# REGULATION OF FRUIT SET OF *PHASEOLUS* CROSSES BY POLLEN SOURCE AND ENVIRONMENT

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# INTRODUCTION

Regulation of early fruit abscission is of interest to physiologists. The common means of studying fruit abscission is to compare retained and abscised fruit to elucidate the mechanism of abscission. A method of predicting fruit abscission at a specific stage of development would allow a study of physiological or morphological changes occuring prior ot the event. Interspecific hybridization may be a useful tool for this purpose because it often leads to seed abortion and subsequent fruit abscission (3, 4, 19, 28, 43, 45).

Phaseolus is the genus of choice because seeds and embryos are relatively large which facilitates analysis, and because breeders are interested in gene transfer with the species so work with interspecific hybrids has been extensive (4, 6, 11, 19, 36). The major barrier to Phaseolus coccineus X P. vulgaris hybridization is seed abortion and subsequent fruit abscission (4, 28). In these crosses fertilization occurs but embryos abort 8 to 24 days after pollination (19, 43, 45).

The overall objective of this work was to determine the causes of failure of the seeds in an interspecific Phaseolus cross, P. coccineus X P. vulgaris. To obtain an answer to this complex problem this project asked a series of questions comparing pre-abortion seed development in the interspecific cross P coccineus X P. vulgaris, with normal seed development in selfed P coccineus:

1) Do the seeds from the crosses develop at the same rate?

2) Does the partitioning of $^{14}C$ photoassimilates change as abortion is commencing?

3) How do the endogenous hormone profiles differ in the seeds from the two crosses?

4) How do exogenous treatments affect the process of abortion?

1. DO THE SEEDS FROM THE TWO CROSSES DEVELOP AT THE SAME RATE?

A) P. coccineus selfed. The embryology of P. coccineus breaks down into 4 stages. A review of this developmental process will follow.

PROEMBRYO (1 to 4 days after pollination).

After fertilization, the zygote divides transversally forming a large basal cell and a smaller terminal cell. Further cell divisions result in a filamentous embryo with little cell differentiation (49).

Just before to the globular stage, the suspensor cells become differentiated at the base of the embryo. These suspensor cells undergo a non-synchronous pattern of cell division while the cells of the embryo-proper are smaller and arranged in a regular fashion (49). At this point cell division in the basal portion of the suspensor has ceased and polytene chromosomes have been observed (8). Any further growth of the suspensor in the basal region is from cell enlargement (49). Another distinguishing characteristic of these cells at this time are wall ingrowths. Such ingrowths are primarily found on the wall adjacent to the integumentary tapetum and

are thought to be associated with movement of materials from the tissues surrounding the suspensor into the suspensor and then to the embryo-proper (49).

Early in development, the preembryo appears to be in direct contact with the liquid endosperm. But as the globular stage is approached, the embryo clearly separates from the liquid endosperm by formation of a layer of cellular endosperm which persists from this point in development on (49).

GLOBULAR (4 to 7 after days pollination)

In the embryo-proper, cell divisions continue resulting in a globular-shaped embryo. Cytologically these cells are all similar to each other in size, cytoplasm density, and vacuolation (49). As the embryo approaches the heart stage, the suspensor has many unique and distinguishing characters: 1) extensive vacuolation of basal cells while terminal cells are highly cytoplasmic, 2) nuclei of suspensor cells are larger than those of the embryo-proper, 3) polytene chromosomes are visible, and 4) wall ingrowths are more wide spread (49).

Development of the cellular endosperm continues rapidly during the globular embryo stage. Normal cell division occurs as does cell differentiation. Wall ingrowths can be seen in those cells that abut the integumentary tapetum but are absent from the wall lining enclosing the liquid endosperm (49).

HEART (7 to 12 days after pollination).

The formation of the cotyledon primordia give the embryo its heart shape. Progressive vacuolation occurs and cell expansion

follows. The embryo-proper turns pale green indicating maturation of chloroplasts. By late in this stage, the embryonic axis is clearly defined.

Cell divisions continue in the terminal region of the suspensor and then cease(49). By this time a basal-terminal gradation in nuclear size exists, with the largest nuclei in the basal region (49). Polytene chromosomes are formed at this stage. Nagl (32) and Walbot (46) have postulated that polyteny provides a mechanism for producing large quantities of RNA without committing energy resources to processes related to cytokinesis. After cell division has ceased, the suspensor continues to grow by cell enlargement.

In the cellular endosperm mitotic activity continues, allowing continued separation of the embryo-proper from the liquid endosperm.

COTYLEDON AND MATURATION (12 to 18 days, and 18 to 36 days after pollination).

This stage is marked by accelerated cell division in the cotyledons which gradually fill the endosperm cavity. The embryonic axis also continues differentiation. At mid-cotyledon stage, starch grains are observed in the axis while few are seen in the cotyledons (49). By late cotyledon stage, procambium is fully differentiated, the root and shoot meristems have formed, and leaf primordia are present (49). The major storage bodies in the cotyledons form as the endosperm cavity becomes filled by the cotyledons.

The suspensor attains its maximum size in the early cotyledon stage. As maturation begins, vacuolation decreases in the suspensor and storage substance accumulation increases.

The endosperm changes markedly from the early cotyledon stage to maturation. Cellular endosperm cells are actively dividing and wall ingrowths appear in the wall lining enclosing the liquid endosperm (49). These ingrowths appear first near the funiculus and progress towards the chalazal end of the seed. Liquid endosperm volume increases 6-fold from heart to mid-cotyledon stage. At the mid-cotyledon stage, cotyledon expansion is extremely fast and absorption of liquid endosperm occurs, as does stretching of the cellular endosperm sheath which finally breaks at maturation.

B) P. coccineus X P. vulgaris.

Embryo development of the interspecific cross P coccineus X P vulgaris is significantly different than that in P. coccineus selfed. However no extensive work on growth has been done on any interspecific Phaseolus cross.

Thomas (45) confirmed that in the cross, P coccineus X P. vulgaris, fertilization occurred but success was much higher if P vulgaris was the seed parent. Differences in embryo survival were due to retarded embryo and endosperm development immediately following the post-fertilization period. This resulted in low pod set and low embryo survival. Although Thomas (45) concluded pollen tube growth was slow, Hawkins and Evans (18), Mok (28), and Alveraz (1) demonstrated that most interspecific Phaseolus crosses did not have retarded pollen tube growth.

PROEMBRYO AND GLOBULAR STAGE

Fertilization occurs 12 to 16 h after pollination (17). The rate of initial embryo and endosperm cell division appears to be

similar to that of P coccineus selfed, but slower than in P. vulgaris selfed. Abnormalities in suspensor cells are noticed 4 days after pollination. By 6 days after pollination a decrease in the quantity of endoplasmic reticulum, plastids, dictysomes, lipid bodies, ribosomes, and a loss of mitochondrial integrity occurs in the suspensor (17).

Embryo and endosperm development remains similar to P coccineus selfed through this stage of development.

HEART STAGE

By the heart stage the suspensor cells show marked disorganization. Wall ingrowths are not extensively developed.

Embryo cells begin to deteriorate by day 6. Numerous mitochondria have lost structural integrity and less endoplasmic reticulum is seen (17). Nuclei show degeneration 10 days after pollination.

Endosperm organelles develop normally until day 8. From then on, mitochondria are fewer and cell disorganization appears by day 10. The inner layer of cell wall projections of the cellular endosperm are poorly formed and their frequency is low in comparison to cellular endosperm of P. coccineus selfed.

COTYLEDON AND MATURATION

Most seeds of P coccineus X P. vulgaris do not develop beyond the beginning of the cotyledon stage. The cotyledons of the developing embryo do not fill the endosperm cavity even though the seed coat continues to grow (16, 17).

## 2. DOES THE PARTITIONING OF $^{14}C$-PHOTOASSIMILATES CHANGE AS ABORTION IS ABOUT TO COMMENCE?

The ability of a seed to accumulate photosynthate has been defined as its sink strength (47b). Sink strength has been further defined by the following equation:

SINK STRENGTH = SINK SIZE X SINK INTENSITY

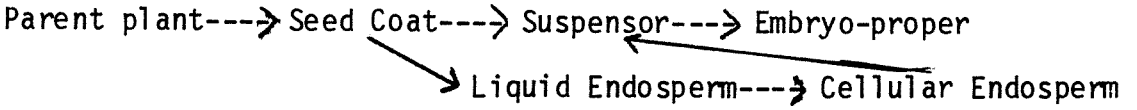
A change in any of these parameters will affect the movement of photosynthate to the developing seed.

Yeung (48) studied the movement of $^{14}C$-sucrose to developing embryos of P. coccineus selfed. When $^{14}C$ sucrose is administered to the embryos via the base of the excised pod or through the endosperm cavity an unexpected labeling pattern emerged. The suspensor is the major uptake site during early stages of embryo development. Even when the labeled solution is introduced into the endosperm cavity at late-heart stage, radioactivity still appears first in the suspensor, then the embryonic axis, and finally the cotyledons. The uptake pattern changes at the mid-maturation stage when the cotyledons become the major site of uptake for the maturing embryo. The following schematic diagram presents a summary of nutrient flow pathways (Yeung, 48):

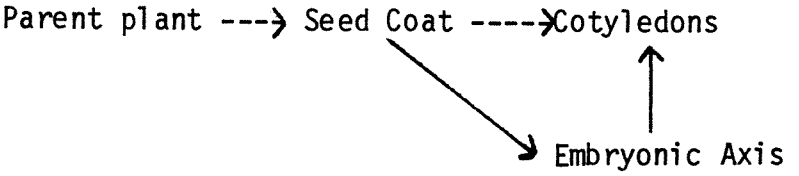
Proembryo and Globular stage

Parent plant----→ Seed Coat----→ Suspensor----→ Embryo-proper

Heart and Early Cotyledon Stage




Maturation Stage




The ability of the embryo to increase its sink strength which increases the photosynthate being imported (48) may be critical to further embryo development. The sink strength of interspecific embryos has not been determined. However, if the interspecific embryos do not import photosynthate, the reason for abortion may be starvation.

3. HOW DO THE ENDOGENOUS HORMONE PROFILES DIFFER IN THE SEEDS FROM THE TWO CROSSES?

There are 5 defined classes of hormones: cytokinins, auxins, gibberellins, abscisic acid, and ethylene. Each of these classes plays a distinctive role in seed development. Ethylene will not be covered in this review.

CYTOKININS

The cytokinins found in the suspensor of P. coccineus selfed (23) at stage A (4-5 mm seed length, heart stage of the embryo) have

activity co-chromatographing with 2-isopentenyladenosine (2iPA), zeatin (Z), and zeatin riboside (ZR), which all are at the same level. Suspensors at stage B (12 mm seed length, cotyledon stage of the embryo) have their cytokinin-like activity centered around zeatin glucoside (ZG). An opposite situation occurs in the embryos: stage A has ZG and ZR; stage B has Z and 2iPA (23).

The very high levels of zeatin-like activity in the suspensor presumably supplies specific forms of cytokinin to the developing embryo until the embryo acquires autonomy (23). Where cytokinin synthesis occurs is unknown.

Nesling and Morris (37) extracted seeds of P. vulgaris and P. vulgaris X P. acutifolius and determined the cytokinin-like activity present. In both the selfed and interspecific seeds the amount of cytokinin-like activity rose to a maximum 14 days after pollination and declined to barely detectable levels by day 22 (37). At all times the total cytokinin-like activity per seed was an order of magnitude lower in the interspecific than in the selfed seed. If expressed on a dry weight basis, cytokinin-like activity was highest in the earliest sampling (day 6) and declined steadily throughout the sampling period.

When seeds of P. vulgaris selfed was divided into testa, embryonic axis, cotyledons, and endosperm and assayed for cytokinin-like activity, the endosperm was the primary source of activity(37). In fact, the cytokinin-like activity obtained from entire ovules could almost entirely be accounted for by the activity present in the endosperm (37).

GIBBERELLINS

Alpi, et al. (2), studied gibberellin-like activity in the embryo and suspensor of selfed P. coccineus at 2 developmental stages: A --heart-shaped embryo, 4 to 5 mm in seed length; B --cotyledonary embryo, 12 mm in seed length. At stage A, the total gibberellin-like activity in the suspensor was about 30 times greater than in the embryo. Gibberellins found in immature seeds of P. coccineus selfed are $A_1$, $A_5$, $A_6$ and $A_8$ (26), $A_{17}$, $A_{20}$ (27), $A_3$ $A_{19}$ (13), $A_{28}$ (15). $GA_1$ has been found in elevated levels in the suspensor (2). By stage B a dramatic decline in total gibberellin-like activity and $GA_1$ was found in the suspensor. In the embryo at stage B, the level had increased 10 times that of stage A. This suggests transport of gibberellins from the suspensor to the embryo prior to cotyledon development.

The role of the gibberellins in the physiological function of the suspensoof P. coccineus selfed was studied by Cionini (10). Intact embryos and embryos deprived of suspensor were grown in vitro. The results indicated that removal of the suspensor had no effect on the embryo when embryo length had reached 5 mm. However, removal of the suspensor reduced growth of younger embryos. The younger the embryo, the greater the response to suspensor removal. $GA_3$ concentrations of $10^{-8}$ to $10^{-6}$ M could replace the suspensor in heart-shaped embryos. Embryos of later stages demonstrated growth reductions with the same treatment (10).

Brady (7) treated suspensors of P. vulgaris selfed with $GA_3$ by incorporating it into the medium of in vitro cultures. After

treatment for 30 min., a large number of nucleolus-like structures appeared. Brady (7) concluded that chromosome function can be experimentally controlled with $GA_3$.

Nagl (33) injected $GA_3$ into the endosperm cavity of developing P. coccineus seeds and stimulated formation of functional chromosome structures --puffs and loops-- and the production of an increased number of micronuclei in the basal suspensor cells and the chalazal giant cells of the endosperm.

AUXINS

Auxin produced in the fruit, particularly in the seed, is thought to be transported out to the surrounding tissues where it stimulates growth by inducing cell expansion (24). This theory has been supplemented with the proposal that auxins create strong physiological sinks. The sinks are then capable of competing for substances necessary for continued growth (25).

Eeuwens and Schwabe (14) studied endogenous growth regulators in developing seeds of Pisum sativum L. cv. Alaska. Six days after bloom, when the pod wall was elongating most rapidly, a high concentration of auxin-like activity was found in the liquid endosperm. Increased auxin-like activity was observed again first in the endosperm and then the embryo 12 to 14 days after bloom, slightly preceding the increase in relative growth rate of the seed. Eeuwens and Schwabe (14) suggested that a hormone-induced influx of metabolites, particularly sugars, into the developing seeds may result in the osmotic uptake of water and consequently the

sharp rise in fresh weight of the seed which follow these peaks of auxin-like activity in the endosperm.

Nagl (35) applied IAA to the endosperm cavity of developing P. coccineus selfed seeds and observed an increase in puffing of the chromosomes in the suspensor tissue. He concluded that IAA may be involved in regulating chromosome activity.

Rogers (40) studied auxin activity in abscising vs retained fruit in cotton. Auxin-like activity in the retained fruit was low at 3 days pre-anthesis and peaked at day 10. The abscising fruit contained lower levels of auxin-like compounds when compared to the retained fruit. Rodgers (40) concluded that retention of mature cotton fruit was dependent on auxin supplied from centers within the fully developed seed. The failure of some reproductive structures within the cotton plant to provide an ample supply of auxin would reduce competitive sink potential and eventually abscision would occur.

ABSCISIC ACID

Quebedeaux (38) and Ciha (9) determined ABA levels in developing seeds of soybean (Glycine max). Levels of ABA were highest at times of greatest growth. Setter (41, 42) determined that ABA moves from the source leaf to the developing soybean seed where ABA and its metabolites accumulate.

Eeuwens and Schwabe (14) measured ABA activity in Pisum sativum seeds over the course of development. The final decline in growth rate of maturing seeds from 20 to 32 days after bloom coincided with a marked increase in ABA activity. ABA activity peaked when

absolute and relative growth rates of the seed had declined to zero. As the seed dried out, ABA activity in the embryos declined to negligible levels.

Tamas, et al. (44) studied pod abscision in P. vulgaris. It was noted that older fruits growing at the base of the raceme aborted less frequently than did the younger ones above. If old fruit were removed, young fruits developed normally. In intact systems, the ABA concentration of young (abscising) fruits was twice as high as young fruits on plants where old fruits had been removed. It was concluded that older fruit have a direct regulatory effect on the ABA content and therefore on younger fruits ability to survive.

Rodgers (39) studying retained vs abscising cotton fruit observed that during the period of early fruit development, ABA-activity was higher in abscising than retained fruit.

4. HOW DO EXOGENOUS TREATMENTS AFFECT THE PROCESS OF ABORTION?

The literature reviewed in this section is broken down into 2 main areas: 1) the environment and 2) exogenous applications ofgrowth regulating substances. Again, the discussion will center on Phaseolus.

A. The environment

Environmental factors affecting embryo abortion are divided into light and temperature.

LIGHT

Embryo abortion can be affected by light-duration, quality and/or quantity. This review will focus on duration and quality.

DURATION OR PHOTOPERIOD

Nagl (34) observed that polytene chromosomes of suspensors of P. vulgaris grown in vitro responded to different photoperiodic conditions by varying gene activity as expressed by appearance or disappearance of loops and puffs. Too short or too long light periods caused condensation of the polytene chromosomes, but the optimal light-dark cycle led to formation of puffs, loops, and micronuclei. Nagl (34) emphasizes that gene activity is controlled by a complex of factors and photoperiod is just one of the factors. He hypothesizes that exogenous factors, such as light and temperature, affect a number of endogenous factors, e.g. phytochrome and phytohormones, thereby changing permeability of membranes for ions and/or altering the nucleohistone complex. Furthermore, it should be noted that these environmental factors might primarily affect the mother plant and thus the nourishment and metabolism of the suspensor.

No studies have been done to measure embryo development directly and its relationship to photoperiod.

LIGHT QUALITY

Light effects on sink strength was studied by Mor, et al. (29, 30). When rose flower buds were darkened and the rest of the plant exposed to high intensity light, the translocation of $^{14}C$ assimilates to the bud was greatly reduced. The decrease in translocation was detected before flower bud atrophy. The light effect was independent of leaf photosynthesis.

Mor, et al. (30) also exposed flower buds of rose to different wavelengths of light. Perception of the light reaction to promote sink activity was found to be in the tip of the bud. The sink activity was increased by exposure of the bud to red light. Far-red light had little effect and blue was not active. However, Mor, et al (30) did find a synergistic effect of red and far-red light. The combination enhanced photmorphogenic events, flower bud development. The conclusion reached was that light quality may affect the unloading of photsynthate in the bud by influencing membrane transport (30).

TEMPERATURE

Specific temperature effects on embryo abortion have not been studied. However, temperature effects on _in vitro_ development of polytene chromosomes in the suspensors of _Phaseolus_ seeds has been studied by Nagl (31, 32) and Brady (7).

Nagl (32) found temperature too high or too low led to condensation of chromosomes in suspensors of _P. coccineus_. Very little RNA synthesis occurs in these chromosomes (31). However, short term increases in temperature induced puffing of the chromosomes (32). The mechanisms of action are unknown.

Brady (7) isolated and cultured _P. vulgaris_ suspensors at 37, 22 and 12 C. After 4 h at either 37 or 12 C, the chromosomes assumed a contracted and banded appearance as well as showing size reduction and total loss of the nucleolus and nucleolus-like structures. When the heat-treated suspensors were returned to 22 C, the chromosomes expanded and the nucleolus-like structures returned after 30 min. (7).

B. Exogenous applications.

Reports of exogenous applications of plant growth regulating substances (PGRS) to developing interspecific Phaseolus crosses are limited. Homna and Heeckt (20) obtained a few hybrid seeds from P. coccineus X P. lunatus by applying N-m tolyphthalamic acid.

Al-Yasari and Coyne (3) applied various PGRS to see if a delay in the time of embryo abortion would result. They found that naphthalene acetimide and potassium gibberellate applied to the pedicle of the flower of P. vulgaris X P. acutifoius improved pod set. Naphthalene acetimide alone or combined with potassium gibberellate stimulated pod growth and delayed embryo abortion. The embryos obtained from the growth regulator treatments were larger than the controls and viable in culture.

Eeuwens and Schwabe (14) applied the growth substances -- $GA_3$, ABA, 1-naphthylacetic acid (NAA), and benzladenine (BA), mixed with lanoline -- 2 days after full bloom to the placentae of young pods (2 to 2.5 cm long). In the pods the seeds were alive or had been killed by piercing with a sharp needle. Growth of pods depended upon live seeds. The growth of the fruits with killed seeds could be restored by application of $GA_3$ + NAA. ABA and BA had no effect on fruit growth. Gibberellins and auxins have been shown to stimulate parthenocarpic fruit development in emasculated flower (Crane, 12). The $GA_3$ + NAA treatment in Eeuwens and Schwabe (14) study also induced parthenocarpic fruit development.

Ibrahim and Coyne (22) developed three different techniques to obtain $F_1$ hybrid seed from P. coccineus X P. vulgaris. First

treatment consisted of applying White's nutrient solution (4% sucrose) to the stigmatic surface prior to pollination. 54% of pods produced continued to grow after 3 weeks but embryo abortion was not decreased.

Techniques two and three did prevent embryo abortion. Treatment two consisted of breaking pedicles of pods when the pod wall tissue had lost some turgidity. Mature, viable seeds were produced by this technique. Treatment 3 involved removing pods from the plant at the time of embryo abortion and placing them in air-tight plastic bags. The bags were kept in a growth chamber for 2 weeks at 20 C and 14 h photoperiod. Technique 3 was more effective in producing mature viable seed and later fertile $F_1$ plants.

Ibrahim and Coyne (22) speculated that inhibiting substances, produced in the leaves of P. coccineus, were translocated to the pods and may have caused embryo abortion. The transport of these inhibitors was reduced in technique 2 and stopped in technique 3.

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# COMPARISON OF DEVELOPMENT OF TWO *PHASEOLUS* CROSSES[1]

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## ABSTRACT

Studies were designed to determine differences in parameters of two *Phaseolus* crosses: *P. coccineus* selfed - which develops normal seeds; and *P. coccineus* X *P. vulgaris* - which aborts embryos when the seeds reach 10 mm in length. Preliminary studies showed pollination at a bud length of a least 12 mm was needed to ensure pollen germination and fertilization. Pod length, (pod length)/(number seeds per pod), and pod thickness of selfed pods were measured daily from 7 to 16 days after pollination. Linear regression analysis indicated that only pod thickness exhibited a linear relationship with seed length. Therefore pod thickness was used to estimate seed length and seed length to estimate embryo development. When the two crosses were compared, the interspecific cross showed 1) slower seed growth with respect to days after pollination; 2) reduced pod length; 3) a higher (pod length)/(number seeds per pod) ratio demonstrating an effect of seeds on pod development; 4) slower embryo development; and 5) an increased volume of liquid endosperm. Pod thickness did not differ.

