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Short Communication

Desiccation of Axes of *Phaseolus vulgaris* during Development of a Switch from a Development Pattern of Protein Synthesis to a Germination Pattern[1]

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ABSTRACT

Immature seeds of *Phaseolus vulgaris* removed from the pod at 32 days of development do not germinate unless first subjected to a desiccation treatment. This change from development to germination caused by premature drying is mirrored in the pattern of protein synthesis by the axes. Rehydrated axes from 32-day-developed seeds cease to synthesize proteins that are uniquely associated with development, but instead synthesize some proteins that are similar to those made in the germinating axes from mature dry seeds. Desiccation of 22-day-developed seeds does not lead to their germination, nor does it cause a switch from a developmental to a germination mode of protein synthesis by the axes. It is proposed that desiccation plays a role in permanently suppressing developmental protein synthesis and in inducing germination protein synthesis.

When immature axes or embryos are removed from the seeds of some species they germinate precociously, without a requirement for desiccation (2, 3, 7). Such precocious germination is accompanied by a cessation of protein synthesis which is identifiably developmental in character, *e.g.* synthesis of storage proteins (4, 7). Maturation drying is the normal final stage of development for most seeds, however, and subsequent hydration of mature dry seeds leads to their germination. It is possible, then, that the drying process plays an important role in switching seeds from a developmental to a germination regime. There is some evidence for this. For example, immature seeds of some legumes and cereal grains will not germinate on water when removed from the mother plant in the fully hydrated state, but will germinate only after drying (5, 7). Furthermore, postgermination events such as hydrolase production by the aleurone layer of barley will not occur unless the grain has been subjected to drying (5). In this paper, we seek to provide an answer to the following questions. If premature drying of developing seeds causes them to germinate upon subsequent rehydration, do these seeds maintain their ability to synthesize developmental proteins, inasmuch as they had not completed their developmental cycle at the time of desiccation? Or, has the drying treatment terminated all developmental events, and is the synthesis of germination proteins the only possibility?

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MATERIALS AND METHODS

Phaseolus vulgaris cv Taylors' Horticultural seeds were purchased from Asgrow Canada Ltd., Brantford, Ontario, and stored at 4°C. Developing seeds were obtained from plants grown under natural and artificial daylight conditions in a greenhouse. Flowering usually occurred after 30 d from seed sowing, and developing seeds were taken from the pods at various intervals following anthesis. The developmental stages were identified according to Walbot *et al.* (9), and seeds were used immediately after harvest or after drying in screw-cap jars over activated silica gel at room temperature.

Incubation Conditions for Radioactive Labeling. Embryo axes (eight) were dissected from fresh or 2-h rehydrated beans and transferred to a 0.5 ml solution of sterile distilled H_2O containing 100 μCi [^{35}S]methionine (1,174 Ci/mmol [Amersham]) in sterile Petri dishes. Labeling was for 3 h at room temperature while the dishes were agitated slowly on a platform shaker.

Protein Extraction and Separation. Embryos were extracted in 2 ml 50 mM Tris-HCl buffer (pH 8.6) containing 20 mM KCl and 10 mM $MgCl_2$. Extracted proteins were precipitated in 10% TCA and after centrifugation dissolved in lysis buffer (8). Two-dimension separation was performed according to O'Farrell (8). Protein samples with approximately equal TCA precipitable counts (1.2–1.5 $\times$ 10^6 cpm) were loaded on the first dimension gels at the basic end (pH range, 8.5–4.3). The second dimension separation was by the method of Laemmli (6) using a 12.5% acrylamide gel at room temperature. Staining was with 0.1% Coomassie brilliant blue R (10), and after destaining gels were prepared for fluorography by soaking in Enhance (New England Nuclear), drying and exposing at −70°C to Kodak X-omat RP film.

RESULTS AND DISCUSSION

Phaseolus vulgaris seeds removed from the pod after 22 and 32 d from anthesis will not germinate when placed in Petri dishes in water. But when seeds are removed after 32 d of development and dried over silica gel, they germinate upon subsequent rehydration. In contrast, seeds desiccated at 22 d of development fail to germinate when rehydrated, and eventually deteriorate (7, and our own observations). Thus 22-d-developed seeds are desiccation-intolerant, and those at 32 d are both desiccation-tolerant, and capable of germinating following premature drying.

