease of intensity mature embryos versus 18 DAP[3]
1	+++	+	
2	++	−	−
3	++	−	−
4	+	−	
5	+	−	
6	+	−	
7	+++	−	++
8	+	+++	
9	+	−	
10	++	−	
11	+++	−	−
12	++	−	
13	+++	+	
14	+++	?	
15	+	−	
16	++	−	
17	++	?	
18	+	+	+++
19	+	+++	++
20	+++	−	
21	+++	+	
22	+++	+	
23	++	+	
24	+++		
25	++	−	
26	+++	−	−
27	+++	−	−
28	only present in 12 DAP		
29	++	−	
30	++	−	
31	enhanced in dried embryos		
32	++	−	
33	+++	−	
34			+++
35	+++	+	
36	++	−	
37	++	−	
38	+++	+	
39	++	−	
40	++	−	

[1] Comparison of the expression of in-vitro-synthesized proteins from 16- to 18-DAP embryos. The number of crosses indicates the different intensities of the proteins on the film
[2] + = polypeptides appearing during in-vitro culture in the presence of ABA, − = polypeptides which appear in vivo at 16–18 d but were not present in ABA-treated embryos in vitro
[3] Only those of polypeptides are marked which differ in their expression in mature embryos compared with 18-DAP embryos: − = expressed at a lower level in mature embryos; ++ or +++ = expressed at a higher or much higher level in mature embryos. Proteins which are not marked do not change

drying needed to take place for tolerance to drying to be manifest. A further observation is that in the barley embryo the onset of desiccation tolerance is acquired before the normal maturation drying of the seeds starts. This is also characteristic for several other seeds (Kermode and Bewley 1987) and indicates that dehydration of the seed itself is not a prerequisite for the expression of desiccation tolerance in the developing embryo.

To address the question whether the acquisition of desiccation tolerance can be related to the expression of specific gene products, protein patterns were compared from a non-desiccation-tolerant and a desiccation-tolerant stage during embryo development. The patterns of in-vivo and in-vitro-synthesized proteins show that a set of 25–30 proteins and mRNAs exists which is synthesized de novo or enhanced during the stage of embryogenesis which leads to the desiccation-tolerant stage (Fig. 4). However, these molecular components are not necessarily related to desiccation tolerance. Their synthesis may result from the action of genes not correlated to desiccation but activated during the same developmental period. The disappearance of certain gene products could also lead to the acquisition of desiccation tolerance, but only very few possible candidates for such a mechanism were identified during these studies (spot. no. 28 in Fig. 4). It has also to be emphasized that mRNAs have to be present in a certain concentration in order to detect their translation products, therefore products derived from low-abundance mRNAs may not be visible.

The observation that a new set of proteins is expressed as embryos develop is in agreement with similar findings describing embryo development in other species (Crouch and Sussex 1981; Quatrano et al. 1983; Galau et al. 1986; Sanchez-Martinez et al. 1986). In cotton and in maize, the best-described systems so far, a set of early and late embryo-specific mRNAs were identified (Galau et al. 1986; Sanchez-Martinez et al. 1986).

In barley the effect of drying per se on mRNA expression was also studied. The rationale of these particular experiments was to reveal if the switch from intolerance to desiccation tolerance can be correlated with a differential response to desiccation of the population of mRNAs extracted at 12 and 16 DAP. The drying treatment applied to a desiccation-sensitive phase can result in a severe decline of translation capacity. For example, in germinating pea axes, desiccation causes modifications of the protein-synthesizing program which reverts to earlier stages of germination (Lalonde and Bewley 1986). The major outcome of our ex-

periments was that the mRNAs of desiccation-intolerant and tolerant embryos remain functional for translation during the drying process. One polypeptide (No. 31 in Fig. 4) was found which seems to be enhanced as a result of the drying treatment. It was visible in the translation products of both the 12- and 16-DAP-derived mRNA samples.

In addition to the experiments reported here for barley, in several other plant systems, precocious germination of excised immature embryos is prevented and embryogenesis continues in the presence of suitable concentrations of ABA (Quatrano 1986). During culture on ABA the embryo also acquires the ability to resist desiccation, as is shown in Fig. 1 b. That desiccation tolerance can be developed in vitro has already been demonstrated for embryonic axes of *Phaseolus* (Long et al. 1981). We emphasize, however, as pointed out by Morris et al. (1985), that the suitable ABA concentration for these experiments needs to be carefully tested. For instance, in our system, proteins most probably related to germination had already appeared on GM medium after a 4-h incubation period, but they were normally suppressed by 100 μM ABA.

Besides repressing the GM proteins, the ABA applied in vitro for 5 d to barley embryos of 12 DAP repressed a set of proteins present in the translation pattern of 16- to 18-DAP mRNAs, and absent or not so abundant at 12 DAP. This restricts the number of protein products associated with the onset of desiccation tolerance. In fact, desiccation tolerance acquired in the presence of ABA during in-vitro embryo cultivation can now be associated with a set of polypeptides smaller than that obtained by comparing mRNAs extracted and translated from 12-DAP and 16- to 18-DAP embryos.

A second group of polypeptides was found to be controlled by ABA during the in-vitro cultivation of barley embryos. This is a small set of protein components whose appearance is stimulated by ABA (see Table 1). That a particular set of mRNAs is induced by ABA corresponds to observations made in several other embryo systems such as maize (Sanchez-Martinez et al. 1986), wheat (Quatrano et al. 1983), cotton (Galau et al. 1986), *Brassica* (Crouch and Sussex 1981), soybean (Eisenberg and Mascarenhas 1985), and *Vicia faba* (Barrat 1986). Most of these investigations, however, focused on embryo storage proteins which are induced by ABA. In the barley system the low number and level of such ABA-induced mRNAs may depend on the particular stage of development studied.