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[2] Lecturer, Professors, respectively

# INTRODUCTION

Interspecific hybridization within the section Euphaseolus of the genus Phaseolus is a way of importing valuable genes into P. vulgaris (3). However, a major difficulty in obtaining these hybrids is a lack of normal seed development. Fertilization does occur but embryos abort 8 to 24 days after pollination (9). The phenomenan of abortion is well documented but the causes are still unclear.

Successful crosses of P. vulgaris wth P. coccineus occurred more frequently when P. vulgaris was used as the female parent (10). The reciprocal cross, P. coccineus X P. vulgaris did not produce mature seed. Comparisons were made of the ratio of nucleii number in the liquid endosperm to nucleii number in the embryo tissue in P. coccineus X P. vulgaris and P. coccineus selfed. Thomas concluded that P. coccineus X P.vulgaris developed slower than P. coccineus selfed because of the retarded development of embryo and endosperm tissue immediately following the post-fertilization period.

From Thomas' data it was obvious the seeds of the crosses we were interested in studying - P. coccineus selfed and P. coccineus X P. vulgaris - were not developing at the same rate. So the traditional means of estimating the seed developmental stage from days after pollination would be inaccurate for our study. In order to design appropriate experiments to compare the differences in seed

development of the two crosses, it was first necessary to have a nondestructive method to identify specific stages of seed development.

The purpose of this study was to develop a nondestructive measurement technique and then to use it to determine if the two crosses have similiar developmental patterns.

## MATERIALS AND METHODS

Leaf-bud cuttings of *P. coccineus* ($F_1$ 'Scarlet Runner Bean' X 'Dutch Knife Case', selfed) were taken from one stock plant. After rooting, the plants were grown to flowering and then used as the female parent in the crosses. Male parents were: 1) the same *P. coccineus* clone and 2) and accession A80-43 of *P. vulgaris* from Wisconsin. Plants were grown and pollinated in a greenhouse maintained at 27 °C/22 °C (day/night). Supplemental cool white fluorescent lighting was used in the first experiment to extend the day to 14 h with predawn irradiance level of 150 uEinsteins $m^{-2}$ $s^{-1}$ measured 0.5m from the light source.

### Experiment 1

To determine whether restricted pollen tube growth occurred the two pollinations were made (2) when flower buds were 10 to 16 mm in length. Pistils were harvested 24 h after pollination, fixed for 24 h in formaldehyde:acetic acid:ethanol (1:1:8) rinsed three times in distilled water, and softened 20 h in 8 N NaOH at room temperature. The pistils were rinsed again, stained for 8 h in 0.1% analine blue

in 0.1 M $K_3PO_4$, and squashed in 2:1 glycerine to water. Observation of pollen tube growth were made with a microscope with blue light fluorescence (1).

Samples of developing fruit from the self pollinated flowers were collected daily from 7 to 16 days after pollination. Measurements of the external parameters - pod length, width, and thickness - were made using calipers on 10 pods per sample. Measurements of pod width and thickness were taken at the mid-point aver the second seed. Pod width was the distance between the sutures and pod thickness was the largest distance perpendicular to the width measruement. The pod was then opened and seed number and length were recorded. Simple linear regressions were calculated with seed length as the dependent variable and each external growth paramenter as the independent variable.

Experiment 2

The measurements were repeated using ten pods each of the interspecific cross and the self. However, samples were collected based on seed length estimated from pod dimeter (see Results form Experiment 1) rather than by days after pollination as in Experiment 1. Liquid endosperm volume (12) and embryo weight were also determined. T-tests were done comparing the two crosses for all parameters at each seed length. Standard errors were calculated for each mean. Significance was determined at the 5% level.

## RESULTS AND DISCUSSION

### Experiment 1

The size of the flower bud of P. coccineus affected pollen tube development. If the bud was less than 12 mm in length, the stigma did not appear receptive for either source of pollen. From 12 to 15 mm uniform pollen tube growth was observed. At 16 mm, flowers of P. coccineus had shed pollen within the enclosed bud ofter contaminating the stigma. When pollen tube growth was observed in buds from 12 to 15 mm in length, no difference was detected between the self and the interspecific pollinations. All pollinated pistils exhibited abundant pollen tubes at the base of the ovary 24 h after pollination. This is in agreement with other workers (5, 6, 9, 10).

Correlation coefficients for seed length against each of the externally measured parameters were significant (Table 1). However all the parameters are not practical to use in the greenhouse. Within the range of seed lengths, from 3 to 10 mm, only pod thickness demonstrates a linear relationship with seed length. This parameter was then tested on a small sample (3 pods/seed length) of the interspecific cross and again a one to one relationship was found. Walbot (11) and Nagl (7) report pod length as a good measure to determine seed length and consequently the stage of development of the embryo. Our study demonstrates that pod thickness may be a better measurement to determine stage of development of the embryo when the pod is in the early growth stages (3 to 10 mm in thickness).

## Experiment 2

The interspecific cross showed an early retardation of seed development (seed length less then 7 mm, Fig 1a). If sampling was done before 13 days after pollination, seeds of the two crosses would not be comparable in development. The two crosses had equal seed lengths by 13 days after pollination, however the interspecific embryo was much smaller than the self embryo. Similiar observations were made by Thomas (10).

Pod lengths were comparable between the two crosses early in seed development (Fig. 1b). However, when the seed was 6 mm or longer, a slowing of development of the pod occurred in the interspecific cross.

This decrease in pod development could have been due to fewer number of seeds. Therefore (pod length)/(no. seeds per pod) was determined (Fig. 1c). The two crosses followed the same development pattern. At the end of sampling, the interspecific cross had significantly greater growth of the pod wall when compared to the number of seeds per pod than the self. This difference may indicate different factors are affecting pod wall development in the two crosses.

Pod thickness vs seed length for the two crosses is shown in Fig 1d. This line was applicable to both crosses and did not seem to change as abortion was occurring in the interspecific cross.

The embryos did not grow at the same rate. By 7 mm the selfed embryo was at the heart-shaped stage of development (12). As the

selfed embryo grew, it increased in weight (Fig 2a), the volume of liquid endosperm leveled off (Fig. 2b), and the embryo moved into the cotyledon stage of development. The interspecific embryo also appeared to be at the heart stage at 7 mm seed length. The interspecific embryo, though, did not increase in weight to the extent of the self (Fig. 2a), the volume of liquid endosperm rose (Fig. 2b), and the cotyledons did not enlarge. The embryo aborts by the early cotyledon stage (4). This pattern of development of interspecific Phaseolus crosses has also been observed by Haq (4) and Nesling and Morris (8).

In summary: 1) Pod thickness is a good nondestructive measurement to use to determine seed length and subsequently the stage of development of the embryo. 2) As the abortion of the interspecific embryo occurs between 9 and 10 mm seed length, the embryo does not increase in weight and the liquid endosperm is not absorbed.

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Table 1: Equations of lines describing different external parameters vs seed length of P. coccineus selfed.

Days after pollination vs seed length

$y = 0.65x + (-2.3)$, $r = .89$

Pod length vs seed length

$y = 0.05x + 1.30$, $r = .91$

(Pod length)/(number seeds per pod) vs seed length

$y = 0.21x + 1.54$, $r = .87$

Pod width vs seed length

$y = 0.40x + 1.71$, $r = .91$

Pod thickness vs seed length

$y = 0.95 + 0.32$, $r = .96$

Figure 1: Comparisons of P. coccineus selfed (PCR X PCR) to P. coccineus X P. vulgaris (PCR X PV) for a) days after pollination, b) pod length, c) (pod length)/(no. seed per pod), and d) pod diameter. The values represent the mean ± SE. SE bars are not present if the SE was smaller than the symbol.

Figure 2: Comparison of P. coccineus selfed (PCR X PCR) to P. coccineus X P. vulgaris (PCR X PV) in a) embryo growth, and b) liquid endosperm volume. The values represent the mean ± SE. SE bars are not present if the SE was smaller than the symbol.

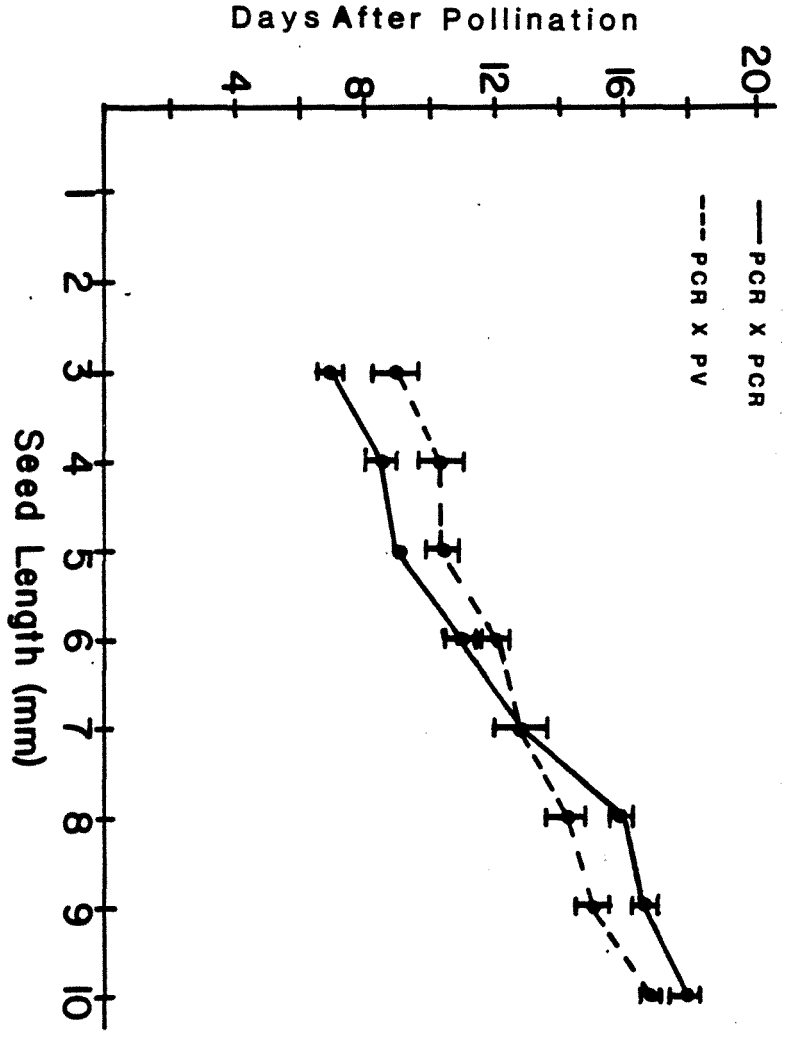

Days After Pollination
4
8
12
16
20
Seed Length (mm)
1
2
3
4
5
6
7
8
9
10
PCR X PCR
PCR X PV

Fig. 1a

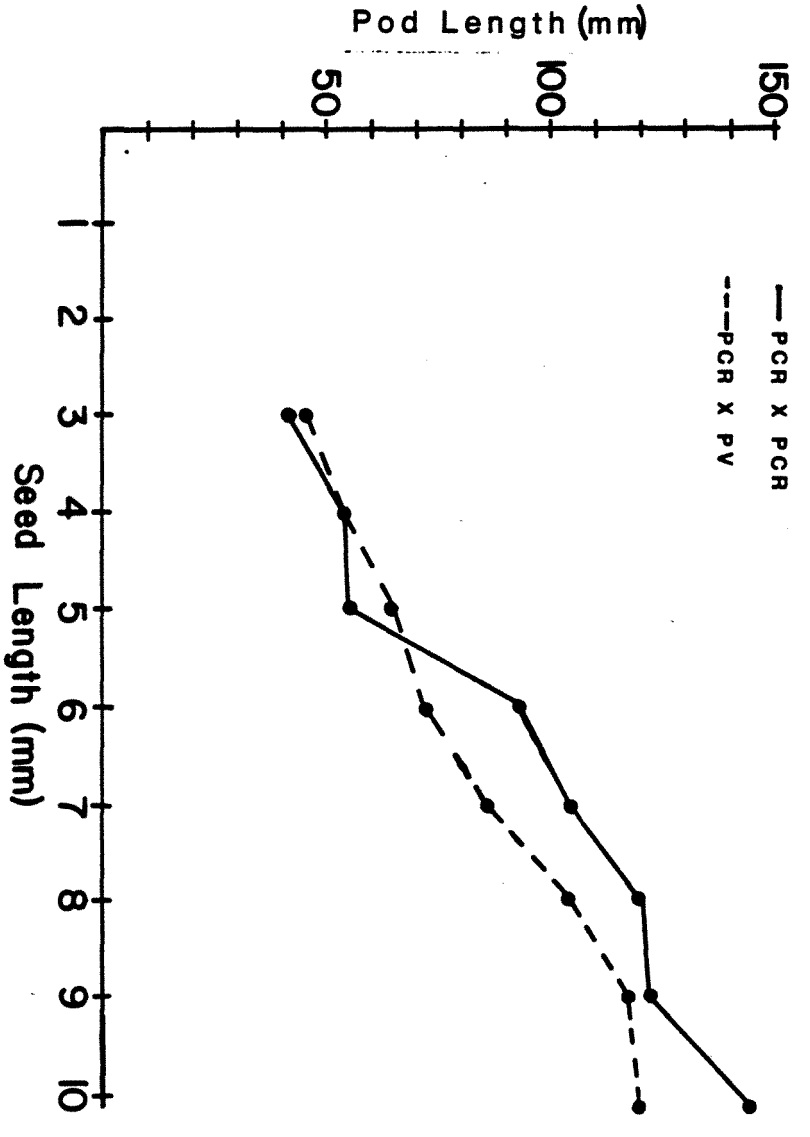
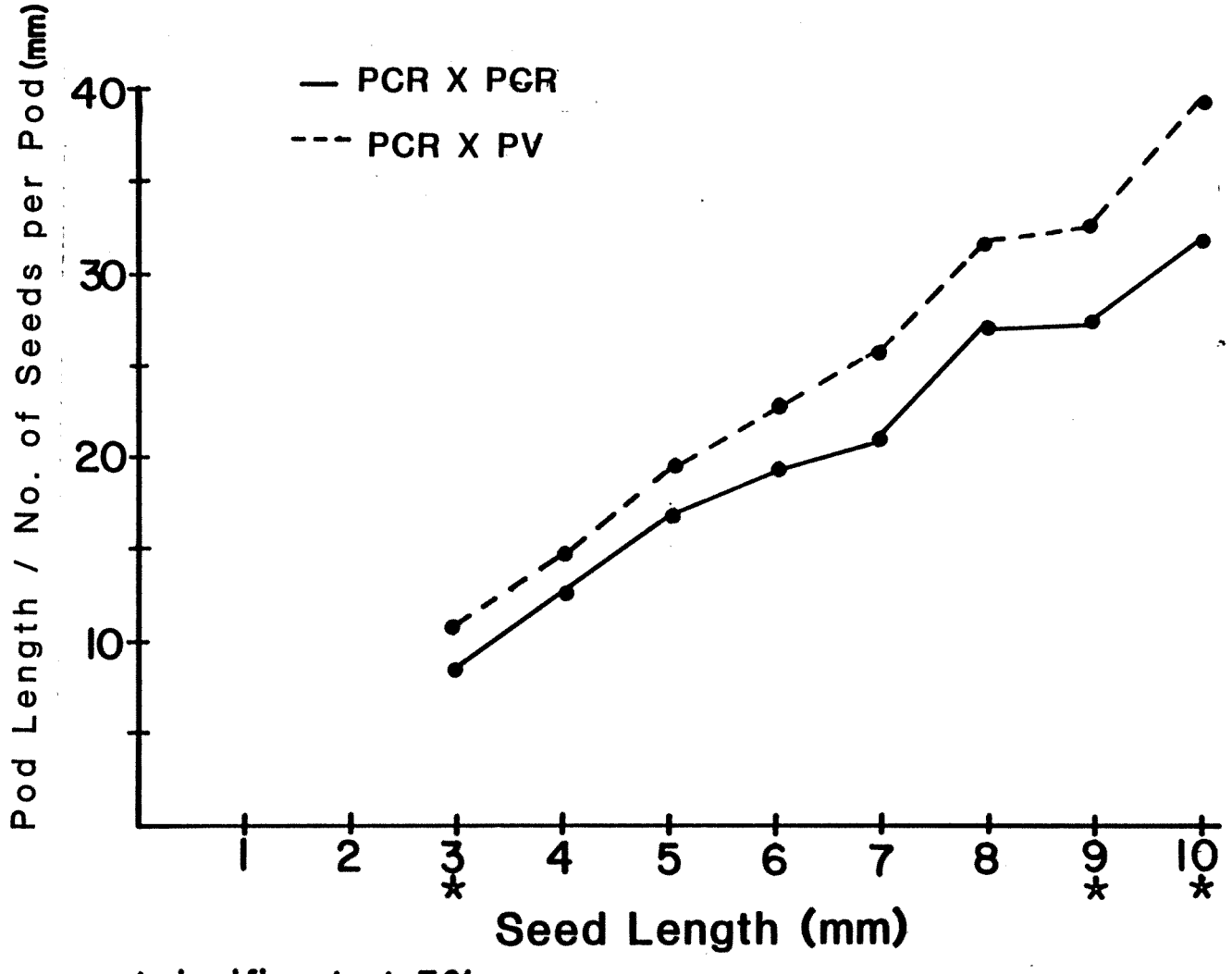

Pod Length (mm)
50
100
150
Seed Length (mm)
1
2
3
4
5
6
7
8
9
10
PCR X PCR
PCR X PV

Fig. 1b

Fig. 1c




*significant at 5%

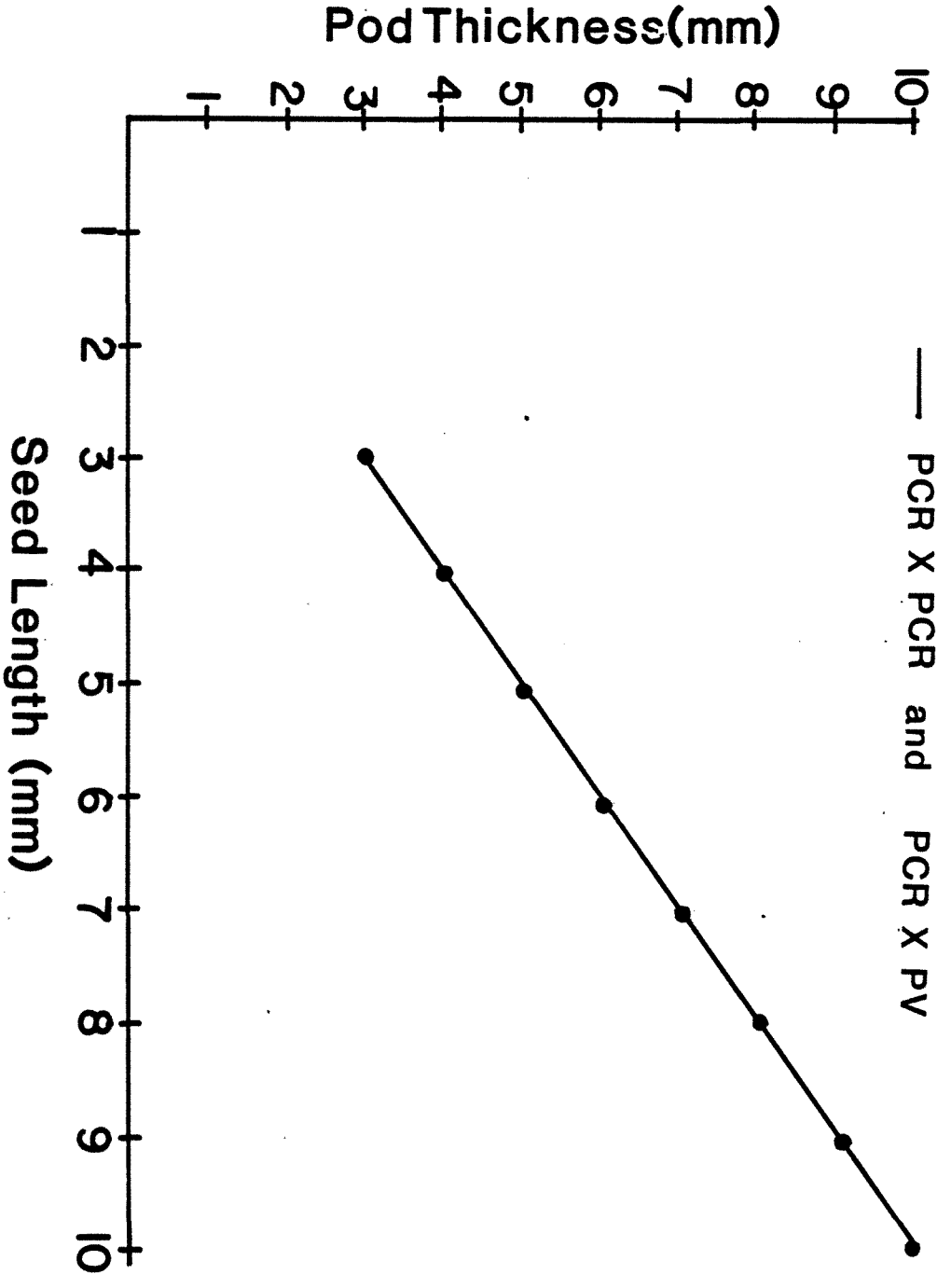

Pod Thickness(mm)
1
2
3
4
5
6
7
8
9
10
Seed Length (mm)
1
2
3
4
5
6
7
8
9
10
PCR X PCR and PCR X PV

