THE EFFECT OF THE SUSPENSOR AND GIBBERELIC ACID ON
PHASEOLUS VULGARIS EMBRYO PROTEIN SYNTHESIS

by

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ABSTRACT

In this thesis the role of the suspensor and gibberellic acid in Phaseolus vulgaris embryo protein content and synthesis was examined. The plant embryo exists in a very specialized environment and this environment must be maintained in tissue culture for continued normal embryonic development. Optimum sucrose concentrations for culture of 0.2 mm and 0.5 mm embryos in Gamborg B5 medium were determined to be 12% and 6% respectively. Protein content and synthesis of various culture combinations of these embryos and their suspensors were examined by polyacrylamide electrophoresis. Two-tenths millimeter embryos required an attached suspensor for maximum protein content. Virtually all protein synthesis was dependent upon an attached suspensor. Maximum protein quantity and synthesis in 0.5 mm embryos were observed when the embryo was cultured attached to the suspensor. Protein levels decreased when it was cultured detached from or without the suspensor. Gibberellic acid of 10^{-7} M elicited the same protein diversity and greater S^{35} -methionine incorporation than the attached suspensor in 0.2 mm embryos. Five-tenths millimeter embryos did not appear to be differentially responsive to various gibberellin concentrations. All of the G1 storage protein subunits and the 32 and 34 kD G2 storage protein subunits were observed in 0.2 mm embryos. The 0.5 mm embryos in gibberellin had all of the G1 and G2 storage protein subunits.

ABBREVIATIONS

E = Embryo cultured in the absence of the suspensor

E/E-S = Embryo of embryo cultured attached to the suspensor

E/E+S = Embryo of embryo cultured with a detached suspensor

GA₃ = Gibberellic Acid

kD = KiloDaltons

S.A. = Specific Activity

S/E-S = Suspensor of embryo cultured attached to the suspensor

S/E+S = Suspensor of embryo cultured with a detached suspensor

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I. INTRODUCTION

The function of the angiosperm suspensor was classically thought to be purely mechanical, i.e., pushing the embryo into the nutrient-rich endosperm (Maheshwari, 1950). However, Gunning and Pate (1969) pointed out that the wall projections found on suspensor cells of numerous angiosperms suggested a transfer (transport) function for this organ due to the increased surface area.

Cionini et al. (1976) found that in Phaseolus coccineus heart-shaped embryos of less than 5 mm length (suspensor excluded), suspensor removal caused a decrease in the percentage of embryos surviving tissue culture. Survival percentages ranging from 44% when grown intact (15 days) to 7% when grown without the suspensor were obtained for 0.5 mm embryos. Larger embryos (2.0 mm to 5.0 mm) had up to a 30% decrease in survival when cultured in the absence of the suspensor. Clearly the suspensor has some effect on the embryo; this study will attempt to determine the suspensor's effect and how it influences the embryo by observing the embryo protein quantity and synthesis.

In a white-seeded variety of the runner bean, Phaseolus coccineus, the suspensor had 30 times the gibberellin activity as the embryo at the heart-shaped stage (Alpi et al., 1975). At the cotyledonary stage, the level of gibberellin activity in the suspensor dropped while the gibberellin activity in the embryo rose until the two tissues had approximately the same levels of activity. At this time, the overall gibberellin level in the embryo had increased about 10 times above that at the heart stage.

Gibberellic acid has been shown to initiate increases in the activity of several enzymes including α -amylase (Hooley, 1982; Varner, 1964; MacLeod and Millar, 1962; Paleg, 1960), β -glucanase (Briggs, 1963; MacLeod and Millar, 1962), phosphatase (Briggs, 1963), and ribonuclease (Hooley, 1982; Kapoor, 1981; Chrispeels and Varner, 1967). Kapoor (1981) found that GA_3 stimulated RNAase activity two- to three-fold in germinating seeds and that this enhancement was due to de novo synthesis. Varner and Ram Chandra (1964) demonstrated that in germinating barley seeds (Hordeum vulgare var. Himalaya), a gibberellic acid-dependent increase in α -amylase activity required de novo protein synthesis. Further work on H. vulgare (Varner et al., 1965) showed the necessity of RNA synthesis for this increase in α -amylase activity. Gibberellic acid also has been shown to enhance RNA synthesis in isolated pea nuclei. These newly synthesized RNAs differed both in base sequence and size from the RNAs of untreated nuclei (Johri and Varner, 1967).