The results presented here indicate that the onset of desiccation tolerance is temporally correlated with the appearance of a small set of polypeptides, the identities of which are clearly of great interest. Future studies will centre on identification of cloned coding sequences for these polypeptides and definition of their roles during the stage of acquisition of desiccation tolerance.

We wish to thank Barbara Eilts and Margret Feck for excellent technical assistance and Margrit Pasemann for typing the manuscript.

References

Barratt, D.H.P. (1986) Modulation by abscisic acid of storage protein accumulation in *Vicia faba* cotyledons cultured in vitro. Plant Sci. **46**, 159–167

Bartels, D., Thompson, R.D. (1983) The characterization of cDNA clones coding for wheat storage proteins. Nucl. Acids Res. **11**, 2961–2978

Bewley, J.D., Krochko, J.E. (1982) Desiccation-tolerance. In: Encyclopedia of plant physiology, N.S., vol. 12B: Physiological plant ecology III, pp. 325–378, Lange, D.L., ed. Springer, Berlin Heidelberg New York

Bochiccio, A., Vazzana, C., Raschi, A., Bartels, D., Salamini, F. (1988) Effect of desiccation on isolated embryos of maize. Onset of desiccation tolerance during development. Agronomie **8**, 29–36

Bonner, W.M., Laskey, R.A. (1974) A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**, 83–88

Crouch, M.L., Sussex, I.M. (1981) Development and storage protein synthesis in *Brassica napus* L. embryos in vivo and in vitro. Planta **153**, 64–74

Damerval, C., De Vienne, D., Zivy, M., Thiellement, H. (1986) Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis **7**, 52–54

Eisenberg, A.J., Mascarenhas, J.P. (1985) Abscisic acid and the regulation of synthesis of specific seed proteins and their messenger RNAs during culture of soybean embryos. Planta **166**, 505–514

Gaff, D.F. (1971) Desiccation-tolerant flowering plants in Southern Africa. Science **174**, 1033–1034

Gaff, D.F. (1980) Protoplasmic tolerance of extreme water stress. In: Adaption of plants to water and high temperature stress, pp. 207–209, Turner, N.C., Kramer, P.J., eds. Wiley-Interscience, New York

Gaff, D.F., Latz, P. (1978) The occurrence of resurrection plants in the Australian flora. Aust. J. Bot. **26**, 485–492

Galau, G.A., Hughes, D.W., Dure, L. III (1986) Abscisic acid induction of cloned late embryogenesis abundant (Lea) mRNAs. Plant Miol. Biol. **7**, 155–170

Jackson, R.J., Hunt, T. (1983) Preparation and use of nuclease-treated rabbit reticulocyte lysates for the translation of eucaryotic messenger RNA. Methods Enzymol. **96**, 50–74

Kermode, A., Bewley, J.D. (1985) The role of maturation drying in the transition from seed development to germination. J. Exp. Bot. **36**, 1906–1915

Kermode, A., Bewley, J.D. (1987) Regulatory processes involved in the switch from seed development to germination: Possible roles for desiccation and ABA. In: Drought resistance in plants, pp. 59–76, Monti, L., Porceddu, E., eds.

Commission of the European Communities, Brussels Luxembourg
King, R.W. (1982) Abscisic acid in seed development. In: The physiology and biochemistry of seed development, dormancy and germination, pp. 157–181, Khan, A.A., ed. Elsevier Biomedical Press
Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680–685
Lalonde, L., Bewley, J.D. (1986) Patterns of protein synthesis during the germination of pea axes and the effects of an interrupting desiccation period. Planta **167**, 504–510
Long, S.R., Dale, R.M.U., Sussex, I.M. (1981) Maturation and germination of *Phaseolus vulgaris* embryonic axes in culture. Planta **153**, 405–415
Morris, P.C., Maddock, S.E., Jones, M.G.U., Bowles, D.J. (1985) Changes in the levels of wheat- and barley-germ agglutinin during embryogenesis in vivo, in vitro and during germination. Planta **166**, 407–413
Quatrano, R.S. (1986) Regulation of gene expression by abscisic acid during angiosperm embryo development. In: Oxford surveys of plant molecular and cell biology 3, pp. 467–477, Miflin, B.J., ed. Oxford University Press Oxford, UK
Quatrano, R.S., Ballo, B.L., Williamson, J.D., Hamblin, M.T., Mansfield, M. (1983) ABA controlled expression of embryo-specific genes during wheat grain development. In: Plant molecular biology, pp. 343–353, Goldberg, R.B., ed. Liss Inc. New York
Raghavan, V. (1976) Experimental embryogenesis in vascular plants. Academic Press, New York
Rappaport, J. (1954) In vitro culture of plant embryos and factors controlling their growth. Bot. Rev. **20**, 201–225
Rogers, S.A., Quatrano, R.S. (1983) Morphological staging of wheat caryopsis development. Am. J. Bot. **70**, 308–311
Ross, J.D. (1984) Metabolic aspects of dormancy. In: Seed physiology, vol. 2, pp. 45–73, Murray, D.R., ed. Academic Press, London New York
Sanchez-Martinez, D., Puigdomenech, P., Pages, M. (1986) Regulation of gene expression in developing *Zea mays* embryos. Plant Physiol. **82**, 543–549
Triplett, B., Quatrano, P.S. (1982) Timing, localization and control of wheat germ agglutinin synthesis in developing wheat embryos. Devel. Biol. **91**, 491–496
Walbot, V. (1978) Control mechanism for plant embryogeny. In: Dormancy and developmental arrest, pp. 113–166, Clutter, M.E., ed. Academic Press, New York

Received 15 December 1987; accepted 4 March 1988