Fig. 1d

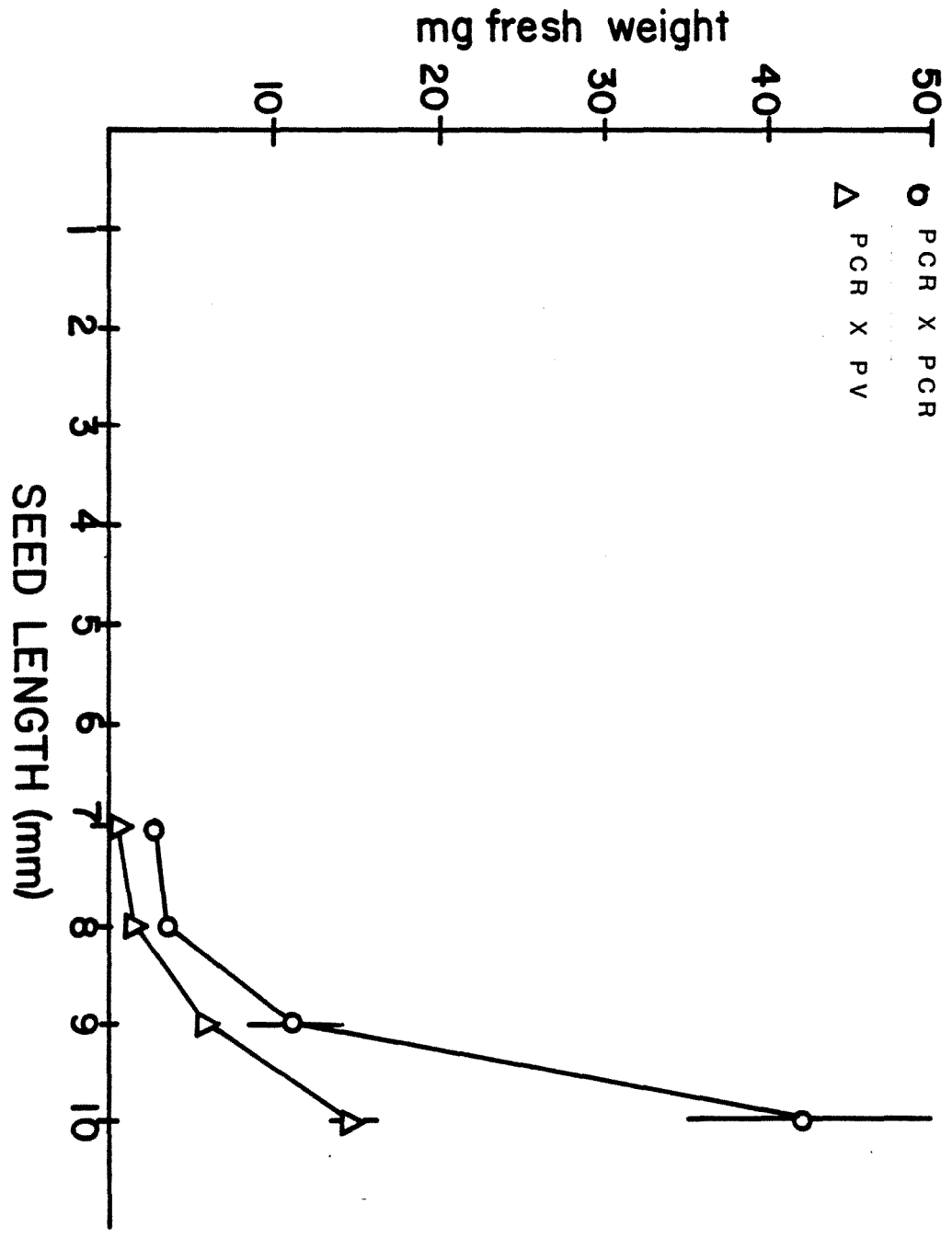
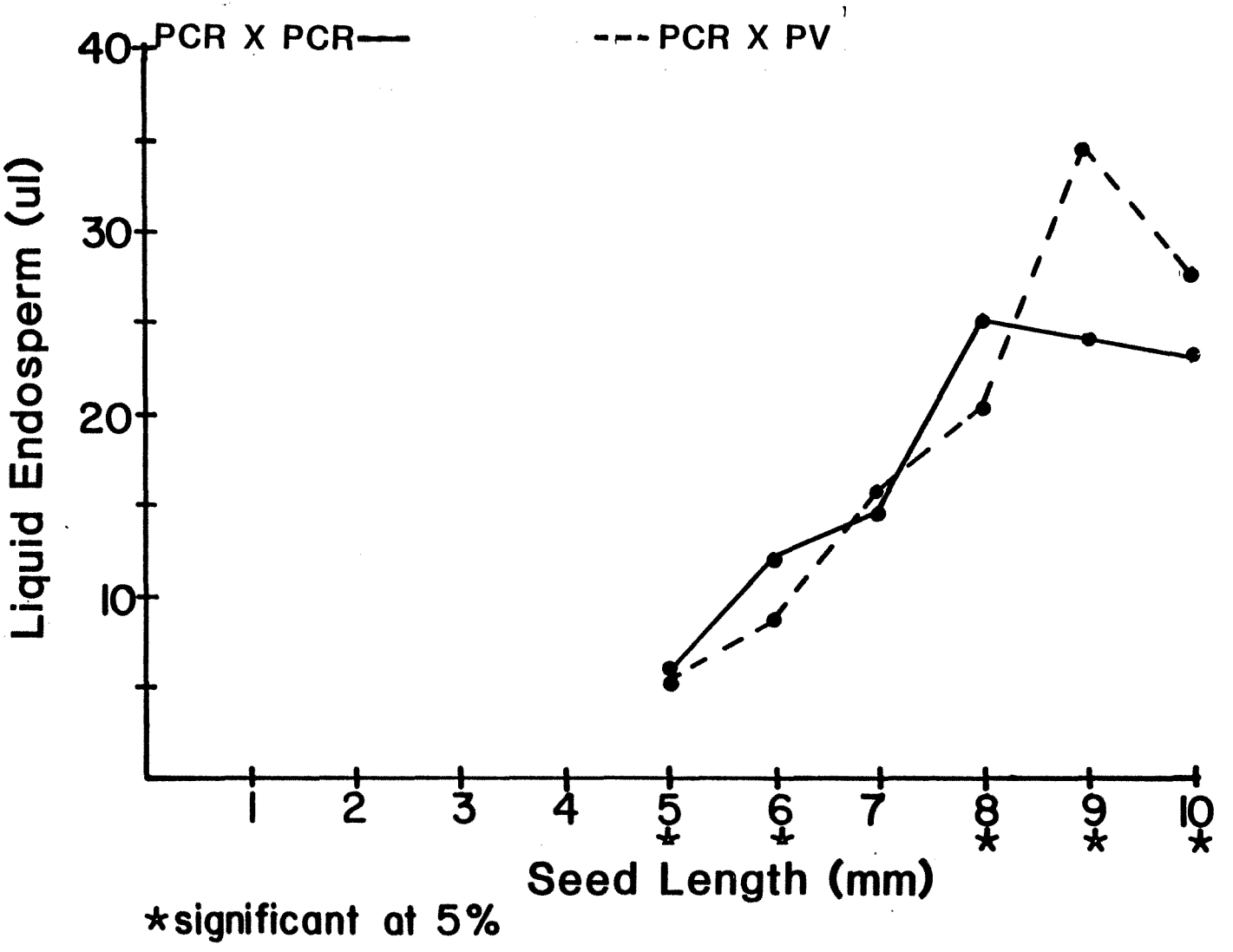

mg fresh weight
10
20
30
40
50
EMBRYO
o PCR X PCR
Δ PCR X PV
SEED LENGTH (mm)
1
2
3
4
5
6
7
8
9
10

Fig. 2a

Fig. 2b




*significant at 5%

# THE EFFECT OF LIGHTING TREATMENTS ON POD ABSCISION OF *PHASEOLUS* CROSSES[1]

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## ABSTRACT

Pod abscision of interspecific *Phaseolus* crosses occurs after 5 days in winter months compared to 16 to 20 days during the summer. Studies were designed to determine if supplemental lighting would delay pod abscision in *P. coccineus* Lam. interspecific, full-sib, and selfed crosses. Supplemental lighting had no effect on pod abscision of the interspecific cross. Full-sib crosses develop to maturity in summer and under metal halide HID lighting in winter. Selfed pods develop to maturity under all treatments. Therefore to obtain seeds of $F_1$ hybrids of the interspecific cross for embryo culture, pollinations must be done in summer in Minnesota.

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[2] Lecturer, Professors, respectively

In certain interspecific Phaseolus crosses attempted under high light conditions in the field or summer greenhouse, pollen tube growth and fertilization occur, pods and embryos develop for two to three weeks, but then the embryos abort (10). Similiar interspecific crosses attempted in the greenhouse during winter months also result in pollen tube growth and fertilization, but pods abscise within one week (Alveraz, personal communication). This early pod abscission prevents embryo rescue and restricts interspecific hybridization of Phaseolus in Minnesota to the summer months, May to mid-September. In this study three Phaseolus crosses: selfed P. coccineus; an intraspecific cross of P. coccineus; and the interspecific cross P. coccineus X P. vulgaris were used to determine whether a) different light sources could prevent early pod abscision under winter greenhouse conditions, and b) early pod abscision could be induced by lowering the light intensity with shading entire plants in the summer.

Seeds from a selfed $F_1$ red-flowered plant of red-flowered P. coccineus cv. ,Scarlet Runner Bean' X white-flowered P. coccineus cv. ,Dutch Knife Case' were planted in the greenhouse. Stem cuttings were taken from one red-flowered plant. These cuttings were grown to flowering plants and then used as female parents. Male parents are listed in Table 1.

Plants were grown and pollinated (3) in a greenhouse in the summer (April to August) at 27 °C/22 °C (day/night) and in the winter (October to March) at 22 °C/18 °C (day/night). The light flux was

measured at the top of the plant canopy at 10 a.m. on sunny days. In the summer no supplemental light was given but two shading treatments were imposed: 33% shade with green saran (1500 uEinsteins $m^{-2}$ $s^{-1}$); or 66% shade with green saran(700 uEinsteins $m^{-2}$ $s^{-1}$). In the winter months, photoperiod was increased to 14 h to induce flowering with : fluorescent light(300 uEinsteins $m^{-2}$ $s^{-1}$); high pressure sodium HID light(800 uEinsteins $m^{-2}$ $s^{-1}$; or metal halide HID light (800 uEinsteins $m^{-2}$ $s^{-1}$).

Crosses were made when the flower buds were 14 to 16 mm in length. To determine whether pollen germination had occurred in all treatments, pistils were collected 24 h after pollination and immediately fixed in formaldehyde:acetic acid:ethanol (1:1:8). The pistils were then rinsed three times with distilled water and placed in 8 N NaOH at room temperature. After 20 h, the pistils were again rinsed and put into 0.1% analine blue in 0.1 M $K_3PO_4$. They were squashed in a 2:1 glycerine:water solution and observed under a microscope utilizing blue light fluorescence. All pollinated pistils exhibited abundant pollen tubes at the base of the ovary 24h after polllination. This is consistant with the observations of other workers (1, 5, 7, 10).

Pods of red-flowered P. coccineus from self pollinations matured normally under all lighting treatments and environments (Table 2). However the pods from the interspecific and full-sib crosses varied in the number of days until abscision. Interspecific pods abscised prior to maturation of the seeds under all lighting

treatments (Table 2). In the winter, with comparable photoperiod and irradiance level, interspecific pods abscised 12 days earlier than in the summer environment. However, summer shading of entire plants did not induce early pod abscision of interspecific crosses. The winter and summer light environments differed in: photoperiod - winter photoperiod was set at 14 h with supplemental lighting while the summer photoperiod exceeded 14 h; intensity - supplemental lighting in winter was half that on a sunny summer day; and quality - winter light is richer in far red light than is summer light (2). Both photoperiod and light quality may play roles in inducing early pod abscision of interspecific crosses. Irradiance level is not the sole factor because when equal - 66% shade in summer and HID light in winter - differences in pod abscision still resulted (Table 2).

Nagl (9) observed that polytene chromosomes of suspensors of P. vulgaris grown in vitro responded to different photoperiods by varying gene activity. Too short or too long light periods caused condensation of polytene chromosomes but the optimal light-dark cycle led to formation of puffs and loops indicating chromosome activity. Haq (4) demonstrated the suspensor of P. coccineus x P. vulgaris developed abnormalities within four days after pollination. Because of the shortened photoperiod during winter months, abnormalities of suspensor cells in the interspecific cross may be expressed earlier leading to early pod abscision.

Pods from full-sib crosses developed to maturity in all summer light environments. However under fluorescent and sodium HID

lighting environments in the winter, the pods from full-sib crosses abscised by six days after pollination (Table 2). Pods growing under the metal halide HID developed to maturity. The phenomenan of early pod abscision of full-sib crosses under selected lighting treatments rules out photoperiod and irradiance levels as causes of abscission. Both of these factors were held constant in the two HID light treatments yet sodium HID lighting induced early abscisasion while metal halide HID did not. Light quality was different in the three light environments used for this study.

Mor et al. (8) studied the effect of different qualities of light on photoassimilate movement to rose flower buds. Red light enhanced movement of $^{14}C$-photoassimilates to the bud while far red light had little effect and blue had no effect. Red plus far red light acted synergistically. They (8) concluded that light quality may affect photosynthate unloading in the bud by influencing membrane transport.

The spectra of the sodium and metal halide HID lamps are different. Metal halide emits more light in the blue region of the visible spectrum while sodium HID is richer in the yellow and red regions of the visible spectrum (2). The balance of increased far red light in the winter and the enhancement of the lower wavelengths by metal halide HID lighting may be factors influencing pod and embryo development. This does not agree with findings of Mor et al. (8). However the process examined in this study, pod abscision, is physiologically different from flower bud development (8).

Our results demonstrate that the quality of the light the plant receives can influence the success of pod set and subsequent embryo development. Early abscission of pods of the interspecific cross continued under all of the supplemental lighting treatments imposed during the winter months. Summer shading treatments also did not alter the time of pod abscision of the interspecific cross. Abscision of pods resulting from full-sib crosses was delayed in the winter months when the mother plants were placed under metal halide lamps. This paper is the first report where full-sib crosses have been shown to be effected by the light environment the plants are grown under.

The practical implications of the photomorphogenic effects on pod abscision should not be overlooked. The plant breeder should evaluate crosses under several environments known to be favorable to seed development. This is so crosses are not overlooked which may be incompatible under winter greenhouse conditions but compatible under summer greenhouse conditions.

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Table 1: Pollen parents of crosses

| SPECIES | FLOWER COLOR | TYPE OF CROSS |
|---|---|---|
| P. COCCINEUS (PCR X PCR) | RED | SELF |
| P. COCCINEUS (PCR X PCW) | WHITE | FULL-SIB |
| P. VULGARIS (PCR X PV) | PURPLE | INTERSPECIFIC |

Table 2: Average ($X \pm SE_X$) number of days after pollination until pod abscision.

| Cross | Summer | | | Winter | | |
|---|---|---|---|---|---|---|
| | Light Treatment | | | | | |
| | None | 33% | 66% | Fluorescent | Na-HID | Metal Halide HID |
| PCR X PCR | MATURITY | MATURITY | MATURITY | MATURITY | MATURITY | MATURITY |
| PCR X PCW | MATURITY | MATURITY | MATURITY | $4.54 \pm 0.19$ | $5.93 \pm 0.36$ | MATURITY |
| PCR X PV | $18 \pm 2.0$ | $18 \pm 2.0$ | $18 \pm 2.0$ | $5.69 \pm 0.52$ | $5.90 \pm 0.36$ | $6.0 \pm 0.55$ |

# PARTITIONING OF $^{14}$C-PHOTOASSIMILATES WITHIN SEEDS OF *PHASEOLUS COCCINEUS* (LAM.) AND *PHASEOLUS COCCINEUS* X *PHASEOLUS VULGARIS* L.[1]

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## ABSTRACT

The objective of this study was to determine partitioning within seeds of $^{14}$C-photoassimilates at three stages of seed development in two *Phaseolus* crosses - *P. coccineus* Lam. selfed, and *P. coccineus* X *P. vulgaris* L. Abortion of the interspecific embryos occurred when the seed reached 10 mm seed length. When expressed as sink strength (% dpm) or sink activity (%dpm/d.wt.) there were no differences in partitioning of $^{14}$C-photoassimilates when whole seeds were analyzed. If the seed was divided into seed coat, liquid endosperm, and embryo, the sink activity of the interspecific embryo was higher than that of the embryo in the selfed seed. Therefore, abortion of these interspecific *Phaseolus* embryos appeared not to be caused by a lack of photoassimilates.

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[3] Lecturer, Professors, respectively

## INTRODUCTION

Interspecific hybrid embryos from the cross *Phaseolus coccineus* X *P. vulgaris* appear to develop normally for two to three weeks and then abort (3). To obtain mature seed, Ibrahim and Coyne (7) developed two techniques that prevented abortion: either breaking the pedicel of the pod when the pod wall had lost some turgidity, or removing the pods from the plant at the time of abortion and placing the excised pods in plastic bags and maintaining them in a growth chamber for two weeks. Excising hybrid embryos before the time of abortion and culturing them on a defined medium can also be used to produce mature $F_1$ plants (1, 4). These studies demonstrate that viable hybrid embryos can be produced and there is no inherent disparity in their genetic constitution precluding plant growth.

Normally, at the cotyledon stage of development, the liquid endosperm nourishes the embryo (10). However, in the interspecific cross, the liquid endosperm is not being absorbed and the cotyledons cease growth (3). Based on these observations Haq (2) suggested that a lack of food reserves may accelerate embryo failure.

Information is lacking on whether photosynthate is limiting to growth of the developing interspecific *Phaseolus* embryo. Therefore it was of interest to study the accumulation of $^{14}C$-photoassimilates in developing seeds of *P. coccineus* X *P. vulgaris*, which does not develop to maturity *in situ*.

## MATERIALS AND METHODS

### Plant Material

A single red-flowered stock plant of *P. coccineus*, resulting from self-pollination of the $F_1$ obtained by crossing *P. coccineus* 'Scarlet Runner Bean' with ,Dutch Knife Case', was the source of plants used for the maternal parents. Asexually propagated cuttings were grown until flowering occurred in the greenhouse (March to June) under normal light condidtions with a day/night temperature of 25 C/20 $^{o}$. Flowers on the raceme at the fourth node of each plant were selfed or cross pollinated with an accession of *P. vulgaris* (A80-43). After fruit set occurred, all other pods, flowers, and lower buds were removed from the plants.

### $CO_2$ Studies

Radiolabeled $CO_2$ treatment was applied at three stages of development when pod thicknesses were 7, 9, or 10 mm. Pod thickness is linearly related (r=.96) to seed length (5), which is used commonly as a measure of embryo development (6, 9, 14).

The stomatal resistance ($r_s$) of the leaf subtending the raceme with the pod of interest was measured. An automatic diffusion porometer (Delta T Devices, model MKII, Cambridge, England) was used to take three readings on the abaxial surface of the leaflet under greenhouse conditions. The nine readings were averaged for statis-

tical analysis.

The same leaf, after $r_s$ was determined, was exposed to $^{14}CO_2$. The leaf was enclosed in a transparent bag. The open end of the bag was tied around a rubber stopper through which the leaf petiole passed. Affixed to the stopper was a vial to which 0.5 uCi of $Na_2{}^{14}CO_3$ was added. The system was closed and excess HCl was added through a septum to release $^{14}CO_2$. After 30 min., excess NaOH was added to the reaction vessel to trap any free $CO_2$. The system was dismantled 15 min later and the plant remained for 2 h under greenhouse conditions.

After the 2 h period, plants were divided into: the peduncle, pod wall tissue, seeds at the pulsed node; and all plant material apical to the pulsed node. The plant tissue was oven dried at 60 C for 7 days. Samples were ground and subsamles were taken. The samples were combusted using an automatic combustion apparatus (Packard 306). Radioactivity was determined with liquid scintillation spectrometry (Beckman LS 9000).

The $^{14}CO_2$ studies were repeated using the same technique except that 1.0 uCi of $Na_2{}^{14}CO_3$ was added to each vial and the seeds were subdivided into three components: liquid endosperm, embryo, and seed coat. Radioactivity was determined in the peduncle, pod wall, and the three seed components.

## Definition of Terms

The accumulation photosynthate in a seed has been defined as its sink strength (15). Sink strength has been further described by

the following equation:

SINK STRENGTH = SINK SIZE X SINK ACTIVITY

The strengths of various sinks in Experiment 1 were calculated as their radioactivity (dpm) expressed as a percentage of the radioactivity of the plant top excluding the source leaf. Sink strengths in Experiment 2 were calculated as their radioactivity (dpm) expressed as a percentage of the radioactivity within the raceme. Sink activity was calculated as the sink strength divided by the dry weight of the plant part (%dpm/d.wt.).

Stages of embryo development have been defined in relationship to the seed length for the self embryo (5). Embryos from seeds of 5 and 6 mm in length are in the heart-shaped stage of development, while 7 to 10 mm seeds contain embryos in the cotyledon stage (5, 14). It was assumed for this study that the interspecific embryos were at the same stage of development relative to seed length as the self.

## RESULTS AND DISCUSSION

When stomatal resistances ($r_s$) were measured, there were no differences at any seed length between the leaves with different crosses developing at the node. Other researchers have demonstrated that mechanically obstructed translocation induced an increase in $r_s$ (8, 11, 13). If the pod with interspecific embryos was obstructing translocation of photosynthate to the pod from the source leaf, an increase in $r_s$ in the source leaf and less carbon fixation would

have been expected. In this experiment, however, the plants fixed similiar amounts of radioactivity (dpm) regardless of the genotype of embryos in the developing pods.

The two crosses were not significantly different in the sink strength (% dpm) of the different plant parts throughout the sampling period (Table 1). Peduncles associated with the embryos imported a similiar percent of radioactivity at 7 and 9 mm seed lengths and increased at 10 mm seed length (Table 1). This may be due to continued initiation of flower buds on the peduncles even after removal of visible buds. The pod wall tissue had a high sink strength at both 7 and 9 mm seed length and dropped by 10 mm seed length for both crosses (Table 1). The pod wall was still elongating at 7 and 9 mm seed length. However, by 10 mm seed length pod wall growth had ceased (5). Though the trend for the self seeds was to increase in sink strength with age, this was not significantly different from the interspecific seeds through sampling (Table 1).

Sink activity (% dpm/g d.wt.) for the plant parts of the two crosses also did not significantly differ throughout sampling (Table 1). Whole seeds from the two crosses imported similiar amounts of $^{14}$C-photoassimilates through sampling, demonstrating a decreasing sink activity with age. The activity of the pod wall of selfed plants decreased through sampling but the pod containing the interspecific embryo had higher sink activity at seed length of 7 and 9 mm and decreased 6 fold at 10 mm seed length which is just before abortion. The peduncle associated with the interspecific embryo

increased in activity 4 fold between 9 and 10 mm seed length.

The fact that partitioning of recently fixed carbon was not significantly different between the selfed and interspecific seed does not necessarily indicate that photosynthate availability was not limiting to the embryo destined to abort. The partitioning of photosynthate within the seed may have differed. Therefore a similiar experiment was conducted but the seed was dissected into the liquid endosperm, embryo, and the seed coat, and the sink strength calculated as a percentage of that which entered the raceme.