The high gibberellin activity in the suspensor and the ability of gibberellin to enhance RNA and protein synthesis in other systems (Hooley, 1982; Kononowicz et al., 1982; Martin and Northcote, 1982; Kapoor, 1981; Sawhney et al., 1977; Srivastava et al., 1975) make it reasonable to examine whether the suspensor may play a physiological role (i.e., regulation of protein synthesis) during Phaseolus embryo development. If it can be established that the suspensor has such a role, then I wish to determine whether GA_3 can be employed to mimic the suspensor in regulating embryo protein content and synthesis.

In this thesis the effect of the suspensor on protein content

and synthesis during two stages of embryo development will be examined.

The following questions will be addressed:

- (1) Does the presence or absence of the suspensor affect embryo protein content or synthesis?
- (2) If there is an effect, could the gibberellin activity in the suspensor be responsible for part or all of the suspensor's effect on the embryo?

II. METHODS AND PROCEDURES

Plants

Phaseolus vulgaris var. Taylor's Horticultural (Courtesy of Asgrow Seed Company) seeds were grown in seven inch pots in a growth chamber at $24 \pm 2^{\circ}\text{C}$. Light intensity ranged from 284 to 384 micro-einsteins/ m^2 -sec on a 12 hour photoperiod. Pods were harvested when 1 to 4 centimeters in length.

Embryo Culture

The pods were surface sterilized with absolute ethanol, opened and the embryos excised with sterile tungsten needles and cultured in 10 μl of sterile Gamborg's B5 medium (Gamborg, 1968) in sterile Falcon Microtest Plates and cultured at $27 \pm 2^{\circ}\text{C}$ on a 12 hour photoperiod.

Determination of Optimum Sucrose Concentration for Embryo Culture

Five-tenths mm embryos (suspensor excluded) were cultured in Gamborg's B5 medium containing 2, 4, 6, 8, 10, 12, and 14% sucrose for 7 days to determine the sucrose concentration yielding optimum growth. Embryos 0.2 mm long were cultured in 6, 8, 10, 12, 14, and 16% sucrose under the same criteria as for the 0.5 mm embryos. Length measurement of both sizes of embryos were made daily.

Protein Labeling

Five-tenths mm embryos were cultured in Gamborg's B5 medium (Gamborg, 1968) containing 6% sucrose and 0.2 mm embryos in medium containing 12% sucrose for 48 hours with (a) the suspensor

attached, (b) the suspensor present but unattached and (c) in the absence of the suspensor. Fifteen of the 0.5 mm embryos or forty of the 0.2 mm embryos of each state were combined for labeling the last hour of culture in 20 μ l of Gamborg's B5 medium with the appropriate sucrose concentration. One μ Ci of S^{35} -methionine was used for each three 0.5 mm embryos and each eight 0.2 mm embryos (S.A. ranged from 1.21 μ Ci/ μ l to 5.59 μ Ci/ μ l).

Exogenously Applied Gibberellic Acid

Three 0.5 mm and 40 0.2 mm embryos were cultured without the suspensors in 10 μ l of medium containing appropriate sucrose concentrations and gibberellic acid (Sigma Chemical) at 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M concentrations for 48 hours. Newly synthesized proteins were radioactively labeled (S^{35} -methionine) during the last hour of culture in 20 μ l of Gamborg's B5 medium with the appropriate sucrose and gibberellin concentrations. The medium also contained 1 μ Ci of S^{35} -methionine (S.A. ranging from 1.21 μ Ci/ μ l to 5.59 μ Ci/ μ l) for each three 0.5 mm embryos and each eight 0.2 mm embryos.

Protein Determination

Protein concentrations were determined by homogenizing three 0.5 mm embryos or suspensors or thirty 0.2 mm embryos or suspensors in 200 μ l of water. Samples were then centrifuged at 7000 X g in a Fisher Model 59 centrifuge for 20 minutes at room temperature. Assays were performed in duplicate on 90 μ l of this homogenate. Protein concentration was determined according to the method of Bradford (1976) using the Bio-Rad Protein Determination Kit.