We established that the pattern of protein synthesis carried out by developing axes is different from that carried out by germinating axes. Figure 1A is a fluorograph of the proteins being synthesized by freshly isolated axes of *P. vulgaris* removed from seeds at 32 d after anthesis. When compared with the pattern of proteins

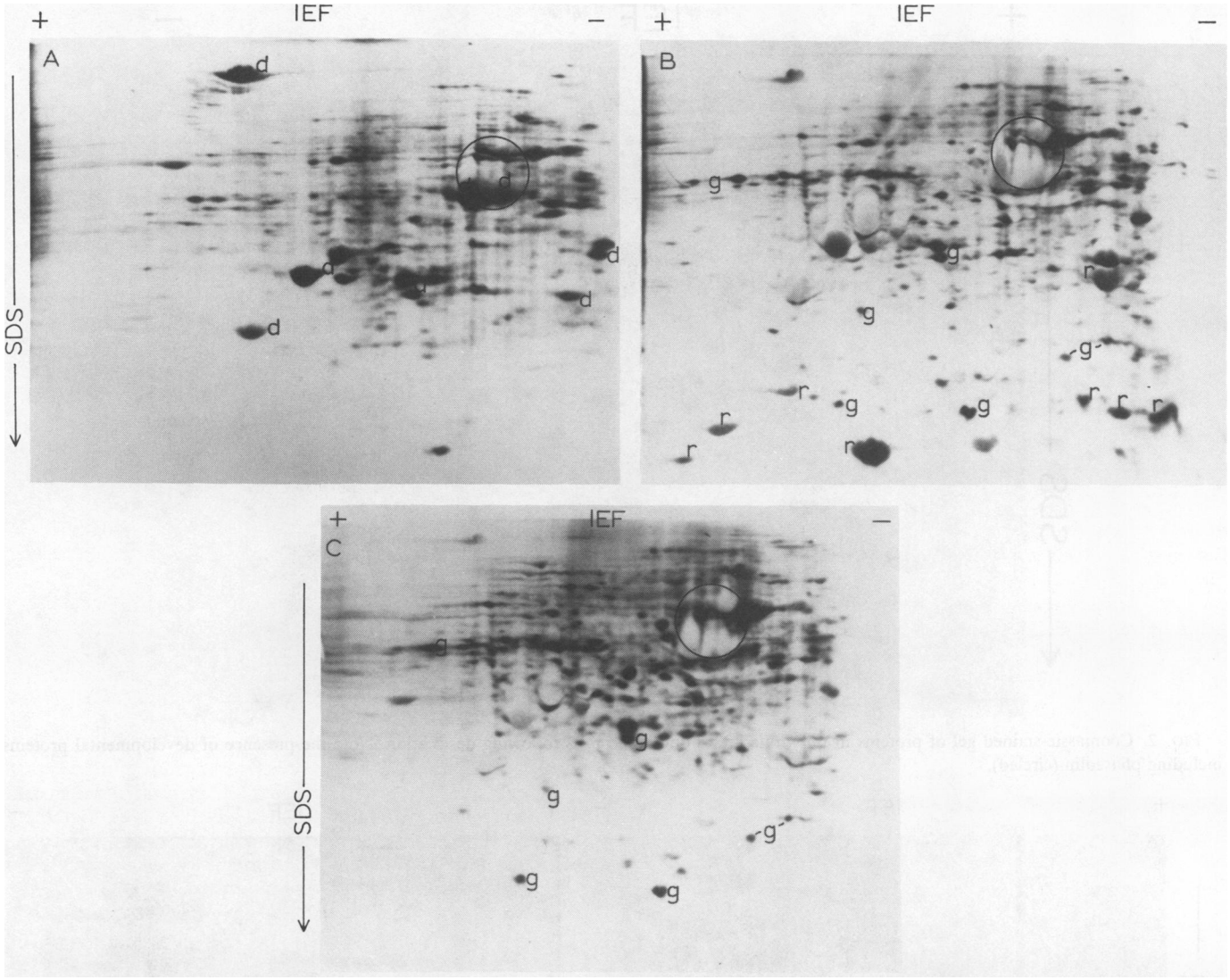

FIG. 1. Fluorographs of proteins synthesized over a 2-h period in: A, isolated fresh axes from 32-d-developed seeds; B, isolated axes from 32-d-developed seeds desiccated over silica gel and then rehydrated; C, isolated axes from seeds germinated for 12 h. Proteins synthesized during germination (C) and not development (A) are marked with a g; those synthesized during development and not germination are marked with a d (including phaseolin—*circled*), and those found neither during germination nor develoment upon rehydration (B) are marked r.

synthesized by 12-h germinated axes (*i.e.* axes removed from fully mature, 45-d-developed seeds) (Fig. 1C), it can be seen that there are certain proteins produced that are unique to development (including phaseolin [7], identified by us from the two-dimensional polyacrylamide gel electrophoresis pattern given by partially purified storage protein), and others that are unique to germination. Some proteins are synthesized that are common to both developing and germnating axes. Presumably many of these are involved in the 'basal' metabolism that is essential for the maintenance of cells during both development and germination.