When the sink strengths are compared for the plant parts of both crosses a different distribution pattern emerged (Table 2). At 7 mm seed length the strongest sinks (% dpm) in the self pollinated plant were the peduncle and pod wall. At the same stage of development in the interspecific cross,the liquid endosperm was the strongest sink. At 9 and 10 mm seed length the strongest sink for both crosses was the pod wall. This pattern demonstrated that sink strength increased in larger (heavier) tissues.

At 7 mm seed length, the sink activity was greatest in the embryo of both crosses (Table 2). The interspecific embryo was obtaining significantly more $^{14}$C-photoassimilates per unit dry weight than the embryo of the self pollinated plant. At 7 and 9 mm seed length. This difference may be large because of the error in weighing embryos of such small size. The liquid endosperm fluctuated in uptake of $^{14}$C-photoassimilates over sampling but no difference was seen between plants with the two crosses. The seed coat

associated with the self decreased in activity throughout sampling whereas the seed coat associated with the interspecific cross peaked in activity at 9 mm seed length.

The ability of the embryo to maintain high sink activity, which indicates photosynthate movement into the embryo, may be of critical importance to embryo development. Development of the interspecific embryo ceases at the cotyledon stage, 7 to 10 mm in seed length (3, 5). The endosperm is not absorbed and the cotyledons do not expand (3, 12). Based on these anatomical observations, Haq (2) suggested that a lack of food reserves may cause interspecific embryo failure. From the data generated in this study, photosynthate availability to the interspecific embryo was not limiting. The interspecific embryo had a significantly higher sink activity than that of the self when the respective seeds are 7 and 9 mm in length.

The liquid endosperm provides nutrients to the developing embryo (10). Liquid endosperm of P. coccineus X P. vulgaris have fewer mitochondria and the inner layer of cell wall projection of the cellular endosperm were in low frequency and poorly formed at the heart-shaped stage of embyro development (seed length of 5 and 6 mm) (3). The embryo in the late heart- early cotyledon stages is nourished by the liquid endosperm (10). Reduction in the uptake of newly fixed carbon by the interspecific liquid endosperm at 9 mm seed length may accentuate developmental abnormalities already present and accelerate embryo failure.

Newly fixed carbon does not seem to be the limiting factor to

embryo growth. Furthermore the partitioning of carbon is the same in both aborting and nonaborting seeds. This evidence leads to the conclusion that abortion of the interspecific embryo is due to other factors.

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TABLE 1: SINK STRENGTH (% DPM) AND SINK ACTIVITY (% DPM/ D.WT.) OF P. COCCINEUS SELFED (PCR X PCR) AND P. COCCINEUS X P. VULGARIS (PCR X PV) AT THREE STAGES OF EMBRYO DEVELOPMENT 7, 9, AND 10 MM SEED LENGTH. EACH NUMBER IS THE MEAN ± SE FOR 4 REPLICATES. SIGNIFICANCE WAS DETERMINED WITH THE T-TEST.

| SEED LENGTH (MM) | PLANT PART | % DPM/ G D.WT. | | % DPM | |
|---|---|---|---|---|---|
| | | PCR X PCR | PCR X PV | PCR X PCR | PCR X PV |
| 7 | APICAL (A) | 9,9 ± 9,0 | 22 ± 13 | 14 ± 11 | 36 ± 17 |
| | PEDUNCLE (R) | 73 ± 24 | 68 ± 11 | 27 ± 14 | 21 ± 7 |
| | POD WALL (PW) | 165 ± 31 | 108 ± 21 | 56 ± 17 | 38 ± 11 |
| | SEEDS (S) | 82 ± 32 | 156 ± 50 | 3 ± 1 | 4 ± 2 |
| 9 | A | 3,2 ± 3,0 | 16 ± 13 | 3 ± 1,2 | 13 ± 6,5 |
| | R | 74 ± 23 | 69 ± 18 | 29 ± 6 | 19 ± 7 |
| | PW | 84 ± 10 | 109 ± 25 | 63 ± 8 | 60 ± 10 |
| | S | 73 ± 28 | 110 ± 20 | 5 ± 2 | 9 ± 2.5 |
| 10 | A | 5,3 ± 1,8 | 13 ± 9 | 7 ± 4 | 36 ± 23 |
| | R | 79 ± 25 | 286 ± 107 | 42 ± 17 | 52 ± 21 |
| | PW | 49 ± 20 | 18 ± 9 | 33 ± 12 | 7.3 ± 3.3 |
| | S | 66 ± 16 | 63 ± 27 | 18 ± 9 | 5.3 ± 1.2 |

TABLE 2: SINK STRENGTH (% DPM) AND SINK ACTIVITY (% DPM/ MG D.WT.) OF P. COCCINEUS (PCR X PCR) AND P. COCCINEUS X P. VULGARIS (PCR X PV) AT THREE STAGES OF EMBRYO DEVELOPMENT 7, 9, AND 10 MM SEED LENGTH. EACH NUMBER IS THE MEAN ± SE FOR 4 REPLICATES. SIGNIFICANCE WAS DETERMINED WITH THE T-TEST AND IS DENOTED WITH *.

| SEED LENGTH (MM) | PLANT PART | % DPM/ MG D.WT. | | % DPM/UL | | % DPM | |
|---|---|---|---|---|---|---|---|
| | | PCR X PCR | PCR X PV | PCR X PCR | PCR X PV | PCR X PCR | PCR X PV |
| 7 | PEDUNCLE (R) | 0.06 ± 0.02 | 0.03 ± 0.01 | | | 32 ± 6.6 | 13 ± 6.6 |
| | POD WALL (PW) | 0.91 ± 0.03 | 0.11 ± 0.04 | | | 27 ± 7.2 | 15 ± 4.0 |
| | SEED COAT (SC) | 0.76 ± 0.17 | 0.23 ± 0.03 | | | 16 ± 5.2 | 8 ± 3.8 |
| | EMBRYO (EM) | 14.70 ± 6.20 * | 132 ± 42 * | | | 9.3 ± 2.7 | 8 ± 4.7 |
| | LIQUID ENDOSPERM (LE) | | | 0.44 ± 0.16 | 3.1 ± 0.16 | 16 ± 4.8 | 55 ± 18 |
| 9 | R | 0.07 ± 0.02 | 0.12 ± 0.08 | | | 25 ± 4.4 | 23 ± 7.4 |
| | PW | 0.10 ± 0.01 | 0.22 ± 0.07 | | | 40 ± 2.0 | 40 ± 11 |
| | SC | 0.36 ± 0.03 | 0.80 ± 0.16 | | | 14 ± 1.2 | 13 ± 2.0 |
| | EM | 1.23 ± 0.45 * | 40.3 ± 14.8 * | | | 7 ± 1.9 | 10 ± 1.3 |
| | LE | | | 3.23 ± 1.30 | 0.66 ± 0.25 | 14 ± 5.2 | 13 ± 3.8 |
| 10 | R | 0.03 ± 0.01 | 0.08 ± 0.05 | | | 13 ± 1.6 | 21 ± 7.2 |
| | PW | 0.09 ± 0.01 | 0.11 ± 0.02 | | | 44 ± 10 | 36 ± 14 |
| | SC | 0.19 ± 0.04 | 0.31 ± 0.11 | | | 13 ± 2.7 | 11 ± 3.4 |
| | EM | 1.01 ± 0.16 | 15.1 ± 9.0 | | | 11 ± 1.2 | 14 ± 5.1 |
| | LE | | | 1.81 ± 1.40 | 1.64 ± 0.33 | 19 ± 5.6 | 17 ± 2.0 |

# CHANGES IN LEVELS OF PLANT GROWTH SUBSTANCES IN ABORTING AND NONABORTING *PHASEOLUS* SEEDS[1]

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## ABSTRACT

Pollinating *Phaseolus coccineus* with *P. vulgaris* predetermines that seeds abort by the time they reach 10 mm in seed length while selfed *P. coccineus* develop normally. Experiments were designed to compare the content of abscisic acid (ABA), indole-3-acetic acid (IAA), zeatin (Z), zeatin riboside (ZR), and dihydrozeatin riboside (diZR) in the different seed components of the aborting and non-aborting seeds. The concentrations of four of the compounds IAA, ABA, ZR, and diZR changed independently in the individual seed parts of both seed types over the sampling period. Z was in insufficient quantities to analyze. ZR concentration peaked in the self embryos at 7 mm seed length, the time of maximum cell division. Inter-specific embryos never reached the same level of ZR. The inter-specific embryos had seven times more ABA and 4.5 times more IAA than the self embryos at 7 mm seed length. In the seed coats, the concentration of IAA was higher in the self than the interspecific pollinated plants. While there was no difference between seed coats of the two types of pollination in the levels of ZR or diZR through-out sampling, the concentration of ABA was lower in the seed coat of

interspecific embryos. The liquid endosperm showed no differences in the levels of ZR, diZR, and ABA in the early sampling periods. However, more IAA was found in the interspecific liquid endosperm. In the later sampling periods there was no difference in the levels of IAA and ZR but significantly more diZR was found in the interspecific liquid endosperm. The peaks in concentration of ZR and diZR as well as the relatively low concentrations of IAA and ABA in the self embryos suggests plant growth substances may function in regulating normal embryo development. The timing of distinct peaks in concentration of IAA and ABA in the interspecific embryo suggest that imbalances of these compounds may function in regulating embryo abortion.

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# INTRODUCTION

In the interspecific hybrid of *Phaseolus coccineus* x *P. vulgaris*, fruit set occurs, the pod and embryo appear to develop normally for two to three weeks after pollination, but then the embryo aborts (28). In a few instances viable seed have matured from this cross (12). Other studies found that interspecific hybrid embryos, excised shortly before the expected time of abortion, can be cultured on a defined medium to a mature plant (1, 9).

The ability of the excised embryo of *P. coccineus* x *P. vulgaris* to grow *in vitro* suggests there is no inherent disparity in the genetic constitution of the hybrid embryo. Rather it is the failure of the support system of the ovule which leads to abortion. This is also supported by *in vivo* observations of cell division, differentiation, and morphogenesis of hybrid embryos, which appear normal although slower in development than the selfed seeds (7, 29).

Interspecific hybridization allows prediction of embryo abortion at a specific stage of development. Because there is no inherent disparity in the genetic constitution of the interspecific hybrid embryo, comparisons can be made between these aborting embryos and embryos that develop normally. Understanding how the interspecific hybrids abort may enable us to predict limitations in yield of beans.

Two factors which may strongly influence embryo abortion are availability of photosynthate and plant growth substances . Since

photosynthate availability is not limiting (10), an imbalance of plant growth substances may induce failure of the embryo.

The data available lack information on plant growth substance levels in specific seed components - seed coat, liquid endosperm, and embryo. It is also important to know whether there is a difference between aborting and nonaborting seed types in plant growth substance concentrations in specific seed components.

Our study compares P. coccineus x P. vulgaris, in which embryos abort at 10 mm seed length, with P. coccineus selfed, in which embryos develop to maturity. The objectives were 1) to determine a profile for the following plant growth substances in each seed part: abscisic acid (ABA), indole-3-acetic acid (IAA), zeatin (Z), zeatin riboside (ZR), and dihydrozeatin riboside (diZR) and 2) to compare each plant growth substance in the seed parts of the two seed types at each stage of development.

## MATERIALS AND METHODS

### Plant Material

A single red-flowered stock plant of P. coccineus , resulting from a self-pollination of the F1 obtained by crossing P. coccineus ,Scarlet Runner Bean' with ,Dutch Knife Case', was the source of stem cuttings. The cuttings were grown in a 2:2:1 (peat:vermiculite:sand) potting mixture in 20 cm diameter pots. Supplemental lighting was given to extend the natural day length to 14 h with predawn lighting at approximately 150 uEinsteins $m^{-2}$ $s^{-1}$ measured 0.5

m from the light source. This lighting treatment was given from January to April. After this time no supplemental lighting was given. Plants were fertilized twice weekly with 200 ppm 20N-20P-20K fertilizer.

Pollinations were started in April and were done under natural day length conditions. The cuttings were used as the female parent. Pollinations consisted of 1) P. coccineus selfed and 2) P. coccineus x an accession of P. vulgaris from Wisconsin (A80-43). The crossing technique used was developed by Buischand (4). After fruit set, the other flowers on the raceme were removed.

Pods were harvested based on pod thickness. Pod thickness was used to estimate seed length which indicated embryo development (10, 30). For each sample, the second seed from two pods was collected. Samples were taken from the two seed types at six seed lengths, 5 to 10 mm. 5 and 6 mm seed lengths (heart-shaped embryos) were divided into liquid endosperm, and the embryo plus seed coat. At 7 to 10 mm seed length (cotyledon stage of embryo development) the seed was divided into three components: liquid endosperm, embryo, and seed coat. After 10 mm in seed length the liquid endosperm had been absorbed by the self seed and cellularized in the interspecific seed.

## Hormone Analysis

### Preparative

After harvest, seed parts were placed on dry ice. Samples were

extracted in cold 80% methanol containing butylated hydroxytoluene (10 mg/l, Calbiochem, LaJolla, CA) and internal standards of 500 dpm of 1- $^{14}$C-IAA and 3000 dpm of 2- $^{14}$C-ABA (51.5 mCi/mmol IAA, 11.3 mCi/mmol ABA, Amersham, Arlington Hts., IL). Grinding of seed parts was done at 4 C in silanized glass centrifuge tubes with a glass homogenizer. Samples were centrifuged for 15 min at 12,000 x g at 4 C. The supernatent was removed and dried in vacuo at 30 C and frozen (-20C) until further analysis.

The plant growth substances - ABA, IAA, Z, ZR, and diZR- were analyzed as outlined in Tables 1 and 2. The samples were dissolved in 3 ml of deionized distilled water and filtered through a 1 um pore size filter (LC type, Millipore Corp.). Filtered samples were placed in 6 ml syringes and injected automatically into a 6 port high pressure injection valve (HPa7000, Valco, Houston, TX). Samples eluted from a preparative PRP-1 column (system 1, Table 2) with a 15 min gradient. The fraction in which standards of ABA, IAA, Z, ZR, and diZR would elute from the PRP-1 column (9.50 to 14.0 min) was diverted to a preparative $C_{18}$ column and was eluted with a linear gradient of 20 min. Three fractions containing the cytokinins (Z, ZR, and diZR), IAA, and ABA were collected at the elution times for previously run standards. The system was automated with a microprocessor (SLIC 1400, Systec Inc., Minneapolis, MN). The three fractions were dried in vacuo at 30 C and stored at -20 C until further analysis.

A second preparative high performance liquid chromatographic

system was used to further purify the cytokinin and IAA fractions. Cytokinin fractions from the initial preparative separation were dissolved in 1 ml 0.1 N acetic acid and loaded into 6 ml syringes. Samples were injected automatically onto a $C_{18}$ column (system 4, Table 2) through a 6-port injection valve (Rheodyne 7126). Z was separated from ZR and diZR (Table 1). The fractions were collected at the retention times of standards and dried as before. The IAA fraction was treated in the same manner except that the linear elution gradient was 0-50% for the first 2 min. (system 2, Table 2).

Quantitation

ABA was quantified by the method of Hein et al. (8). Samples were methylated with diazomethane (23) and dried under $N_2$. ABA was determined by GLC separation with a SE30 capillary column (30 m x 0.32 mm, J&S Scientific, Ranch Cordova, CA) and electron capture detection (Hewlett Packard 63Ni). Samples were dissolved in ethyl acetate. One ul of the sample was injected automatically (Hewlett Packard 7671A autosampler) at 240 C into a quartz inlet liner packed with 3% SP2100 on Supelcoport 100 to 120 mesh, split 20:1, run at a column temperature of 240 C, and detected at 260 C. Recovery was calculated for each sample using isotope dilution. Radioactivity from $^{14}C$-ABA from each vial prepared for GLC analysis was determined with liquid scintillation spectrometry. Recovery averaged 64% $\pm$ 12% (SE).

ZR/diZR samples from the second preparative step were manually injected onto a cation exchange column (system 6, Table 2). Two UV

detectors were connected in series, the first monitoring at 254 nm (model 440, Waters Assoc.) and the second at 210 nm (SF770, Schoeffel). The two compounds were quantified by measuring the peak heights of the 254 nm response and comparing to an external standard curve. The ratio of the peak heights of the cytokinins from the two detectors for plant samples was compared to the ratio for standards. The ratio from the plant samples had to be within $\pm$ 10% of the mean ratio calculated for the standards or the peak was considered impure.

ZR and diZR authentication was accomplished using gas chromatography-mass spectrometry (GC-MS) . Trimethyl silyl derivatives of dry standards and plant samples, after HPLC analysis were prepared immediately preceeding injection on the GC-MS. ZR and diZR were derivitized at 55 C for 0.5 h with N,O-bis(trimethylsilyl)trifluroacetamide-trimethyl-chlorosilane (99:1, 8 ul; Regis Chemical Co., Morton Grove, IL) with 2 ul of pyridine as solvent. Mass spectra were obtained using a LKB 9000 GC-MS operating at an ionization potential of 70 eV which interfaced to a PDP 8e computer. All samples were run on a 60 cm x 2 mm glass column packed with 3% OV-1 on 100 to 120 mesh Supelcoport with the temperature programmed from 150 to 290 C at 10 C/min and a helium flow rate of 30 ml/min.

Zeatin samples were run on a cation exchange column (system 5, Table 2) and were treated the same as ZR/diZR samples. No Z was detected in any of the plant samples.

IAA was quantified using the method of Hein et al.(8). Samples

were injected onto an anion exchange column (system 3, Table 2) with an autoinjector (WISP 710 B, Waters Assoc.). The column was eluted first through a fluorescence monitor (FS 950, Kratos, Westwood, NJ) and then through an oxidative amperometric detector (LC5A, Bioanalytical Systems, W. Lafayette, IN). IAA was quantified by measuring the peak height from the fluorescence detector (Data Module, Waters Assoc.) and compared to an external standard curve from IAA standards. The ratio of the peak height of IAA from the two detectors for plant samples was compared to the ratio for standards. The ratio from the plant samples had to be within $\pm$ 10% of the mean ratio calculated for the standards or the peak was considered impure. Loss of IAA was calculated as for ABA. Recovery averaged 21% $\pm$ 8% (SE).

IAA samples were authenticated with GC-MS. IAA was derivitized with BSFTA (Regis Chemical Co., Morton Grove, IL) for 0.25 h at 50 C (16). The system used for GC-MS was as described above except that the temperature program was 150 C to 270 C at 10 C/min.

# RESULTS

Analysis of selected ZR, diZR, and IAA samples by GC-MS verified that the HPLC procedures used for isolation and quantitation had yielded pure compounds. Samples of Z were not subjected to GC-MS analysis because it was not present in significant quantities. Mass spectra for TMS-ZR samples contained ions (relative intensities in parentheses) of m/z 639 (M+,3.1), 624(4.0), 549(8.0), 536(10.6), 320(7.8), 230(9.2), 201(19.6), and 73(TMS, 100) which corresponded with other published results (5). Major ions in mass spectra of TMS-diZR samples included m/z 641(M+, 1.7), 626(4.7), 322(35.4), 294(7.5), 230(38.7), 162(10.9), 73(TMS, 100) which corresponded with other published results (32). Mass spectra for bis-TMS-IAA samples contained ions of m/z 319(M+, 26.0), 304(5.5), 202(bis-TMS, 100), 73(18.4) which corresponded to published spectra (16).

Sample quantitation on the GC-MS was done only for the cytokinins and was accomplished using selected ion current monitoring. Samples were calibrated against standards based on peak intensity of specific ions at m/z 320 and 201 for ZR and m/z 322 and 230 for diZR. The quantities of the two compounds from GC-MS corresponded to the quantities calculated from peak heights from HPLC. The GC-MS verified that the isolated compounds were pure and demonstrated the validity of the HPLC procedures.

## Cytokinins

At 7 mm seed length, the embryos of *P. coccineus* were entering

the cotyledon stage of development and cell division was occurring (10, 33). Subsequently a rapid gain in weight was observed in the selfed embryo but not in the interspecific embryos (Fig. 1a). In conjunction with cell division, the level of ZR for embryos from self-pollinations peaked at 7 mm seed length, at 1600 ng ZR/mg f.wt., and then declined rapidly to 26 ng ZR/ mg f.wt. at the end of sampling (Fig. 1b). The concentration of ZR was 644 ng ZR/mg f.wt. in the interspecific embryo at 7 mm seed length (Fig. 1b).

Levels of diZR in the embryo were lower than ZR. The selfed embryo profile of diZR was similiar to ZR: the concentration of diZR peaked for the self embryo at 7 mm seed length at 10 ng diZR/mg f.wt., and then declined to 0.5 ng diZR/mg f.wt. at 10 mm seed length (Fig. 1e).

Levels of ZR in the seed coat were the same throughout the sampling period for both crosses (Fig. 2b). Concentrations were lower than those measured for the embryos. The concentration of diZR in the seed coats of the self at 7 mm seed length was the same as in the interspecific (Fig. 2c). However, the concentration of diZR in the self seed coats increased to 6 ng diZR/mg f.wt. at 8 mm seed length and remained at that level through the sampling period. At 8 mm seed length the weight of the interspecific seed coat was higher than the self (Fig. 2c), but the diZR concentration was significantly lower (Fig. 2c). At 9 and 10 mm seed length, the interspecific seed coat weight was greater (Fig. 2a) but there was no difference in concentration of diZR (Fig. 2c).

The volume of liquid endosperm was comparable for the two seed types until 9 mm seed length (Fig. 3a). At this point in development, the self embryo began an exponential phase of growth and absorbtion of the liquid endosperm occured. The interspecific embryo did not gain weight and did not expand into the seed cavity. Therefore the liquid endosperm was not absorbed and this was reflected in the larger volume seen at 9 and 10 mm seed length (Fig. 3a).

The concentraton of ZR in the liquid endosperm was the same for the two seed types until 10 mm seed length (Fig. 3b). ZR concentration was then significantly higher in the interspecific than in the self liquid endosperm (Fig. 3b).

The concentration of diZR was significantly lower than the concentration of ZR in the liquid endosperm for both seed types. (Fig. 3c). The profiles of the two compounds also were very different. From 5 to 7 mm seed length, the concentration of diZR was the same for both crosses (Fig. 3c). However at 8, 9, and 10 mm seed length, the liquid endosperm of the interspecific cross had significantly higher levels of diZR (Fig. 3c).

IAA

At 7 mm seed length, the interspecfic embryo had 4.5 times more IAA than the self embryo. By 8 mm seed length, IAA concentration was twice as high in the interspecific embryo. However, by 9 and 10 mm seed length the embryos from the two crosses were comparable in IAA concentration (Fig. 1d).