Acrylamide Electrophoresis

Embryos and suspensors (numbers reported in previous sections) were homogenized separately in 100 μ l of sample buffer (LeStourgeon and Beyer, 1977) and centrifuged at 7000 X g in a Fisher Model 59 Centrifuge for 20 minutes at room temperature. The supernatants were electrophoresed on 10% SDS polyacrylamide slab gels (LeStourgeon and Beyer, 1977). The gels were stained with coomassie blue G250, destained in 7-10% acetic acid, impregnated with Enhance (New England Nuclear) and dried. Fluorography was performed as described in Bonner and Laskey (1976) using Kodak X-R5 film. Exposure was from 1 to 30 days at -70°C . The standards were bovine serum albumin (MW 66,000 daltons), ovalbumin (MW 45,000 daltons), trypsinogen (MW 24,000 daltons), β -lactoglobulin (MW 18,400 daltons), and lysozyme (MW 14,300 daltons), all of which were purchased from Sigma Chemical.

RESULTS

Determination of an Optimal Sucrose Concentration

Many plant embryos, when cultured, do not complete the remaining developmental stages but instead germinate (Long et al., 1981). This precocious germination has been shown to be retarded or completely blocked by a decrease in osmotic potential (higher sucrose concentration). The smaller the embryo the more negative the osmotic potential of the culture medium must be for proper developmental progression (Yeung and Brown, 1982; Rietsema et al., 1953).

To determine the optimal sucrose concentration necessary for normal developmental appearance, 0.5 mm embryos were cultured in Gamborg's B5 media containing 2, 4, 6, 8, 10, 12, and 14 percent sucrose (Figure 1B); 0.2 mm embryos were cultured in media containing 6, 8, 10, 12, 14, and 16 percent sucrose (Figure 1A). Mean percent increase in length was determined after 7 days in culture.

The 0.2 mm embryos, being smaller, were tested over a higher concentration range than were the 0.5 mm embryos. Although the differences in 0.2 mm embryos cultured in various sucrose concentrations between 6 and 16% were not significant (standard errors overlapped), 12% sucrose medium yielded the greatest mean percent increase in length. Embryo color and cotyledon shape remained normal at this sucrose concentration and it was selected for all subsequent cultures of 0.2 mm embryos.

Five-tenths mm embryos cultured in 2% sucrose medium formed callus, usually at the point of embryo-suspensor attachment. This accounts for the extremely large increase in length recorded

Figure 1. Effect of sucrose concentration on embryo elongation after seven days of culture. Mean percent increase is reported for each concentration. A. —■— 0.2 mm embryos; B. —●— 0.5mm embryos. Embryos were cultured attached to suspensors but only embryo length was measured (n = 10)

for embryos cultured at this concentration of sucrose. There was a sharp drop in mean percent embryo elongation when embryos were cultured in 4% sucrose Gamborg's medium. The embryos cultured in 6% sucrose medium had a slightly higher mean increase in embryo length. All successive concentrations (8, 10, 12 and 14%) steadily declined in mean percent embryo elongation until 14% sucrose Gamborg's B5 medium had only slightly more than a 50% increase in length. Six percent sucrose Gamborg's B5 medium was chosen for 0.5 mm embryo cultures. Embryos cultured in this medium, in addition to having the greatest mean percent increase in embryo length other than 2% sucrose (which formed callus), also most closely resembled the freshly excised embryos in color and cotyledon shape.

Effect of the Suspensor on Protein Synthesis

In order to determine whether the suspensor has an effect on embryo protein content and synthesis, 0.5 mm and 0.2 mm embryos were cultured for forty-eight hours with the suspensor attached to the embryo, with the suspensor detached from but cultured in the same drop of medium as the embryo, or in the absence of the suspensor. At the end of the culture period, total protein quantity (Bradford, 1976) was determined in both the embryo and the suspensor. Protein synthesis was examined by incubation of embryos in S^{35} -methionine followed by electrophoresis and fluorography.