We then determined if drying at this tolerant stage of desiccation leads to a switch, upon subsequent rehydration, from a pattern of protein synthesis which is typical to development to one which is more closely identifiable as being associated with germination. The pattern of protein synthesis in rehydrated axes after isolation from desiccated 32-d-developed seeds is shown in Figure 1B. It is evident that several major developmental proteins, including the storage protein phaseolin, are no longer synthesized, even though they are present in the axis, as shown by Coomassie-stained gels from which the autoradiographs were made (Fig. 2; and, incidentally, they are also present in the axis of mature seeds—data not shown). Some newly synthesized proteins can be identified as being exclusively germination proteins (Fig. 1, B and C), which are not present during development (Fig. 1A). A unique set of proteins appear to be synthesized upon rehydration that are not present during either germination or development (Fig. 1B). These we have called rehydration proteins, and they might be produced as a reaction to premature desiccation. We do not know of their function, but speculate that they could be associated with mechanisms put into effect to 'repair' damage elicited by drying (1).

In a control experiment, intact 32-d developing seeds were incubated in radioactive precursor under the same conditions as the isolated axes, and then the axes removed only immediately prior to protein extraction and separation. The pattern of protein synthesis exhibited by these intact aces was identical to that of the isolated axes, as shown in Figure 1A (data not presented). Excision alone, therefore, does not cause any changes in axis protein synthesis.

Freshly isolated axes from 22-d-developed seeds synthesize a variety of proteins that are similar to those made at 32 d, including storage protein (Fig. 3A). The pattern is distinctly different from that of germination protein synthesis (Fig. 1C). Upon rehydration

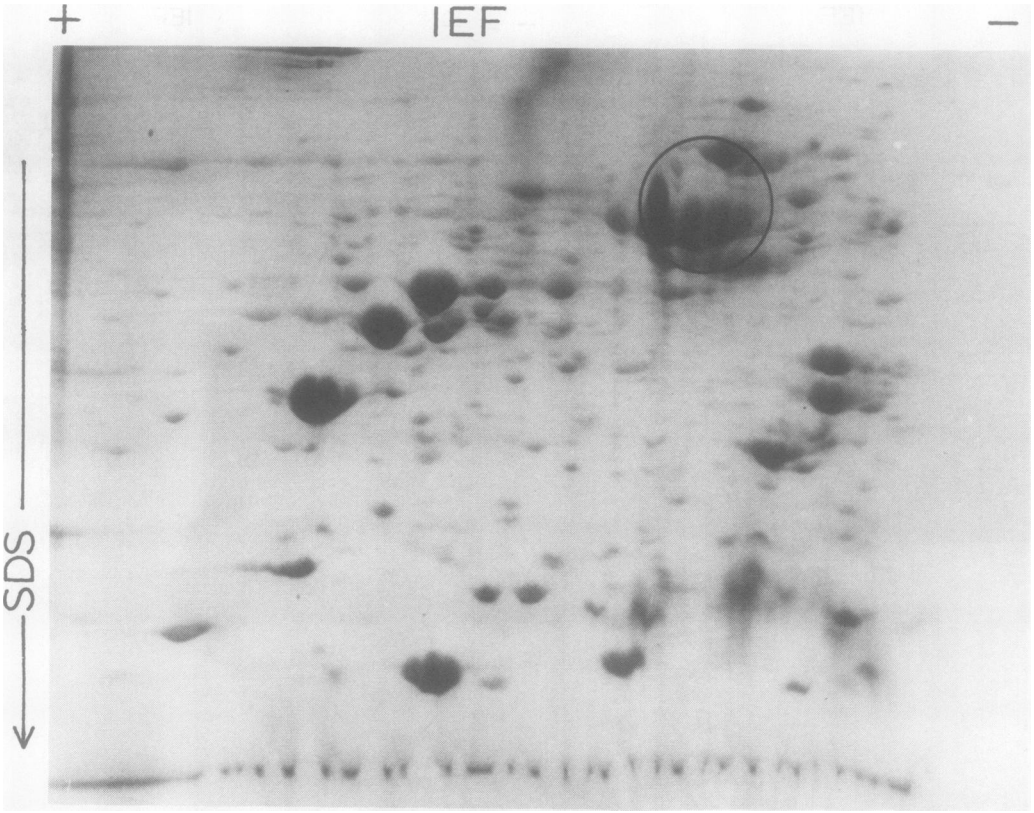

FIG. 2. Coomassie-stained gel of proteins in rehydrated 32-d-developed axes following desiccation. Note the presence of developmental proteins, including phaseolin (circled).