During the sampling period, the concentration of IAA was significantly higher in the seed coats after self pollination (Fig. 2d). The peak in concentration of IAA in the seed coat of the self was observed at 7 mm seed length and was 12 times higher than in the seed coat from the interspecific cross. At 8 mm seed length, the peak IAA concentration in the seed coat was observed in the interspecific cross. Peak IAA concentration in interspecific seed coat was not as high as the peak in the self seed coat. At 9 and 10 mm seed length the seed coats from self pollination were twice as high in IAA than are the seed coats from the interspecific cross (Fig. 2d).

Higher concentrations of IAA were observed in the liquid endosperm from the interpsecific cross at 5 and 6 mm seed length (Fig. 3d). From 7 to 10 mm seed length the concentration of IAA in the liquid endosperm was comparable for the two seed types.

ABA

The concentration of ABA in the embryos of the interspecific cross was significantly greater than the self at 7, 8, and 9 mm seed length (Fig. 1e). At 7 and 8 mm seed length, the interspecific embryo was seven times higher in ABA than the self embryo. Five times more ABA was found in the interspecific embryo at 9 mm seed length.

The levels of ABA in the seed coats from the self were constant throughout the sampling period (Fig. 2e). At 8 and 9 mm seed

lengths there were significantly higher concentrations of ABA in the seed coats from self-pollinations than in the seed coats from interspecific crosses (Fig. 2e). At these two sampling times the seed coats of interpsecific crosses were significantly heavier than those of self-pollinations (Fig. 2a).

There were no significant differences in ABA levels in the liquid endosperm until 9 mm seed length. At this point there was more ABA in the self liquid endosperm (Fig. 3e). The level of ABA in the liquid endosperm was constant over the sampling period for the interpspecific cross but increased for the self (Fig. 3e).

## DISCUSSION

Levels of different plant growth substances in seeds have been reported as mass of the plant growth substance per unit weight of the seed or as plant growth substance per single seed (3, 6, 18). Expressing levels of a plant growth substances in this manner implies a uniform distribution throughout the seed. However, the tissues comprising the seed have different functions in embryogenesis (21, 33). Furthermore, the concentration of the plant growth substance is likely to affect each tissue differently depending on the stage of development. The results presented here demonstrate unequal distribution of IAA, ABA, ZR, and diZR in seed parts. Therefore, to obtain maximum information on the roles of plant growth substances in seed development, the seed should be divided into its component parts for analysis.

Embryo failure is predetermined for the interspecific embryo by 4 days after pollination when abnormalities in the suspensor are observed (7). By the globular stage of embryo development of P. coccineus, the suspensor develops wall ingrowths and polytene chromosomes to aid in transport (33). In comparison, the suspensor associated with the interspecific embryo lacks differentiaton of cell wall ingrowths and polytene chromosomes (7).

The nourishing function of the suspensor lasts into the heart stage of embryo development (21) and is then supplemented by the liquid endosperm (21, 33). As coytledons grow and fill the seed cavity, the liquid endosperm is absorbed by the embryo. The interspecific cotyledons do not fill the seed cavity and the liquid endosperm is not absorbed (7). A difference in the plant growth substance profiles may relate to differences in embryo survival.

In embryo development, rapid cell division occurs at he beginning of the cotyledon stage of devleopment, 7 mm seed length for P. coccineus selfed embryos. At this stage the self embryo had a higher ZR concentration than the interspecific embryo. The occurrence of cytokinins has been associated with tissues with high rates of cell division e.g. root apical meristems (25) and the fruit and seeds of many species (13). Short and Torrey (26) found cytokinin production by pea root callus closely correlated with mitotic activity.

Nesling and Morris (18) extracted seeds of P. vulgaris and P. vulgaris x P. acutifolius to determine cytokinin-like activity.

Throughout the samplng period, the total cytokinin-like activity per seed was an order of magnitude lower in the interspecific than the selfed seed. When expressed on a dry weight basis, the cytokinin-like activity was highest earliest and declined steadily throughout sampling for both crosses.

The ZR concentration in P. coccineus self embryo follows the pattern described for seeds by Nesling and Morris (18). Elevated ZR levels correspond to the period of rapid cell division in the embryo. The interspecific embryo does not increase in ZR until 8 mm seed length which may be too late in development to induce an accelerated rate of cell division.

The difference in magnitude of ZR and diZR in the embryos and the similarity of the profiles of the two compounds in the self embryo indicates that diZR may be acting in conjunction with ZR but what that function is can not be elucidated by this study. Mok et al.(17) added naturally occurring cytokinins to callus cultures derived from P. vulgaris, P. lunatus, and the interspecific hybrid. ZR and diZR were the most active cytokinins in inducing callus growth. ZR was active at lower concentration than diZR for callus from P. lunatus and the interspecific hybrid (17).

Levels of the cytokinins in liquid endosperm were not significantly different until 8 mm seed length (Table 3). If the liquid endosperm was not functioning properly due to a deficiency in cytokinins as suggested by Nesliing and Morris (18), the deficiency would be expected early in development. Because the deficiency was

not detected early in our study, this suggests if the liquid endosperm does not function properly it is not due to a lack of cytokinins.

Auxin produced in the fruit, particularly in the seed, is thought to be transported out to the surrounding tissues where it stimulates growth by inducing cell expansion (14). This theory has been supplemented with the proposal that auxins create strong physiological sinks. The sinks are then capable of competing for substances necessary for continued growth (15). Throughout sampling in the P. coccineus self seed, IAA was highest in the seed coat. Exogenously applied auxin was shown to be transported from developing seeds to other parts of the plant (2). Hein (8) has demonstrated high levels of IAA in the seed coat of soybean and concluded that the high levels of IAA present in the transport pathway into and out of the seed , may be functioning in aiding movement of substances.

The suppressed levels of IAA in the interspecific seed coat may be associated with restricted transport of substances critical for seed development. This hypothesis is supported by the elevated levels of IAA in the embryo and liquid endosperm. IAA has been shown to be produced in the seed (14). If transport out of the seed is hindered, a build-up of IAA in the embryo and liquid endosperm might be expected. Therefore the elevated levels of IAA in the embryo and liquid endosperm and low level in the seed coat support Hein s hypothesis that IAA is needed in the immediate transport pathway to facilitate movement to the seed.

The elevated levels of IAA in the interspecific embryo may be high enough to be inhibitory to embryo growth. The practice of chemically thinning fruitlets involves the application of synthetic auxins, in high concentration to induce abscision (19). Elevated levels of IAA in the embryo of the interspecific cross may induce embryo failure.

Elevated levels of ABA are found in the interspecific as compared to the self embryo until 10 mm seed length. The drop in ABA correlated with the time of abortion. ABA has been correlated with abortion and abscision of young fruit (30). Rodgers (22) studying retained vs. abscising cotton fruit observed that during the period of early fruit development, ABA-like activity was higher in abscising than retained fruit. Tamas (28) studied pod abscision in P. vulgaris. Older pods growing at the base of the raceme abscised less frequently than young pods above. If older pods were removed, young pods developed normally. In the intact system, ABA concentration of young (abscising) pods was twice as high as young pods on plants where older pods had been removed (28). A high concentration of ABA prior to the abortion of the interspecific embryo may be an early signal from the plant for embryo abortion and/or it may indicate a block in metabolism of ABA. Setter et al. (24) determined that $^{14}$C-ABA moved from the source leaf to developing soybean seeds where ABA and its metabolites phaseic acid, dihydrophaseic acid, and aldopyranoside of dihydrophaseic acid accumulated. Walton (31) has reported that nonimbibed P. vulgaris seeds contain more ABA

metabolites that ABA. The elevated ABA level in the interspecific embryo may indicate that movement of ABA into the seed is not hampered. Once ABA is in the interspecific embryo, inability of the embryo to metabolize ABA may maintain the higher level.

As yet no specific functions for the four plant growth substances in seed development have been demonstrated in situ. Cytokinins have been implicated in the cell division process. The profile for ZR in the self embryo supports the hypothesis but that for diZR did not. It has been proposed that IAA is synthesized in the seed and moved to other parts of the plant. The profiles of IAA concentration in the interspecific seed parts imply that a block in transport occurs and increased levels of IAA in the embryo result. ABA has been implicated in abortion or abscision of fruits and again the profile of ABA concentration in the interspecific embryo supports this hypothesis.

How the four plant growth substances analyzed in this study interact to bring about embryo failure in the interspecific hybrid is not known. In order to better understand the interaction, a series of questions have been posed: Is ZR necessary for cell division in the embryo and is this a limiting factor to interspecific embryo growth? What is the function of diZR in seed development? Is the lack of IAA in the interspecific seed coat related to restricted transport of compounds through the seed which are necessary for continued growth of the embryo? Is ABA metabolism blocked in the interspecific embryo? If the self embryo was exogenously

treated with ABA and/or IAA, would embryo abortion result?

This list can be made substantial and depending on an investigator's interest other equally provocative questions can be asked. The complexity of the interaction is just beginning to be investigated.

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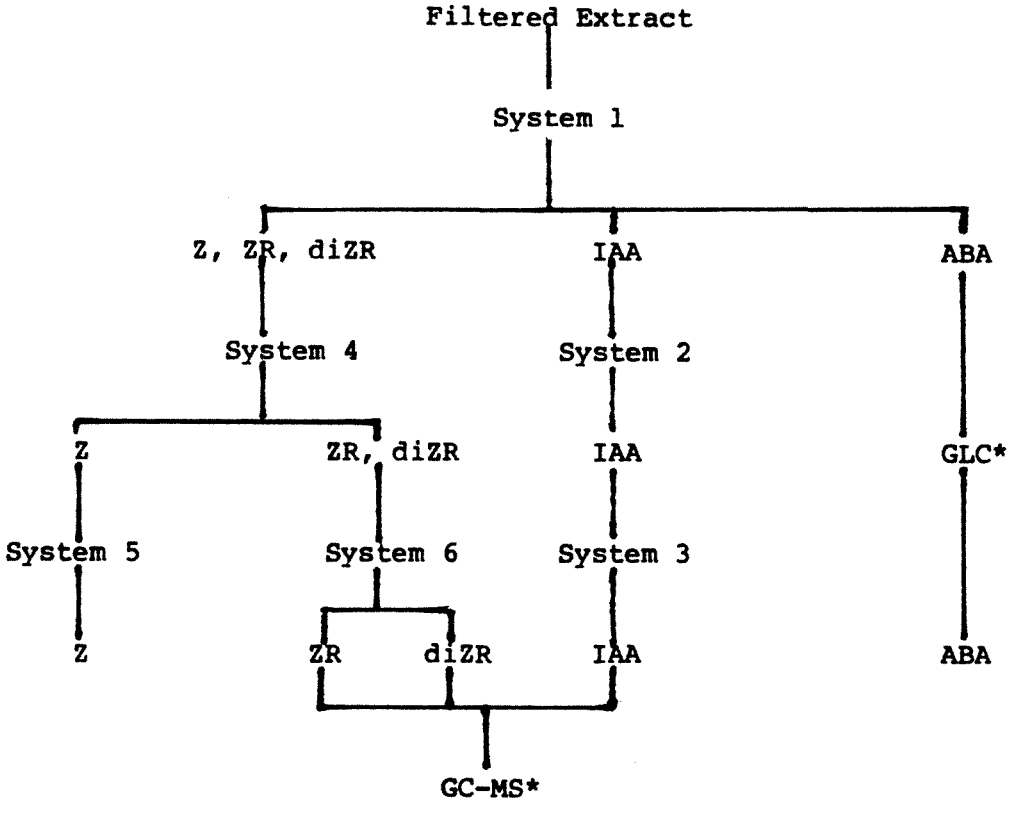
Table 1: Flow diagram of plant growth substance analysis done. Each system (1-6) is defined in Table 2.




*GLC and GC-MS not included in Table 2. Refer to text.

Table 2: Explanation of plant growth substance analysis systems (see Table 1 for reference to specific plant growth susbstance).

| System # | Column type, Particle Size | Sample Solvent, Injection Volume | Solvent A |
|---|---|---|---|
| 1 | PRP(10 um) 10cm X 10 mm Hamilton, Reno, NV<br>RSiL(10 um) 15 cm X 10 mm Alltech Assoc. | water, 3 ml | 0.1 M $NaH_2PO_4$<br>0.1N acetic acid |
| 2 | Nucleosil(7 um) 15 cm X 4.2 mm, Macherey-Nagl Co. Duren, Germany | 0.1 N acetic acid | 0.1 N acetic acid |
| 3 | Zorbax SAX(7 um) 15 cm X 4.2 mm, DuPont, Wilmington, DE | 0.2 N citrate, 0.5 N $NaH_2PO_4$ 0.2 N EDTA 10% MeOH, 50 ul | 0.2 N citrate 0.5 N $NaH_2PO_4$ 0.2 N EDTA 10 % MeOH |
| 4 | Nucleosil(7 um) 15 cm X 4.2 mm, Macherey-Nagl Duren, Germany | 0.1 N acetic acid | 0.1 N acetic acid |
| 5 | Vydac 401 TB(10 um) 20 cm X 4.2 mm Separations Group, Hesperia, CA | 0.1 M $NH_4OAc$ 10% MeOH pH 5.0, 150 ul | 0.1 M $NH_4OAc$ 10% MeOH pH 5.0 |
| 6 | Same as system 5 | 0.005 M $NH_4OAc$ 10% MeOH pH 5.0, 150 ul | 0.005 M $NH_4OAc$ 10% MeOH pH 5.0 |

Table 2 continued

| System # | Solvent B | Flow Rate (ml/min) | Linear Gradient (0-%B) | Retention Time (min) |
|---|---|---|---|---|
| 1 | 0.1 M $Na_2HPO_4$<br>50% EtOH | 2.0 | 0-100%, 15 min | IAA-10.4<br>CK-11.2<br>ABA-12.2 |
| | 0.1 N acetic acid<br>in EtOH | 2.5 | 0-50%, 20 min | CK-29.4<br>IAA-34.1<br>ABA-36.5 |
| 2 | 40% $CH_3CN$<br>10%EtOH<br>0.1 N acetic acid | 1.0 | 0-50%, 2 min<br>isocratic | 14.4 |
| 3 | ------- | 1.2 | isocratic | 7.0 |
| 4 | same as system 2 | 1.0 | 0-28%, 2 min<br>isocratic | Z-9.4<br>ZR,diZR-11 |
| 5 | ------- | 2.0 | isocratic | 10.0 |
| 6 | ------- | 2.0 | isocratic | ZR-8.7<br>diZR-11.5 |

Table 2 continued

| System # | Peak Width at 1/2 Peak Height (mm) | Detector |
|---|---|---|
| 1 | 5<br>6<br>5 | UV 254 nm |
| | 5<br>4<br>4 | UV 254 nm |
| 2 | 4 | UV 254 nm |
| 3 | 2 | Fluorescent-excitation 254 nm, emmission 340 nm<br><br>Oxidative amperometric,+0.95V vs Ag/KCl |
| 4 | 3<br>3 | UV 254 nm |
| 5 | 3 | UV 254 nm, UV 210 nm |
| 6 | 3<br>3 | UV 254 nm, UV 210 nm |

Abbreviations: CK-cytokinins; EtOH-ethanol; MeOH-methanol; $NH_4OAc$-ammonium acetate.

Figure 1: a) Weight (mg f. wt.) and the concentrations of b)ABA, c) IAA, d) ZR, and e) diZR in self (PCR X PCR) and interspecific (PCR X PV) embryos from 7 to 10 mm in seed length.

Figure 2: a) Weight (mg f.wt.) and the concentrations of b)ABA, c) IAA, d) ZR, and e) diZR in self (PCR X PCR) and interspecific (PCR X PV) seed coats from 7 to 10 mm in seed length.

Figure 3: a) Volume (ul) and the concentrations of b) ABA, c) IAA, d) ZR, and e) diZR in self (PCR X PCR) and interspecific (PCR X PV) liquid endosperm from 5 to 10 mm in seed length.

Table 3: Qualitative description of the relationship of the interspecific cross (PCR X PV) to the self (PCR X PCR) based on tissue type and plant growth substance. The results are given as:

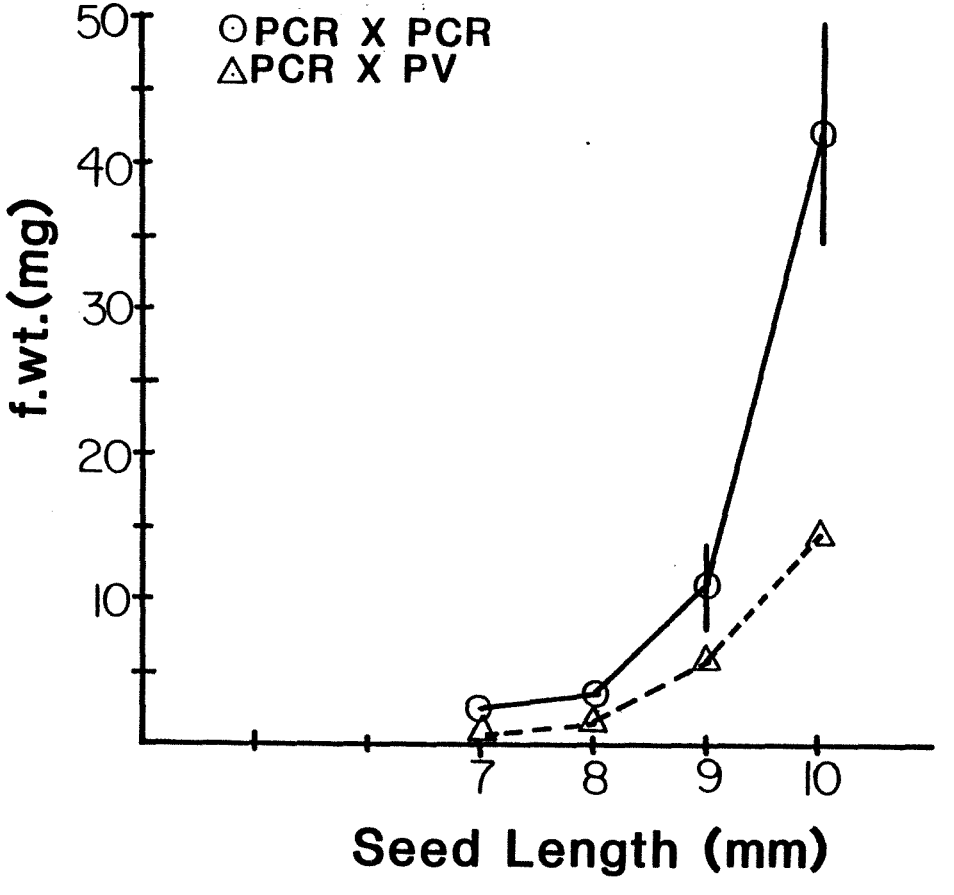
The interspecific cross is greater, less, equal to the self in plant growth substance concentration (ng/mg f.wt.).

(* - difference significant at the 5% level, NS - not significant)

| Seed Length (mm) | Plant Growth Substance | Tissue Type: Liquid Endosperm | Embryo | Seed Coat |
|---|---|---|---|---|
| 5 | ABA | less (NS) | | |
| | IAA | greater (*) | | |
| | ZR | less (NS) | | |
| | diZR | less (NS) | | |
| 6 | ABA | greater (NS) | | |
| | IAA | greater (*) | | |
| | ZR | equal (NS) | | |
| | diZR | equal (NS) | | |
| 7 | ABA | equal (NS) | greater (*) | greater (NS) |
| | IAA | less (NS) | greater (*) | less (*) |
| | ZR | equal (NS) | less (*) | greater (NS) |
| | diZR | equal (NS) | greater (NS) | greater (NS) |
| 8 | ABA | less (NS) | greater (*) | less (*) |
| | IAA | greater (NS) | greater (*) | less (*) |
| | ZR | greater (NS) | greater (*) | equal (NS) |
| | diZR | greater (*) | greater (*) | less (*) |
| 9 | ABA | less (*) | greater (*) | less (*) |
| | IAA | equal (NS) | greater (NS) | less (*) |
| | ZR | greater (NS) | greater (NS) | equal (NS) |
| | diZR | greater (*) | greater (*) | less (NS) |
| 10 | ABA | less (*) | greater (NS) | equal (NS) |
| | IAA | less (NS) | greater (NS) | less (*) |
| | ZR | greater (*) | greater (NS) | equal (NS) |
| | diZR | greater (*) | greater (NS) | less (NS) |

Fig. 1a

EMBRYO



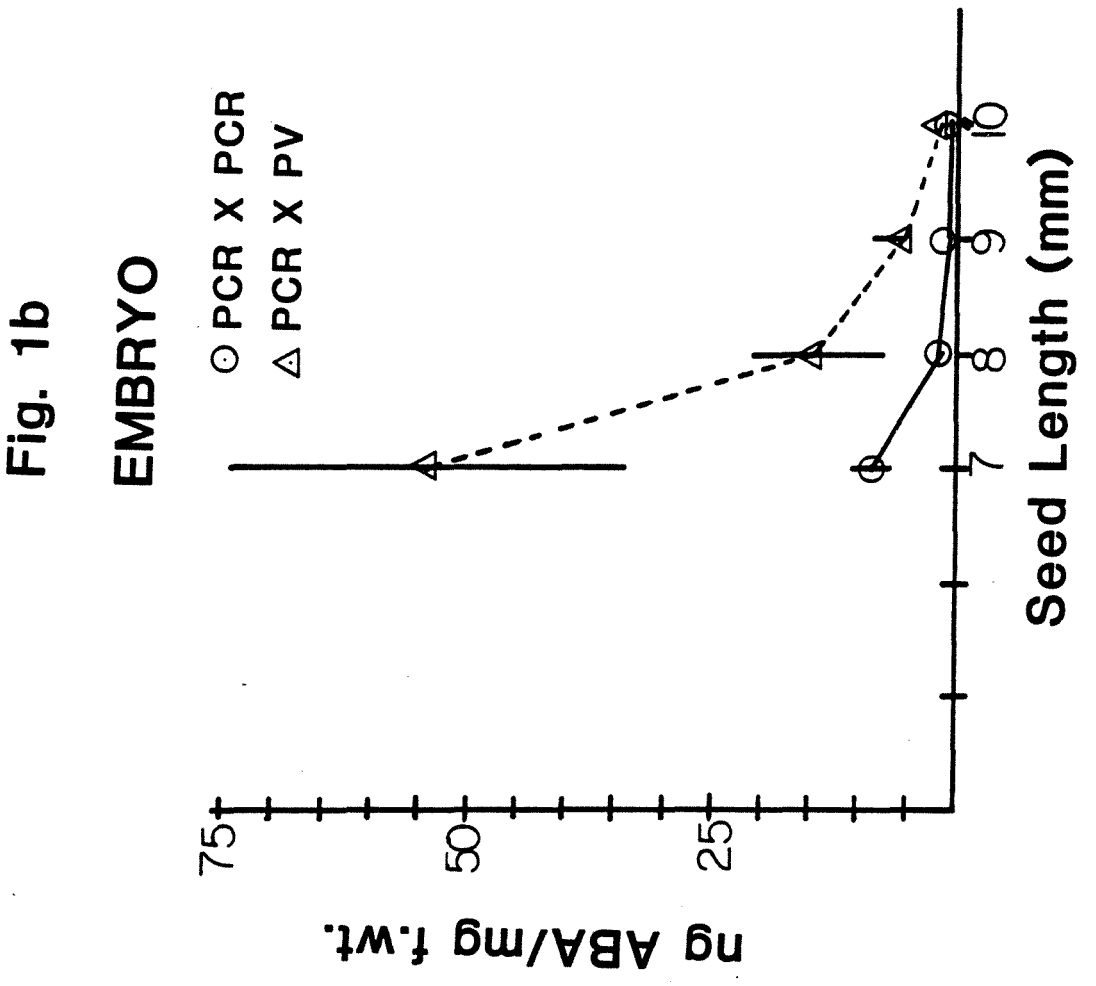


Fig. 1b
EMBRYO
⊙ PCR X PCR
△ PCR X PV
ng ABA/mg f.wt.
75
50
25
7
8
9
10
Seed Length (mm)