0.2 mm Embryos

Protein Content

Of the cultured 0.2 mm embryos, maximum protein quantities were

observed in both embryos and suspensors when they were cultured attached ($0.190 \pm 0.031 \mu\text{g}$, $0.135 \pm 0.020 \mu\text{g}$; embryo and suspensor, respectively)(Figure 2A). These levels were significantly lower than that of fresh tissues ($0.359 \pm 0.076 \mu\text{g}$, embryo; $0.293 \pm 0.071 \mu\text{g}$, suspensor). Severance of the suspensor from the embryo caused a further reduction in protein quantity in the embryo ($0.150 \pm 0.032 \mu\text{g}$, detached; $0.190 \pm 0.031 \mu\text{g}$, attached) but had no affect on the suspensor ($0.133 \pm 0.014 \mu\text{g}$, detached; $0.135 \pm 0.020 \mu\text{g}$, attached). Two-tenths millimeter embryos cultured in the absence of the suspensor showed the least amount of protein ($0.130 \pm 0.024 \mu\text{g}$) of the embryos examined.

When proteins of the 0.2 mm embryos cultured with the suspensor attached (E/E-S) were examined on SDS polyacrylamide gels (Figure 2B), both the number of bands present on the gel and the staining intensity of these bands were greater than when 0.2 mm embryos were cultured with the suspensor detached (E/E+S). The 0.2 mm embryo cultured in the absence of the suspensor (E) had the fewest bands and the lowest staining intensity of any of the 0.2 mm embryos cultured. The suspensor banding pattern appears the same whether the suspensor is cultured attached to the embryo (S/E-S) or severed from the embryo (S/E+S).

Protein Synthesis

The major proteins synthesized in both embryos and suspensors cultured under the various experimental conditions appear to remain relatively constant. There are marked differences in the amount of incorporation which corresponds to the total protein content.

Radioactive amino acid incorporation (Figure 2C) in 0.2 mm embryos

(E) and suspensors (S) peaked when the tissues were cultured attached (E-S). This incorporation was radically reduced when the embryo (E) and suspensor (S) were detached (E+S) or the embryo was cultured alone (E). At the early stage (0.2 mm), protein bands corresponding to 60, 47, 43, and 36 kD molecular weight are the major proteins being synthesized in embryos cultured attached to their suspensors. Except for the protein of 60 kD, whose intense synthesis is observed in embryos cultured detached from their suspensor, the synthesis of these proteins is drastically reduced or absent in embryos cultured either detached from their suspensors or alone. Peptides which migrate at the same molecular weight as these newly synthesized peptides were observed in the Coomassie Blue stained gel of proteins extracted from all cultured embryos. The bands at 43 and 36 kD stained in embryos cultured attached to their suspensors and in embryos cultured with a detached suspensor but not in embryos cultured alone. In the fluorograph this band was synthesized only in the embryo which was cultured attached to its suspensor.

0.5 mm Embryos

Protein Content

The 0.5 mm embryos and suspensors had maximum quantities of protein when cultured attached to each other ($2.508 \pm 0.553 \mu\text{g}$, $1.882 \pm 0.315 \mu\text{g}$; embryo and suspensor, respectively, Figure 3A). These quantities were not significantly different from the protein quantities of fresh tissues ($3.251 \pm 0.398 \mu\text{g}$, $2.290 \pm 0.214 \mu\text{g}$; embryo and sus-

Figure 3A. Effect of the suspensor on protein quantities (μg per organ) of 0.5 mm embryos. E/E-S, signifies the embryo when cultured attached to the suspensor; S/E-S, signifies the suspensor when cultured attached to the embryo; E/E+S, signifies the embryo when cultured with but detached from the suspensor; S/E+S, signifies the suspensor when cultured with but detached from the embryo; and E signifies the embryo cultured alone. For each condition, $n = 5$. Standard error is reported in each case. Organs were cultured 48 hours in Gamborg's B5 medium with 6% sucrose.

- B. Coomassie Blue stained 10% SDS polyacrylamide slab gel demonstrating the suspensor's effect on specific protein quantities and distribution. The numbers to the right, represent molecular weights of standard proteins in 10^4 Daltons. $n = 15$. (—) mark the calculated mobilities of the G1 and G2 proteins' subunits molecular weights (53, 49, 47, 43 and 34, 32, 30 kD, respectively). (►) mark the proteins referred to in the thesis.
- C. Fluorograph of the gel shown in B. E' was exposed 5 days, the remainder of the wells were exposed for 9 days.