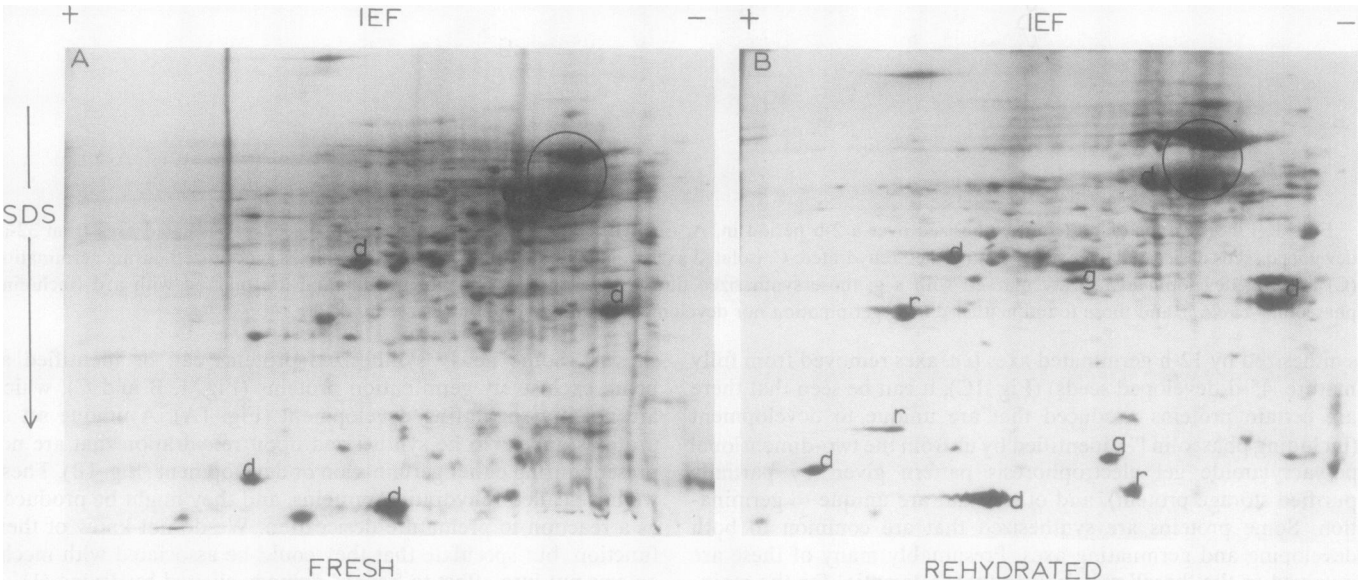

FIG. 3. Fluorographs of proteins synthesized over a 2-h period in: A, isolated fresh axes from 22-d-developed seeds; B, isolated axes from 22-d-developed seeds desiccated over silica gel and then rehydrated. Notations as in Figure 1.

of axes isolated from desiccated 22-d-developed seeds, the pattern of protein synthesis has features that are characteristic of both the developmental and the germination pattern (Fig. 3B), with some proteins produced that are not identifiable as being part of either pattern. Thus, at 22 d, desiccation does not completely terminate developmental protein synthesis nor exclusively elicit germination protein synthesis.

CONCLUSIONS

Desiccation at 32 d of development (in the desiccation-tolerant phase) causes seeds of *Phaseolus vulgaris* to germinate when subsequently rehydrated. This change in direction from development to germination is also mirrored in the pattern of protein synthesis exhibited upon rehydration, in that some protein synthesis uniquely associated with development ceases, and other syntheses associated with germination commence. Presumably, then, desiccation results in the loss from the axis of mRNAs for developmental proteins, and moreover, permanently suppresses the production of these messages. On the other hand, there is induction of those mRNAs for germination proteins. Alternatively, but seemingly less likely, the mRNAs for both development

and germination are present in the cells of the axes, with only the latter being selectively translated. At 22 d of development, when the seed is still intolerant of desiccation, drying neither promotes germination, nor does it successfully switch the pattern of protein synthesis from a developmental to à germination mode. Thus, between 22 and 32 d of development, the axis acquires both a tolerance of desiccation and an ability of the genome to respond positively, as far as germination is concerned, to this stress. Whether desiccation acts directly upon the genome, or indirectly by eliciting changes in the hormone or hormone receptor complement of the developing seed, is a subject for future studies.

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