Fig. 1c

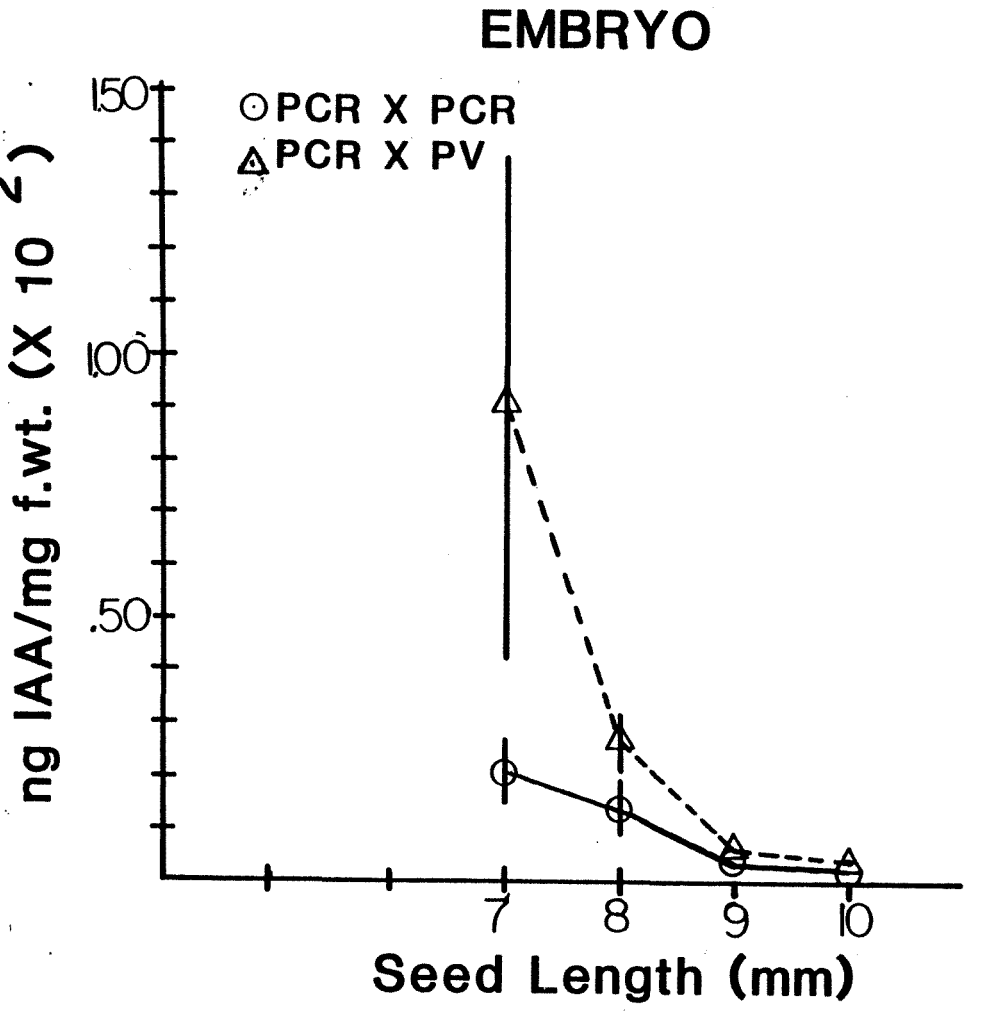

EMBRYO
⊙PCR X PCR
△PCR X PV
150
100
50
ng IAA/mg f.wt. (X 10 2)
7
8
9
10
Seed Length (mm)

Fig. 1d

EMBRYO

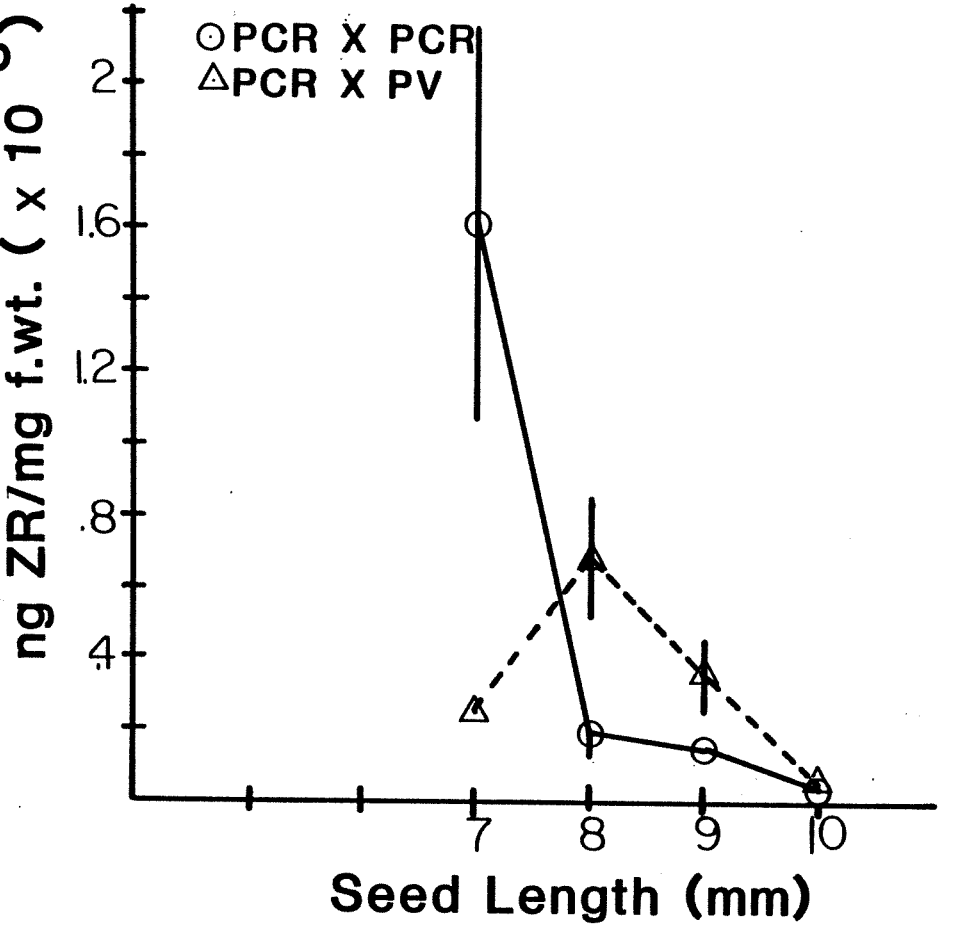


Fig. 1e

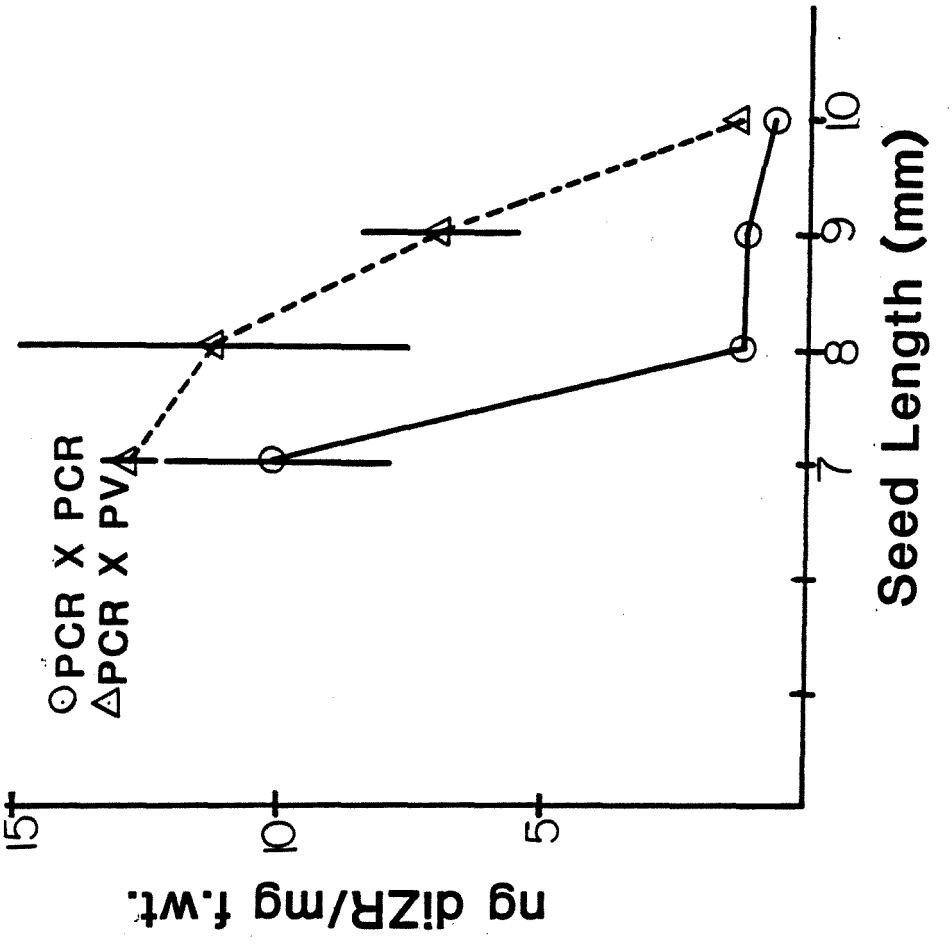

EMBRYO
⊙PCR X PCR
△PCR X PVA
ng diZR/mg f.wt.
15
10
5
7
8
9
10
Seed Length (mm)

Fig. 2a

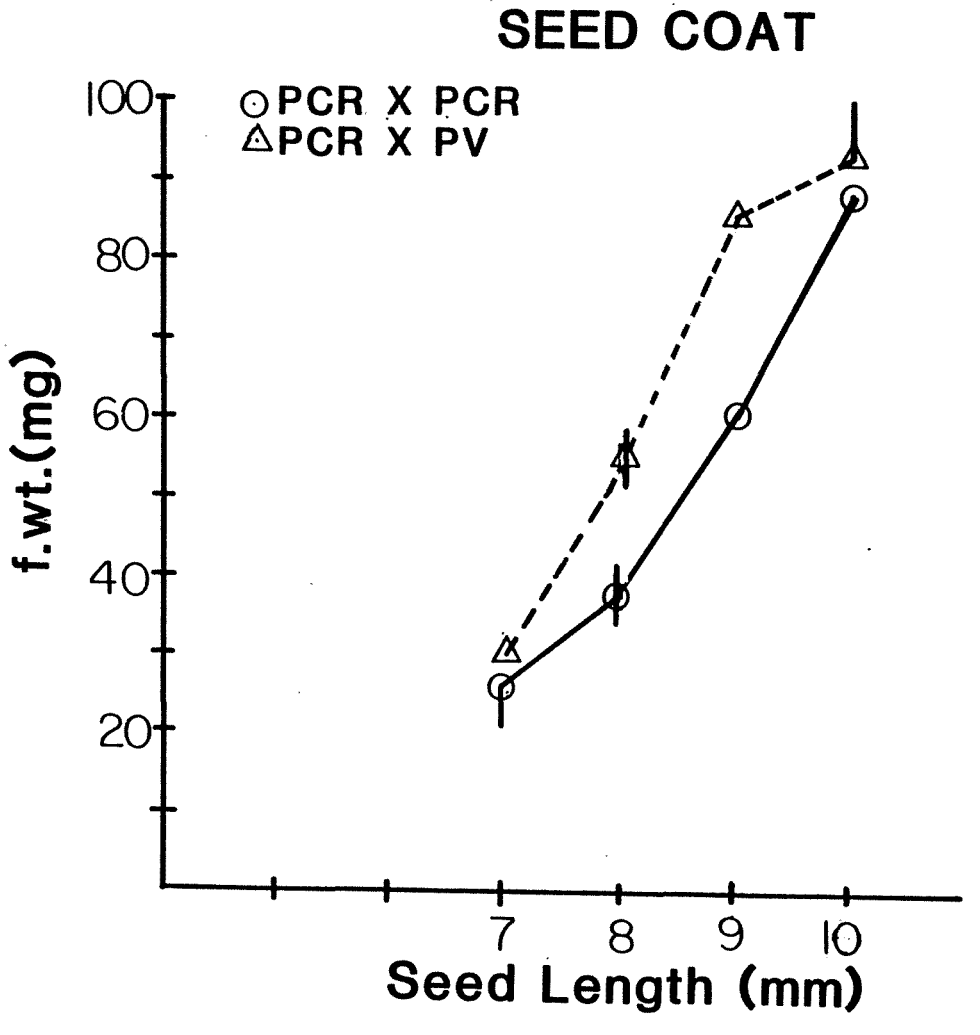

SEED COAT
⊙PCR X PCR
△PCR X PV
100
80
60
40
20
f.wt.(mg)
7
8
9
10
Seed Length (mm)

Fig. 2b

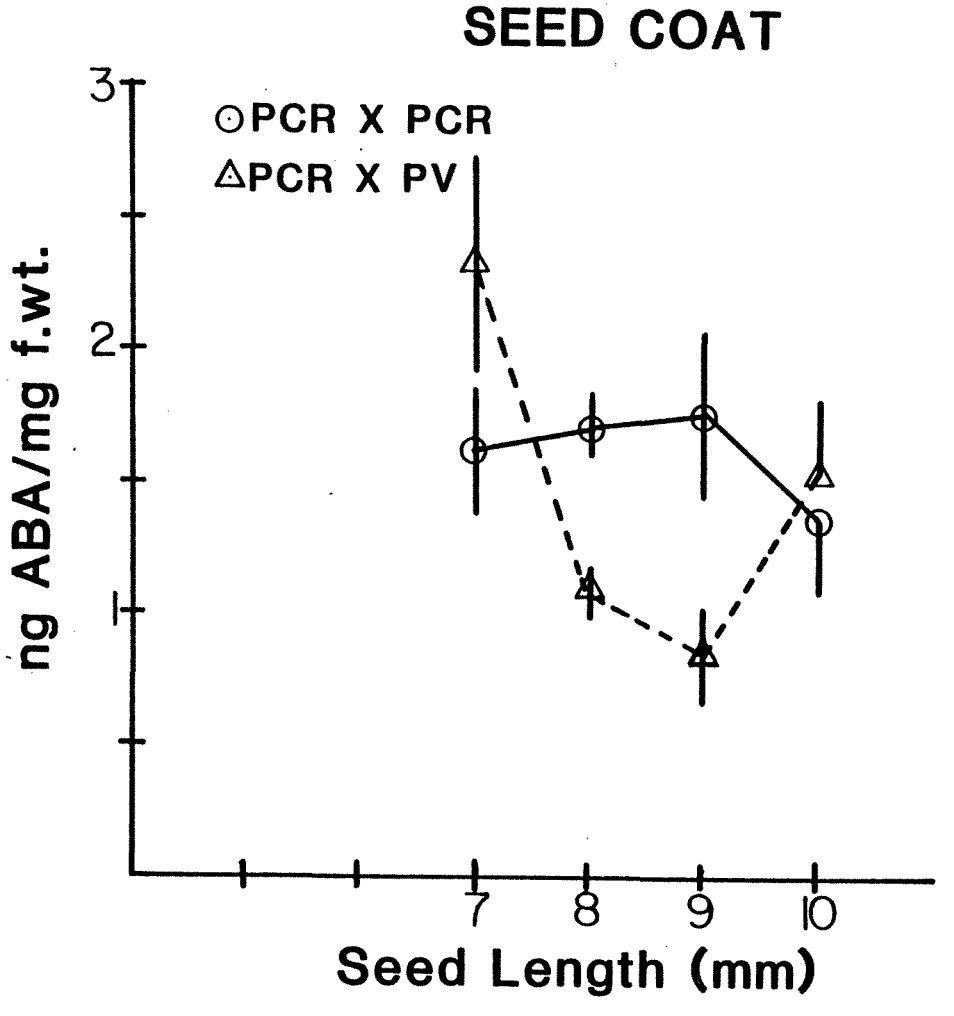

SEED COAT
⊙PCR X PCR
△PCR X PV
ng ABA/mg f.w.t.
3
2
1
7
8
9
10
Seed Length (mm)

Fig. 2c

SEED COAT

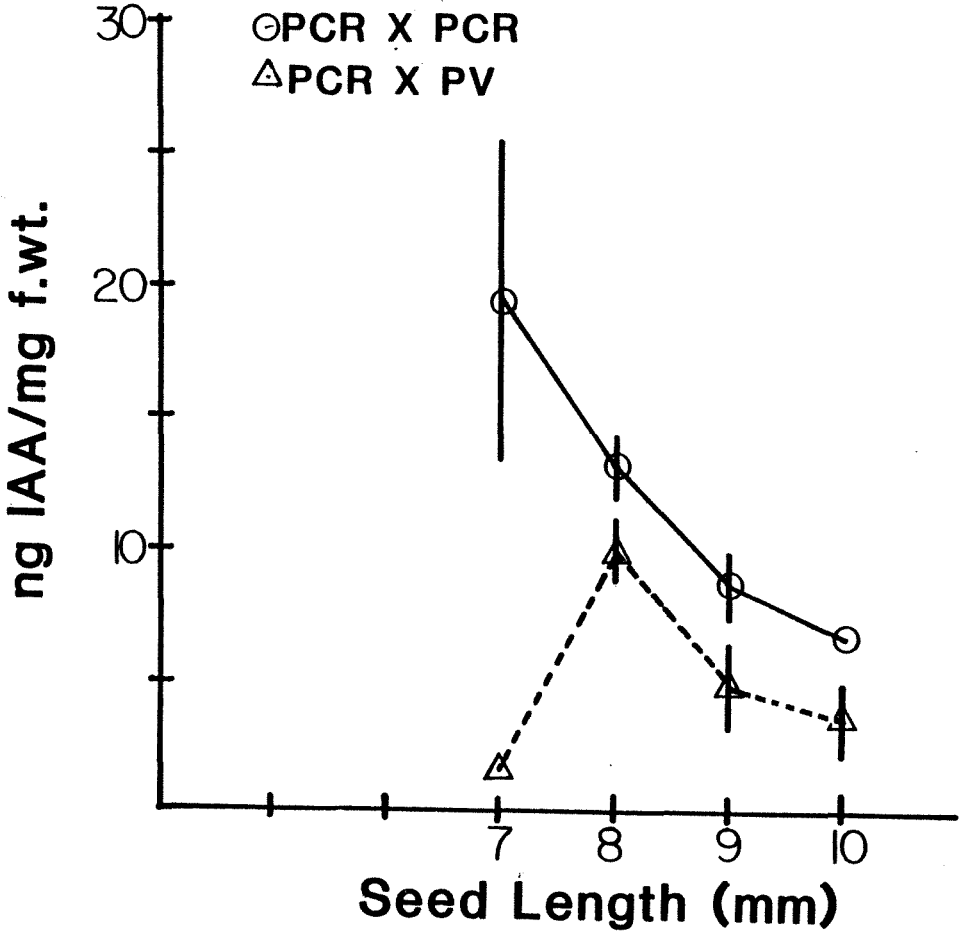


Fig. 2d

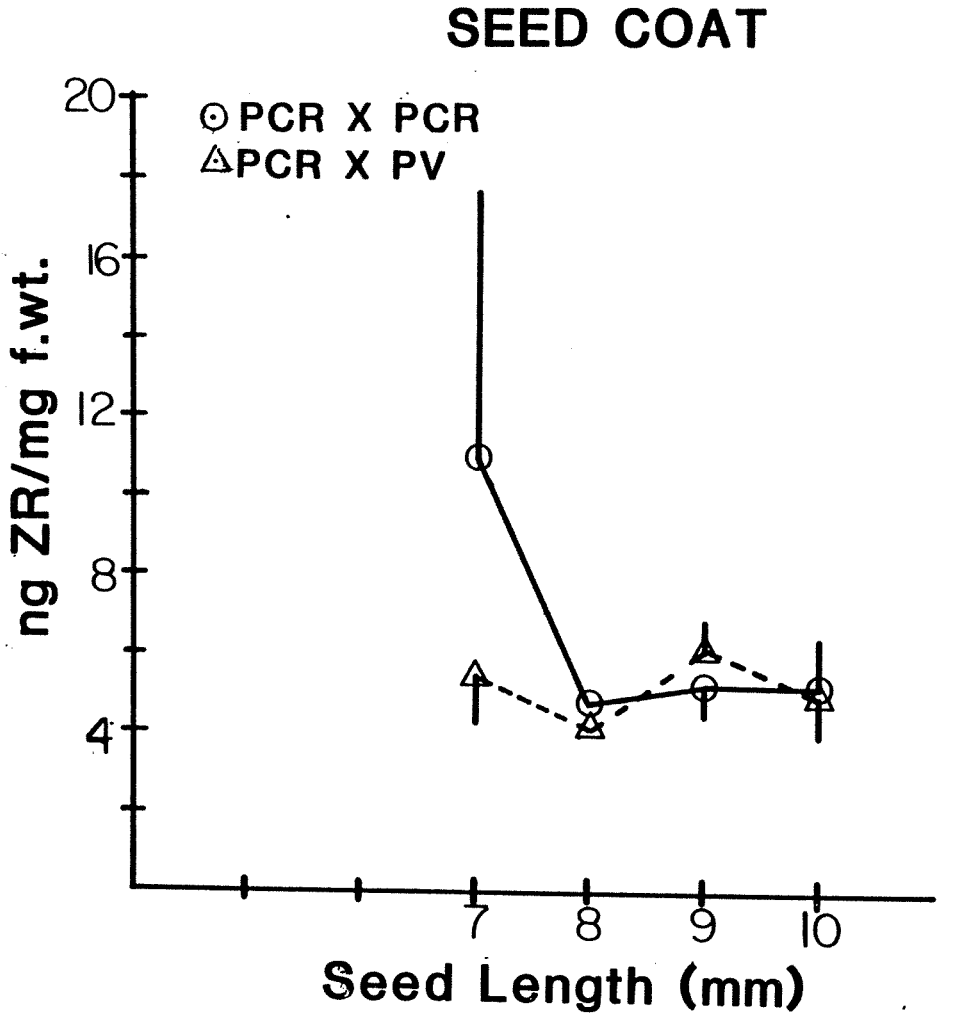

SEED COAT
⊙ PCR X PCR
△ PCR X PV
20
16
12
8
4
ng ZR/mg f.wt.
7
8
9
10
Seed Length (mm)