"Gut, Pulp"

detached 1.

sensor, respectively). Five-tenths mm embryos cultured with but detached from their suspensors had a lower protein quantity ($1.910 \pm 0.252 \mu\text{g}$) than those cultured attached to the suspensor ($2.508 \pm 0.553 \mu\text{g}$). The embryos cultured in the absence of the suspensor showed a sharp reduction in protein ($1.044 \pm 0.304 \mu\text{g}$) which was significantly lower than that of fresh embryos or those cultured in the presence of the suspensor.

Suspensors cultured detached from embryos had less than half the protein of the suspensors cultured while still attached to the embryo ($0.723 \pm 0.194 \mu\text{g}$, $1.882 \pm 0.315 \mu\text{g}$, respectively; Figure 3A).

An examination of the Coomassie Blue stained gel of proteins extracted from 0.5 mm embryos indicated that in general there is a decrease in stain intensity in all of the bands on the gel in the following order: E/E-S > E/E+S > E (Figure 3B). This also is true of any given band from lane to lane.

Protein Synthesis

Embryos (E) cultured attached suspensors (E-S) show intense labeling with S^{35} -methionine (Figure 3C). Embryos (E) cultured with detached suspensors (E+S) show lower levels of incorporation, while embryos (E) cultured without suspensors had lowest levels of radio-labeling. Figure 3C shows two proteins at about 30-32 kD being synthesized in embryos under all culture conditions. A very faint band at 30,000 daltons was observed in 0.2 mm embryos (embryos of embryos cultured attached to suspensors) but not in embryos cultured detached from or in the absence of the suspensor (Figure 3C). The major proteins being synthesized in 0.5 mm embryos remain those at molecular weights

of 60, 53, 47, 43 and 36 kD which were observed in 0.2 mm embryos. Unlike the 0.2 mm embryos in which the synthesis of these proteins was observed only, or to the greatest extent in embryos cultured attached to their suspensors, the 0.5 mm embryos synthesized these proteins under all of the culture conditions examined.

Effect of Gibberellic Acid (GA_3) Concentration on Protein Quantity and Protein Synthesis

It has been previously demonstrated that the suspensor has a high level of gibberellic acid activity. Results in the previous section indicate that the presence of the suspensor attached to the embryo positively influences S^{35} -methionine incorporation into the embryo's total protein. The role of gibberellins in the regulation of protein content and protein synthesis in Phaseolus embryos was examined. Embryos were cultured without their suspensors in concentrations of gibberellic acid of 10^{-4} M to 10^{-8} M for 48 hours. Total protein content was determined and protein synthesis was assayed as previously described.

0.2 mm Embryos

Protein Quantity

Embryos cultured in 10^{-4} M gibberellic acid had slightly higher protein levels than those of embryos cultured without exogenous gibberellin but significantly lower levels than those of fresh embryos (0.146 ± 0.013 μ g, 0.130 ± 0.024 μ g, 0.359 ± 0.076 μ g, respectively) (Figure 4A). Embryos cultured in 10^{-5} M gibberellic acid had protein quantities (0.203 ± 0.024 μ g) which were significantly greater than

Figure 4A. Effect of gibberellic acid concentrations on protein quantities (μg per organ) of 0.2 mm embryos. Molar concentrations of GA_3 in which embryos were incubated are labeled at the bottom of the appropriate histogram. In each case standard error is reported ($n = 5$).

- B. Coomassie Blue stained 10% SDS polyacrylamide slab gel demonstrating gibberellic acid's effect on specific protein quantities and distribution. The concentrations are labeled at the bottom of each well. ($n = 40$) The numbers to the right represent molecular weights of standard proteins in 10^4 Daltons. (-) mark the calculated mobilities of the G1 and G2 proteins' subunits molecular weights (53, 49, 47, 43 and 34, 32, 30 kD, respectively). (►) marks the specific peptides synthesized in response to GA_3 .
- C. Fluorograph of the gel shown in B. Exposure was for 4 days.

B **C**

embryos cultured alone and without exogenous gibberellin. Two-tenths mm embryos cultured in gibberellic acid showed maximal protein quantities when cultured in 10^{-6} M (0.287 ± 0.021 μ g) or 10^{-7} M (0.288 ± 0.034 μ g) gibberellin (Figure 4A). These quantities are significantly higher than those found in embryos cultured attached to suspensors in medium lacking gibberellin (0.130 ± 0.024 μ g), and are slightly lower but statistically indistinguishable from those of freshly excised uncultured embryos (0.359 ± 0.076 μ g). The 10^{-8} M GA_3 caused a response slightly lower than 10^{-6} M or 10^{-7} M, giving 0.266 ± 0.038 μ g of protein. When comparing these quantities with those observed in 0.2 mm embryos cultured with the embryo and suspensor attached, 10^{-6} , 10^{-7} and 10^{-8} M GA_3 gave significantly higher protein quantities (Figure 4A). It is interesting to note that while the protein content of 0.2 mm embryos cultured attached to the suspensor (0.190 ± 0.031 μ g) was significantly lower than fresh tissue (0.359 ± 0.076 μ g), the protein content of those embryos cultured without their suspensors in 10^{-6} , 10^{-7} or 10^{-8} M gibberellic acid was not significantly different from fresh tissue.

The Coomassie Blue stained gel of protein extracted from embryos cultured in various GA_3 concentrations (Figure 4B) demonstrates that although the banding pattern for each well on the gel is identical, the staining intensity indicating protein quantity increased from 10^{-4} to 10^{-7} M and then decreased at 10^{-8} M gibberellic acid.

Protein Synthesis

Figure 4C shows that 0.2 mm embryos cultured in 10^{-4} M and 10^{-5} M GA_3 had relatively low levels of radioactive incorporation although

substantially higher than that of embryos cultured without exogenous gibberellic acid (Figure 2C, E). The 10^{-6} and 10^{-7} M GA_3 -treated embryos had high levels of label incorporation with the 10^{-8} M GA_3 -treated embryos having only slightly lower levels of incorporation although higher than that of embryos cultured with the suspensor.

In addition to the general increases noted above, increased synthesis of specific proteins in the range of 65, 49 and 34 kD are seen at the lower gibberellin concentrations. The 65 kD band appears in all concentrations but is enhanced in 10^{-6} , 10^{-7} and 10^{-8} M GA_3 . A protein band of about 34 kD appears in the 10^{-7} and 10^{-8} M gibberellic acid concentrations (Figure 4C).

0.5 mm Embryos

Protein Quantity

Five-tenths mm embryos cultured in 10^{-8} M gibberellin (Figure 5A) showed the same protein concentration as that of the freshly excised uncultured embryos (3.222 ± 0.388 ug; 3.251 ± 0.398 ug, respectively), both of which were significantly higher than embryos cultured alone without gibberellin (1.044 ± 0.304 ug). Concentrations of GA_3 higher than this level had quantities slightly lower than 10^{-8} M GA_3 -treated embryos and fresh tissue but significantly higher protein levels than found in embryos cultured in the absence of the suspensor and without any exogenous gibberellin (10^{-7} M, 2.543 ± 0.363 ug; 10^{-6} M, 2.406 ± 0.282 ug; 10^{-5} M, 2.148 ± 0.317 ug; and 10^{-4} M, 2.660 ± 0.298 ug). All concentrations yielded protein quantities which were indistinguishable from the protein quantities of the embryo cultured attached to the suspensor (E/E-S).

B **C**

Protein Synthesis

The general pattern and intensity of amino acid incorporation is the same for embryos cultured in gibberellic acid concentrations between 10^{-4} M and 10^{-8} M. Specific proteins in the ranges of 30, 49 and 65 kD are synthesized in response to low gibberellic acid concentrations and are absent at concentrations 10^{-6} M and above.