Fig. 2e

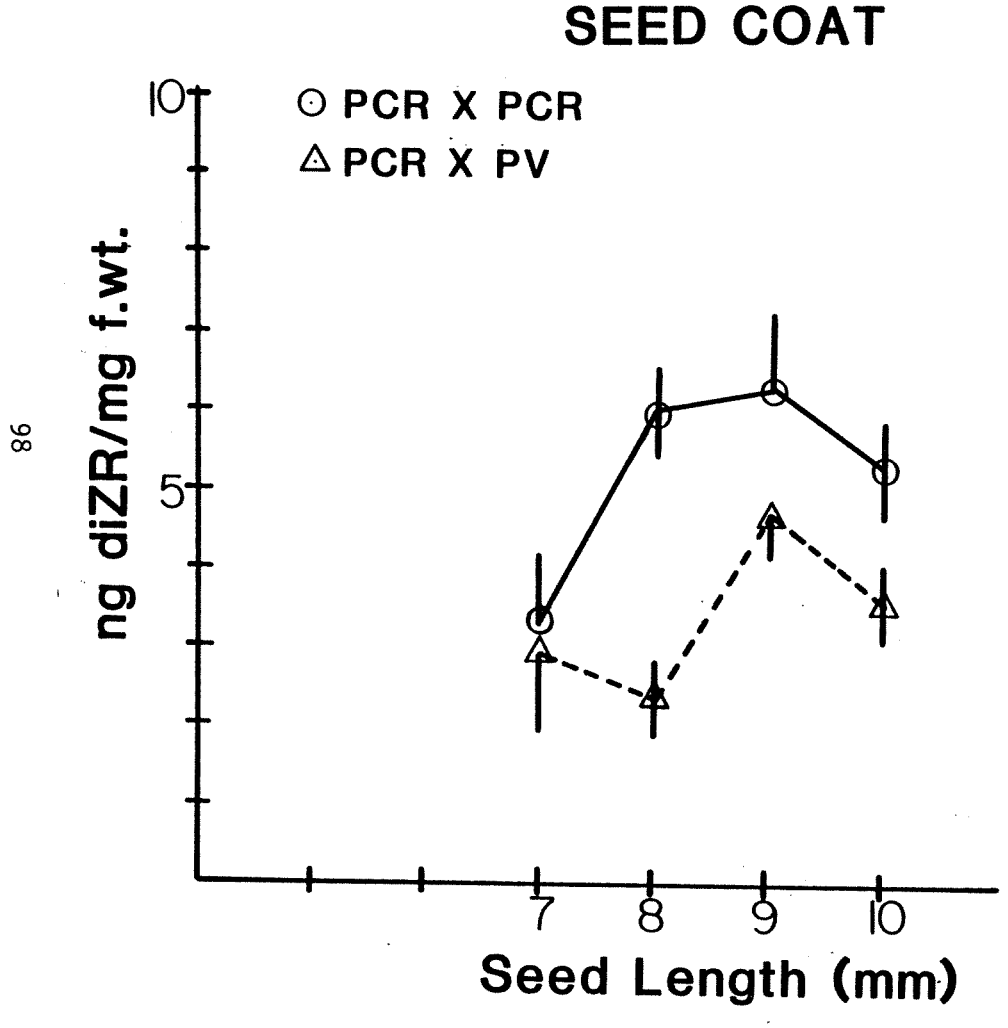

SEED COAT
⊙ PCR X PCR
△ PCR X PV
ng diZR/mg f.wt.
10
5
7
8
9
10
Seed Length (mm)

Fig.3a

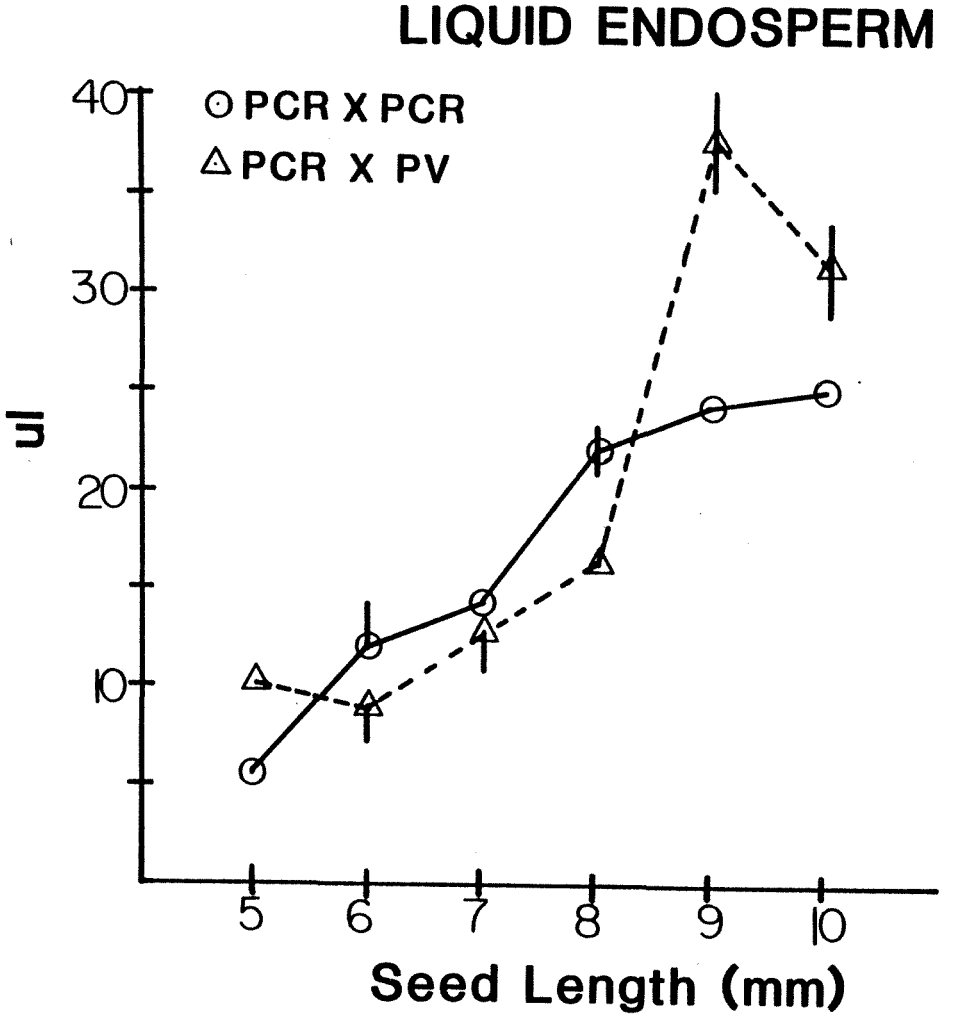

LIQUID ENDOSPERM
PCR X PCR
PCR X PV
40
30
20
10
SI
5
6
7
8
9
10
Seed Length (mm)

Fig. 3b

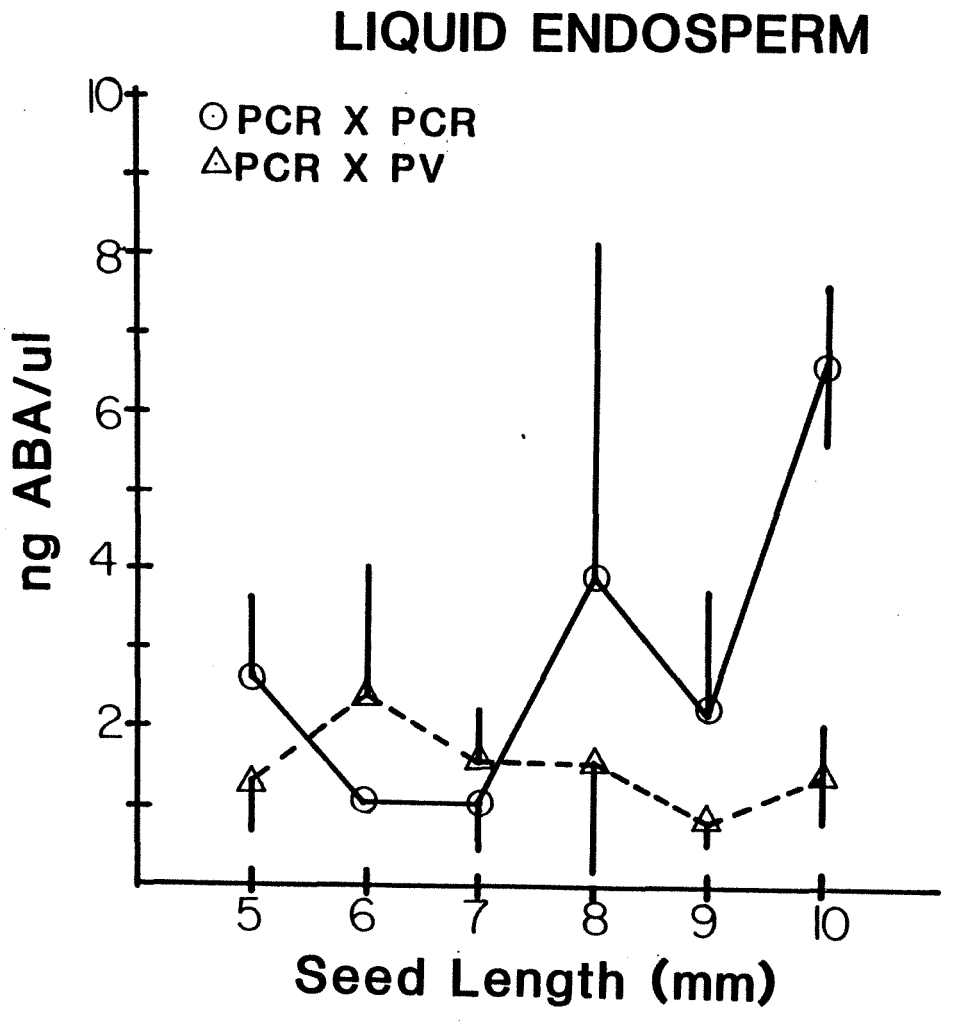

LIQUID ENDOSPERM
PCR X PCR
PCR X PV
ng ABA/ul
10
8
6
4
2
5
6
7
8
9
10
Seed Length (mm)

Fig. 3c

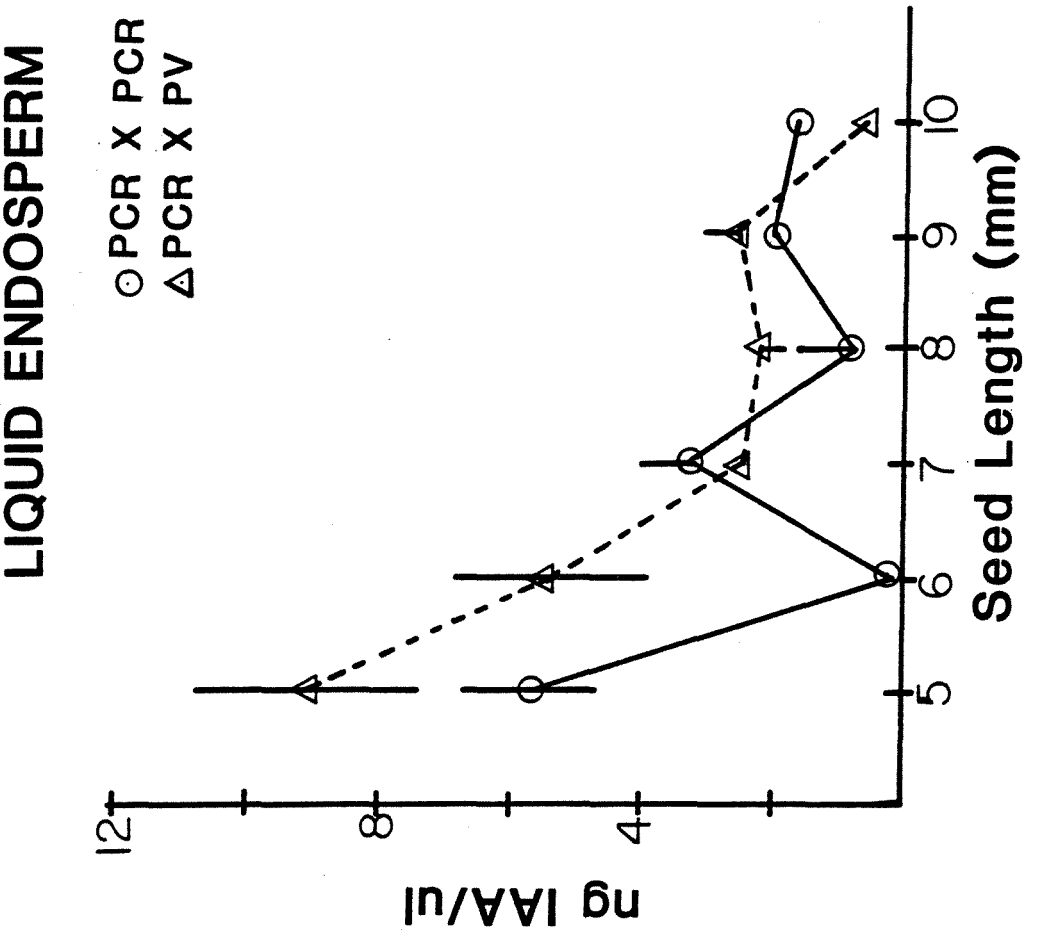

LIQUID ENDOSPERM
⊙PCR X PCR
△PCR X PV
ng IAA/ul
12
8
4
5
6
7
8
9
10
Seed Length (mm)

Fig. 3d

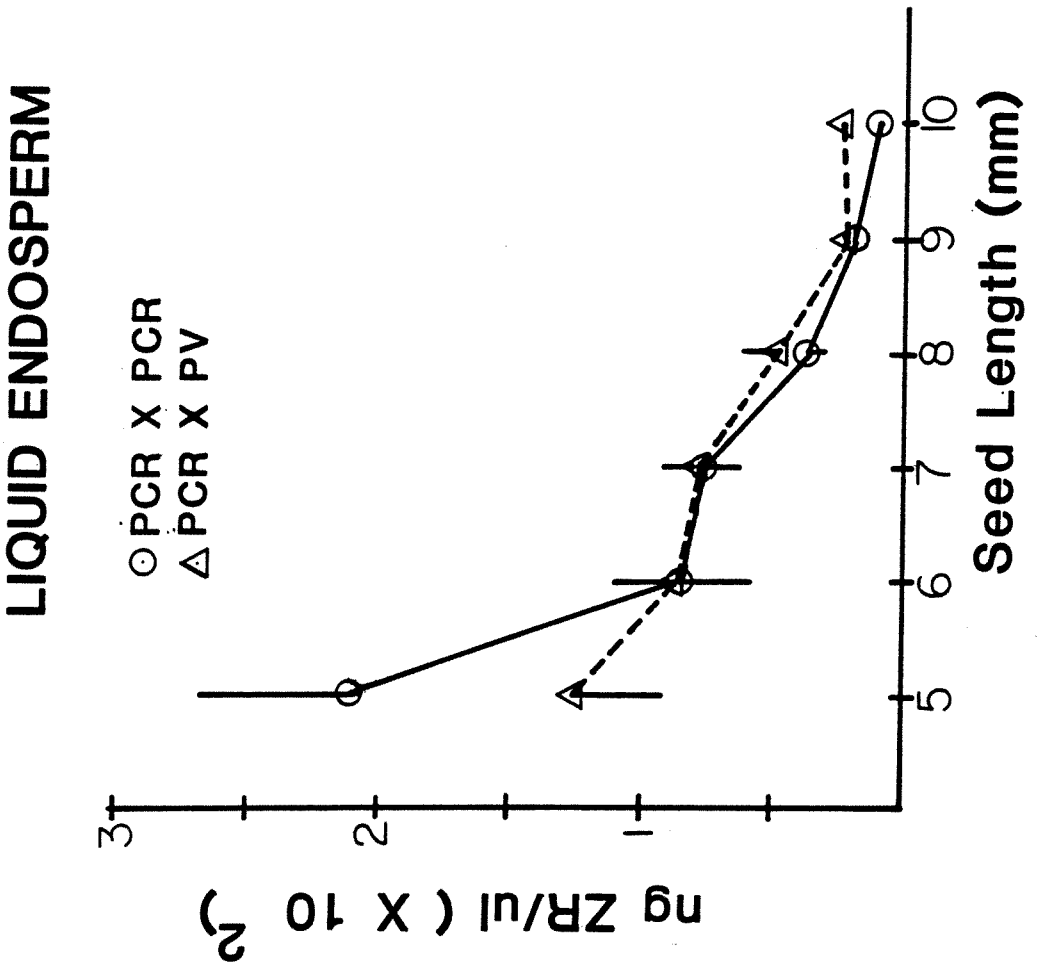
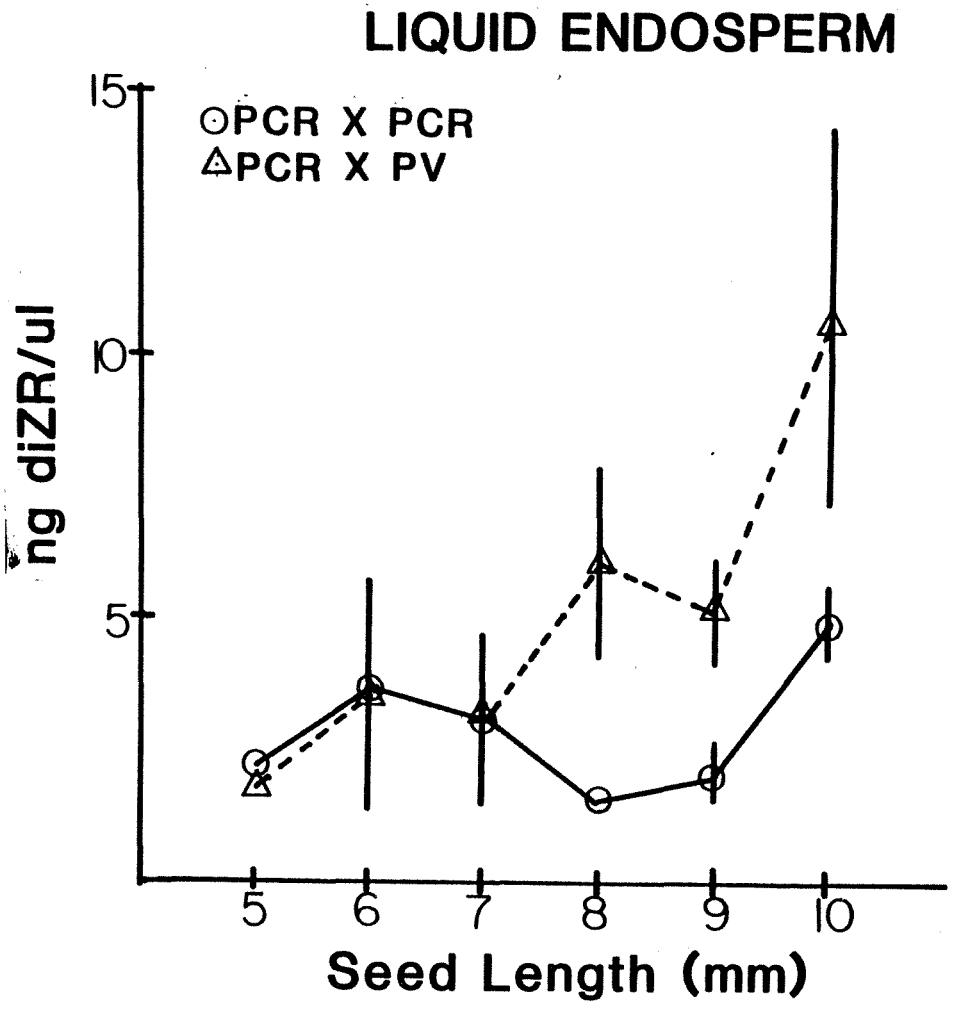

LIQUID ENDOSPERM
⊙PCR X PCR
△PCR X PV
ng ZR/ul ( X 10 2)
3
2
1
5
6
7
8
9
10
Seed Length (mm)

Fig. 3e


LIQUID ENDOSPERM
⊙PCR X PCR
△PCR X PV
ng diZR/ul
15
10
5
5
6
7
8
9
10
Seed Length (mm)

APPENDIX I

The graphs within Appendix I are an extention of the paper on plant growth substances. The data is the same as for the plant growth substances in the paper, however, it is expressed as the compound/tissue type or the compound/seed. It is interesting to note that there is no difference in concentration of the compounds when expressed on a whole seed basis. The interpretation of the other graphs is more difficult.

FIGURE LEGANDS

Fig. 1: Concentration of ABA in the seeds of the self (PCR x PCR) and the interspecific (PCR X PV).

Fig. 2: Concentration of IAA in the seeds of the self (PCR x PCR) and the interspecific (PCR X PV).

Fig 3: Concentration of ZR in the seeds of the self (PCR x PCR) and the interspecific (PCR X PV).

Fig 4: Concentration of diZR in the seeds of the self (PCR x PCR) and the interspecific (PCR X PV).

Fig. 5: Concentration of ABA in the embryo of the self (PCR X PCR) and the interspecific (PCR X PV).

Fig. 6: Concentration of IAA in the embryo of the self (PCR X PCR) and the interspecific (PCR X PV).

Fig. 7: Concentration of ZR in the embryo of the self (PCR X PCR) and the interspecific (PCR X PV).

Fig. 8: Concentration of diZR in the embryo of the self (PCR X PCR) and the interspecific (PCR X PV).

Fig. 9: Concentration of ABA in the seed coat of the self (PC X PC) and the interspecific (PC X PV).

Fig. 10: Concentration of IAA in the seed coat of the self (PC X PC) and the interspecific (PC X PV).