DISCUSSION

The role of the angiosperm suspensor in the development of the embryo has not been thoroughly examined. Based on the presence of finger-like projections between the suspensor and the sterile seed tissue (Schnepf and Nagl, 1970) and on the observation that these projections are found in plant cells with a transfer function (Gunning and Pate, 1969), it has been postulated that the suspensor may serve the function of transferring compounds synthesized either in the suspensor or in other parts of the seed or plant to the developing embryo (Clutter and Sussex, 1968; Brady, 1973). If the suspensor plays such a role, then it must do so early in the development of the embryo because in the Phaseolus vulgaris embryo the suspensor reaches its maximum size at the late heart stage and then begins to degenerate (Walbot et al., 1972). Gibberellin, a plant hormone which has been shown to play a role in the development of several plant embryos (Kefford and Rijven, 1966; Dure and Jensen, 1957), has been demonstrated to be present in high quantities in the suspensor of early Phaseolus embryos, and its concentration decreases in later development (Alpi et al., 1975). Therefore, the effect of the suspensor in vitro and of gibberellins on protein synthesis and content in two early stages of embryogenesis in Phaseolus vulgaris has been examined.

The plant embryo exists in a very specialized environment, and this environment must be maintained in tissue culture for continued normal embryonic development (Raghavan, 1976). In Phaseolus (Yeung and Brown, 1982; Smith, 1973), as with several other plant embryos

(See Raghavan, 1976 for a review), the osmotic potential of the endosperm surrounding the embryo and that of the embryo itself is high early in development and decreases as development proceeds. The results presented in this thesis demonstrate that for 0.2 mm Phaseolus vulgaris 4 days post-anthesis early heart stage embryos 12% sucrose in the culture medium produced maximal growth without either precocious germination or callus formation. Three days later at seven days post-anthesis, the sucrose concentration at which maximal normal development was observed dropped to 6% sucrose. These results are strikingly similar to those obtained for Datura (Rietsema et al., 1953) and Hordeum (Norstog, 1961).

The suspensor clearly affects protein quantities. With both embryo sizes, the presence of an attached suspensor resulted in the highest quantities of protein. When the suspensor was severed, both sizes of embryo showed a decrease in protein content. A further decrease was observed when the suspensor was removed.

Protein synthesis in 0.2 mm cultured embryos was strongly affected by the attached suspensor. Amino acid incorporation virtually ceased when 0.2 mm embryos were cultured with a severed suspensor or without the suspensor. Five-tenths mm embryos were not as dependent upon suspensor attachment as were those of 0.2 mm. Removal of the suspensor from these embryos, moderately reduced protein synthesis. Even 0.5 mm embryos cultured without suspensors showed substantial S³⁵-methionine incorporation.

Low gibberellin concentrations almost doubled the protein content of 0.2 mm embryos cultured without their suspensors. The protein content

of embryos cultured with attached suspensors (E/E-S) was significantly lower than that of fresh embryos, but these quantities are statistically indistinguishable from fresh tissues. If the suspensor supplied the developing embryo with gibberellin, and as it has been demonstrated GA_3 affects the protein content of the embryo, a large culture medium volume (10 μ l) without any exogenous GA_3 would dilute the gibberellin of the suspensor so that even with the embryo attached, the resultant protein quantities would be lower than that attained in vivo. If one assumes that the major route of the GA_3 to the embryo is from the suspensor directly into the embryo and not into the surrounding tissue or through the endosperm, then the difference in protein between the embryos cultured attached to the suspensor and those cultured separated from their suspensor may be explained.

Cionini et al. (1976) found that these same low gibberellin concentrations (10^{-8} M to 10^{-6} M) which enhance protein content of 0.2 mm embryos cultured in vitro also increased survival of heart-stage embryos of Phaseolus coccineus cultured in the absence of the suspensor.

Five-tenths millimeter embryos cultured in all gibberellin concentrations examined had a higher quantity of protein than that observed in embryos cultured in the absence of the suspensor and were indistinguishable from E/E-S. The 10^{-5} M and 10^{-6} M gibberellin-treated embryos had significantly lower protein quantities than 10^{-7} or 10^{-8} M gibberellin-treated or freshly excised embryos.

The synthesis of several bean embryo proteins has been closely examined (Murray and Crump, 1979; Sun et al., 1978; Barker et al., 1976; and Derbyshire and Boulter, 1976). Those proteins which have received the most attention are the storage proteins. These have been

divided into two major groups. The G1 (glycoproteins I) proteins have been extensively examined by the laboratory of T. C. Hall (Brown et al., 1981; Sun et al., 1981; Brown et al., 1980; Ma et al., 1980; Mutschler et al., 1980; Hall et al., 1978; Sun et al., 1978; Stockman et al., 1976; Sun and Hall, 1975; and McLeester et al., 1973) in the cultivar Tendergreen, and less so by the Sussex laboratory (Sussex, personal communication) in the cultivar Taylor's Horticultural. This latter cultivar was used in the present study. Although there is some disagreement as to the genetics of this system, these authors do agree that the major subunits of the G1 proteins have molecular weights of 53, 47 and 43 kD and that a minor component with a molecular weight of 49 kD also exists but is not always expressed. The G2 (glycoproteins II) proteins have also been examined by the Hall laboratory (Mutschler et al., 1980; Sun and Hall, 1975; and McLeester et al., 1973). These proteins are composed of peptides separating into 3 bands on polyacrylamide gels with molecular weights of 30, 32 and 34 kD (McLeester et al., 1973).

Most of the work done on these proteins up to the present has concentrated on the appearance of stained bands on polyacrylamide gels as an indication of when these proteins were synthesized. The one exception (Sun et al., 1978) indicated that the synthesis of these proteins began at the 7 mm bean stage (at 12 days post-anthesis). The work reported here demonstrates that at the 0.2 mm stage embryo (2.0 mm bean), 4 days post-anthesis, all of the G1 peptides are synthesized in embryos cultured forty-eight hours attached to their suspensors, but their synthesis in embryos cultured in the presence of but detached

from their suspensor or in embryos cultured without their suspensor is much reduced. These same peptides were observed to be intensely synthesized by embryos cultured in gibberellin in the absence of their suspensors at all of the concentrations examined. This may indicate the synthesis of all of these proteins is under the regulation of GA_3 .

The G2 proteins show a similar response. The 32 kD subunit is synthesized in 0.2 mm embryos cultured attached to their suspensor but not in embryos cultured under other culture conditions. In 0.5 mm embryos both the 32 kD subunit and a 30 kD subunit are synthesized. This same synthetic pattern (30 and 32 kD) is observed in 0.2 mm embryos in all concentrations of GA_3 examined. At low levels of gibberellic acid the synthesis of a third peptide of 34 kD is observed. The synthesis of this band is most intense at 10^{-7} M GA_3 but is substantially reduced at both 10^{-6} and 10^{-8} M gibberellin and is absent in GA_3 concentrations higher than 10^{-6} M. The 32 kD band has been demonstrated by Hall to be the peptide most abundant in the young embryos (Sun et al., 1978). The synthesis of the 32 kD band is much greater than that of the 30 or 34 kD bands and the synthesis of all of these peptides is most responsive to a gibberellin concentration of 10^{-7} M.

Although there are a few peptides whose synthesis appears to be gibberellin activated, gibberellin appears to stimulate protein synthesis in general rather than altering the amino acid incorporation into particular protein bands. This also has been observed in germinating castor bean seeds (Martin and Northcote, 1982) and in lettuce hypocotyls (Srivastava et al., 1975).

From these data one concludes that the 0.2 mm embryos are strongly

affected by the suspensor and that this effect can be mimicked by low gibberellin concentrations. This mimicry results in the same protein pattern as that induced by the suspensor. The 0.5 mm embryo is influenced by the suspensor but is not dependent upon it for the maintenance of protein synthesis. Equally evident is the fact that gibberellin does something to alleviate the lack of the suspensor. Exactly how protein synthesis is influenced in the 0.5 mm embryos by gibberellin is not clear. The 0.5 mm embryo is at that stage where growth is changing from an increase in cell number to an increase in cell size. Further, this is a time of the elegant altering of hormone balances (Yeung and Brown, 1982; Hsu, 1979). Free abscisic acid (ABA) levels are 1.5 times higher in the 0.5 mm (7 days post-anthesis) embryos than in the 0.2 mm embryos (4 days post-anthesis) (Hsu, 1979). At later stages peaks of free ABA coincided with inhibited development (both cell and tissue, Haddon and Northcote, 1976) and enhanced protein synthesis. These peaks coincided with a decrease in RNA synthesis. Haddon and Northcote (1976) also reported that gibberellic acid could not overcome the inhibitory effect of ABA. It is quite possible that factors other than those discussed here are involved in the 0.5 mm embryo response. Further work is needed to determine exactly how development is controlled and what role gibberellin plays in the development of 0.5 mm embryos remains to be clarified.

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