Fig. 11: Concentration of ZR in the seed coat of the self (PC X PC) and the interspecific (PC X PV).

Fig. 12: Concentratioin of diZR in the seed coat of the self (PC X PC) and the interspecific (PC X PV).

Fig. 13: Concentration of ABA in the liquid endosperm of the self (PC X PC) and the interspecific (PC X PV).

Fig. 14: Concentration of IAA in the liquid endosperm of the self (PC X PC) and the interspecific (PC X PV).

Fig. 15: Concentration of ZR in the liquid endosperm of the self (PC X PC) and the interspecific (PC X PV).

Fig. 16: Concentration of diZR in the liquid endosperm of the self (PC X PC) and the interspecific (PC X PV).

Fig. 1

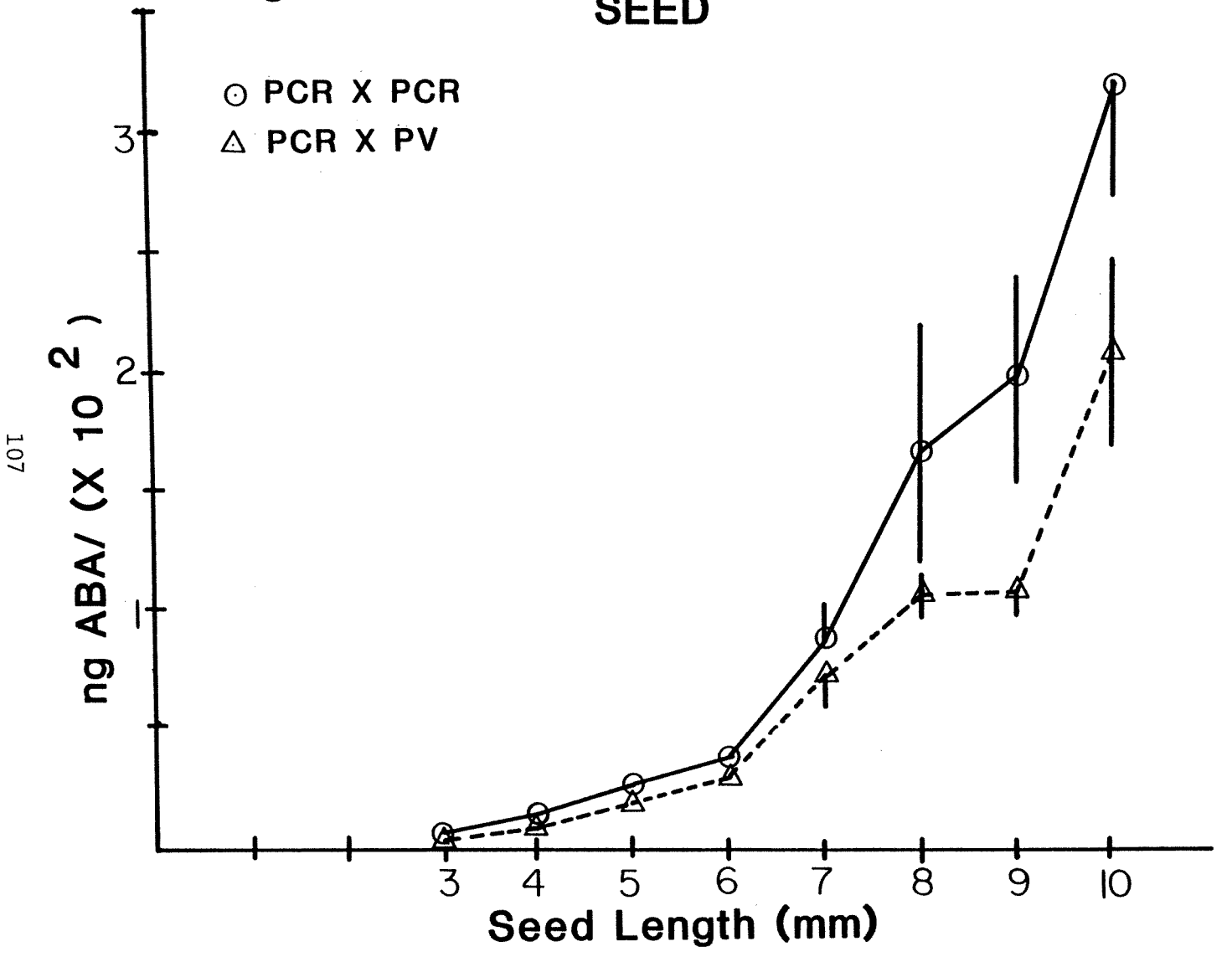

SEED
⊙ PCR X PCR
△ PCR X PV
3
2
1
ng ABA/ (X 10 2 )
3
4
5
6
7
8
9
10
Seed Length (mm)

Fig. 2

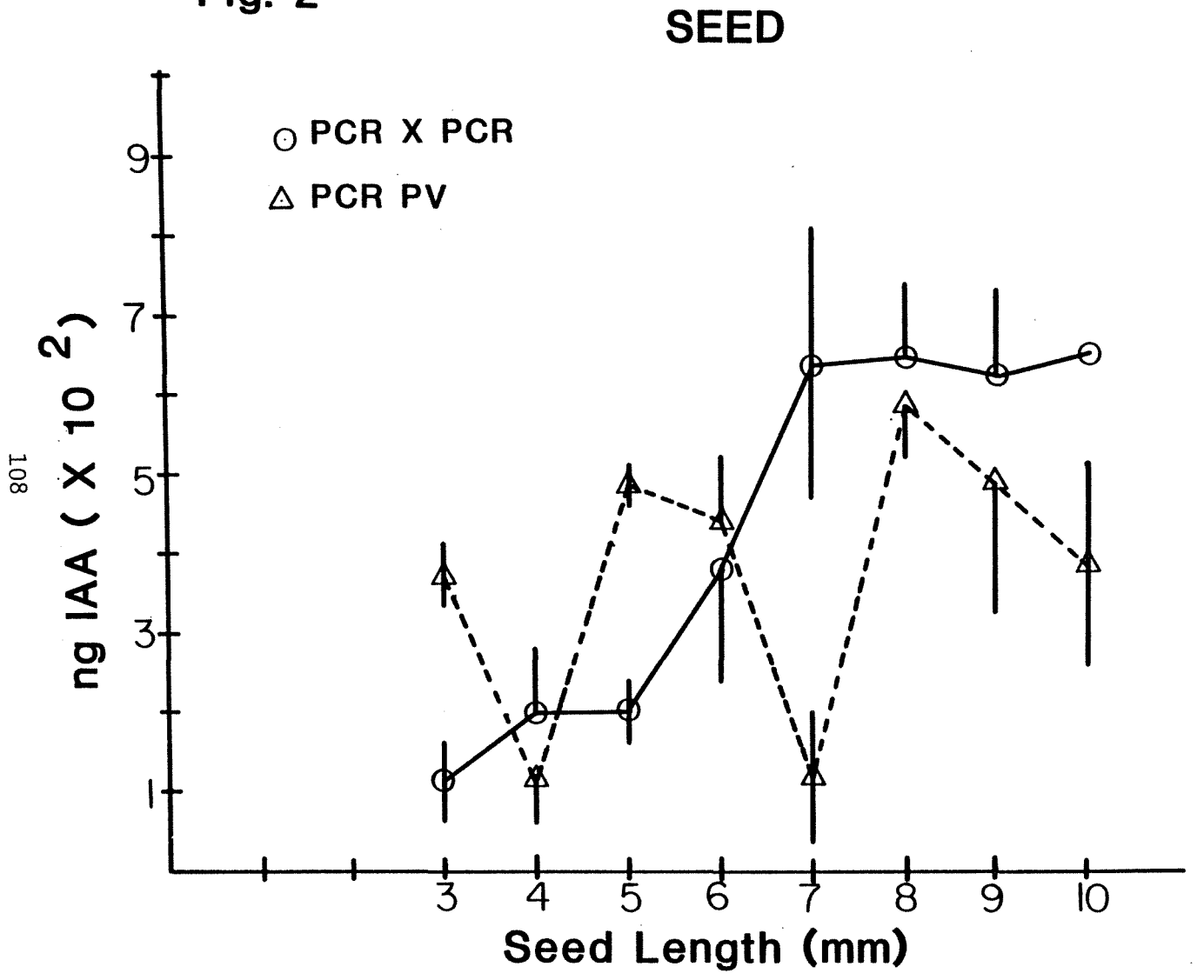

SEED
PCR X PCR
PCR PV
ng IAA ( X 10 2)
108
9
7
5
3
1
3
4
5
6
7
8
9
10
Seed Length (mm)

Fig. 3

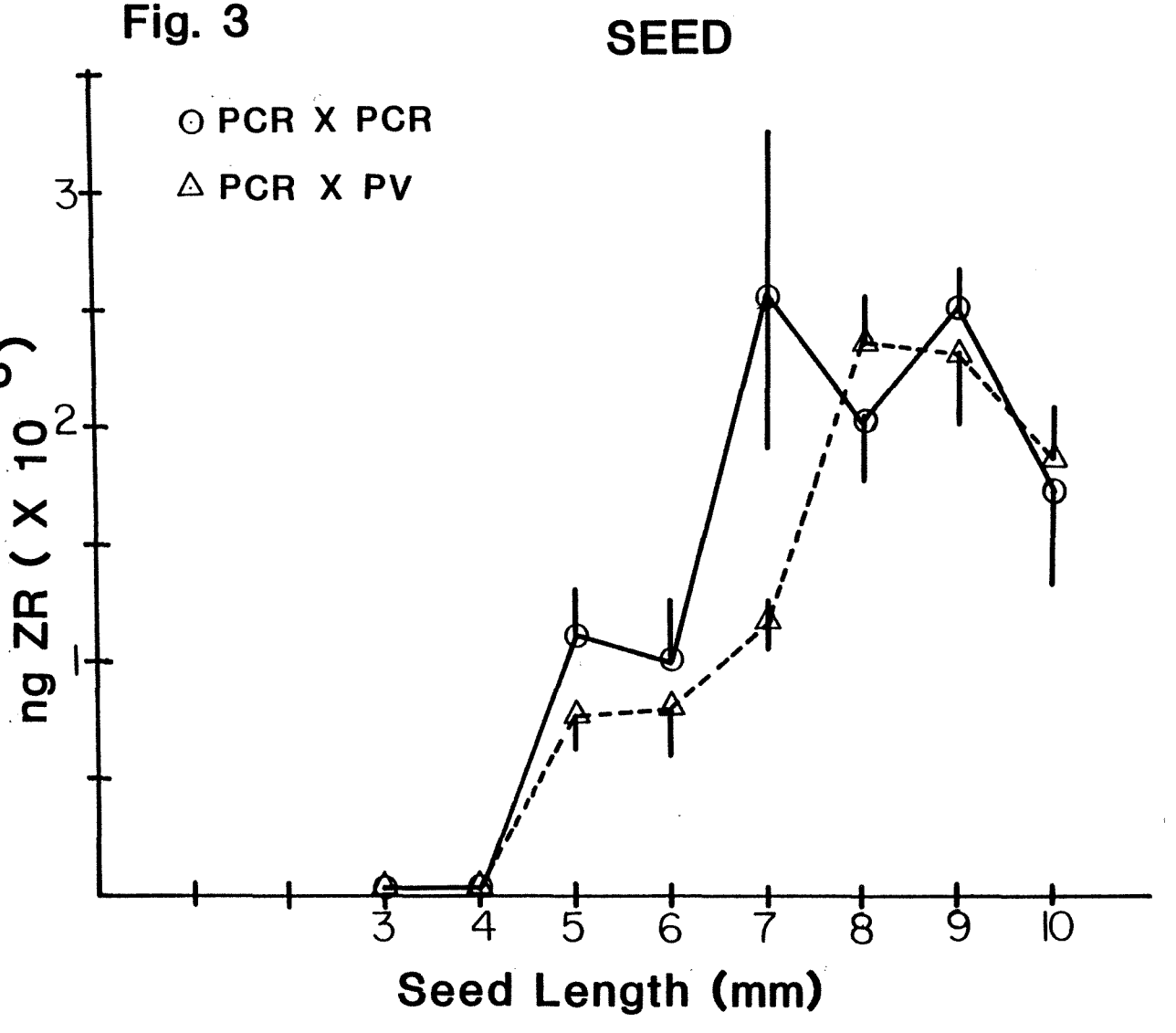


Fig. 4

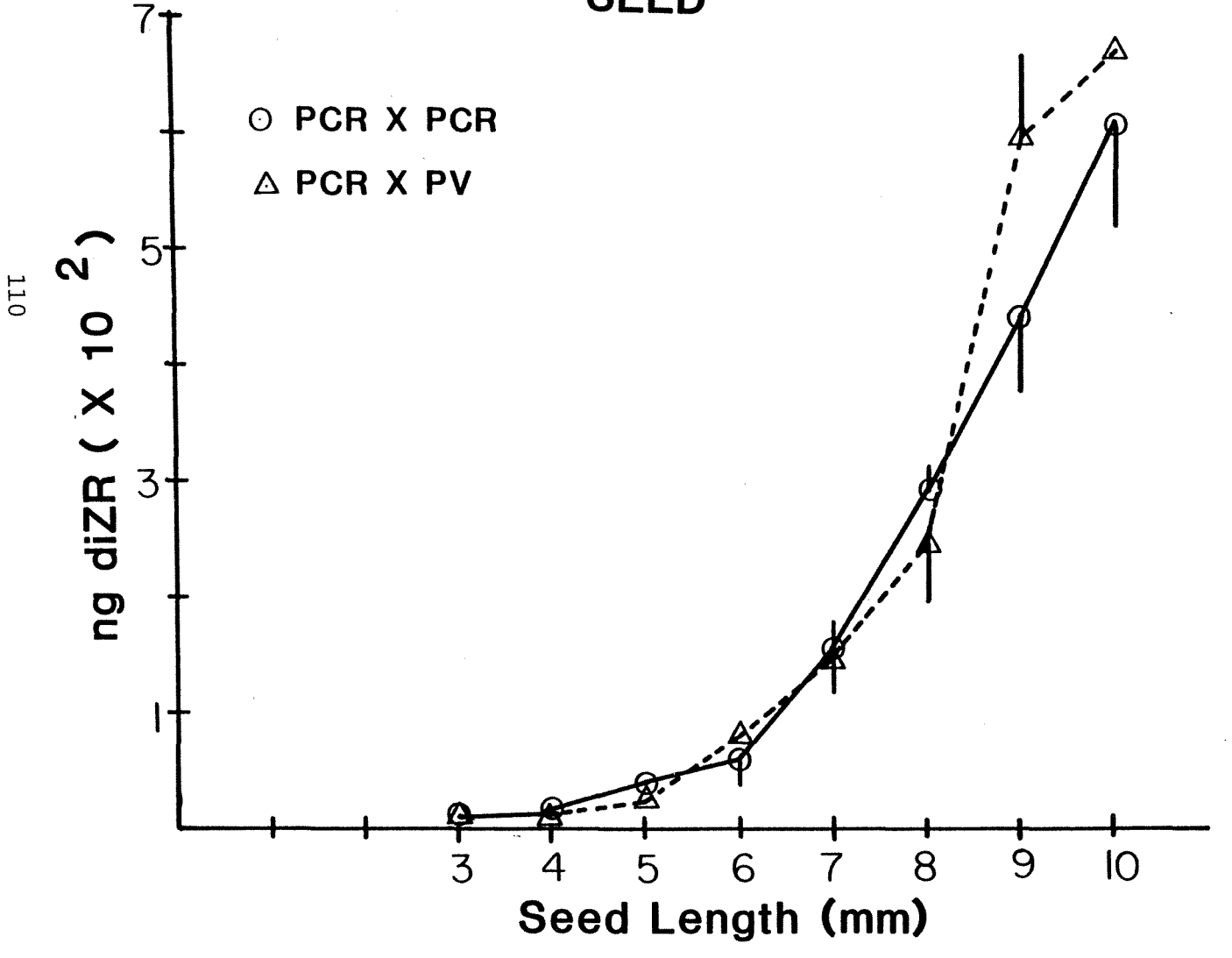

SEED
PCR X PCR
PCR X PV
ng diZR ( X 10 2)
7
5
3
1
3
4
5
6
7
8
9
10
Seed Length (mm)

Fig. 5

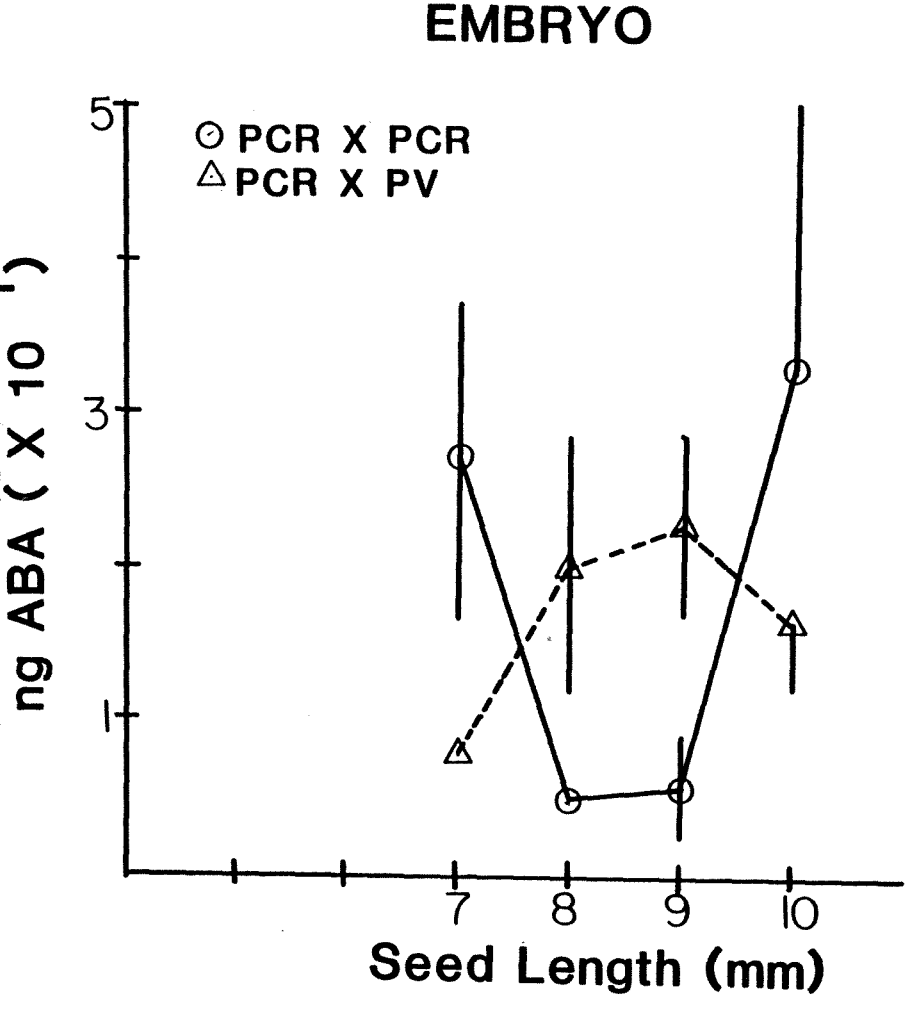


Fig. 6

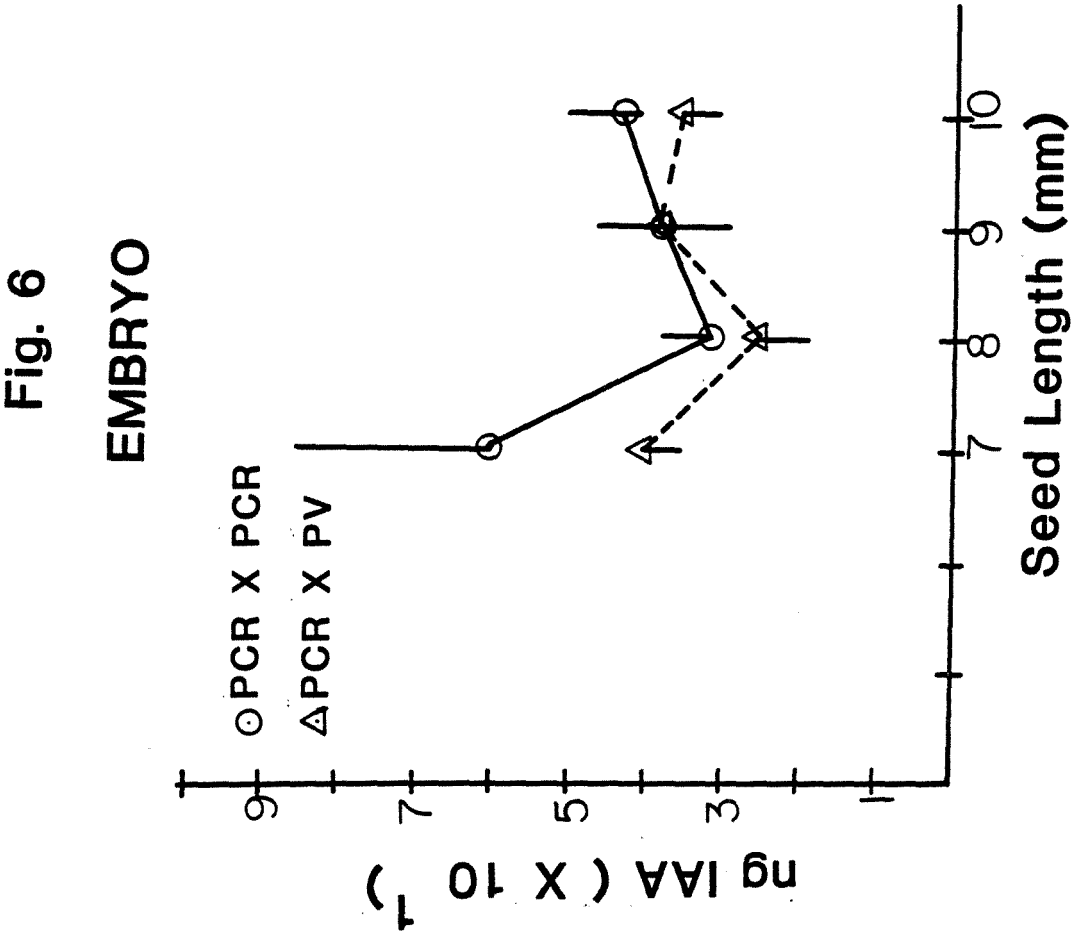

EMBRYO
⊙PCR X PCR
△PCR X PV
ng IAA ( X 10 -1 )
9
7
5
3
1
7
8
9
10
Seed Length (mm)

Fig. 7

EMBRYO

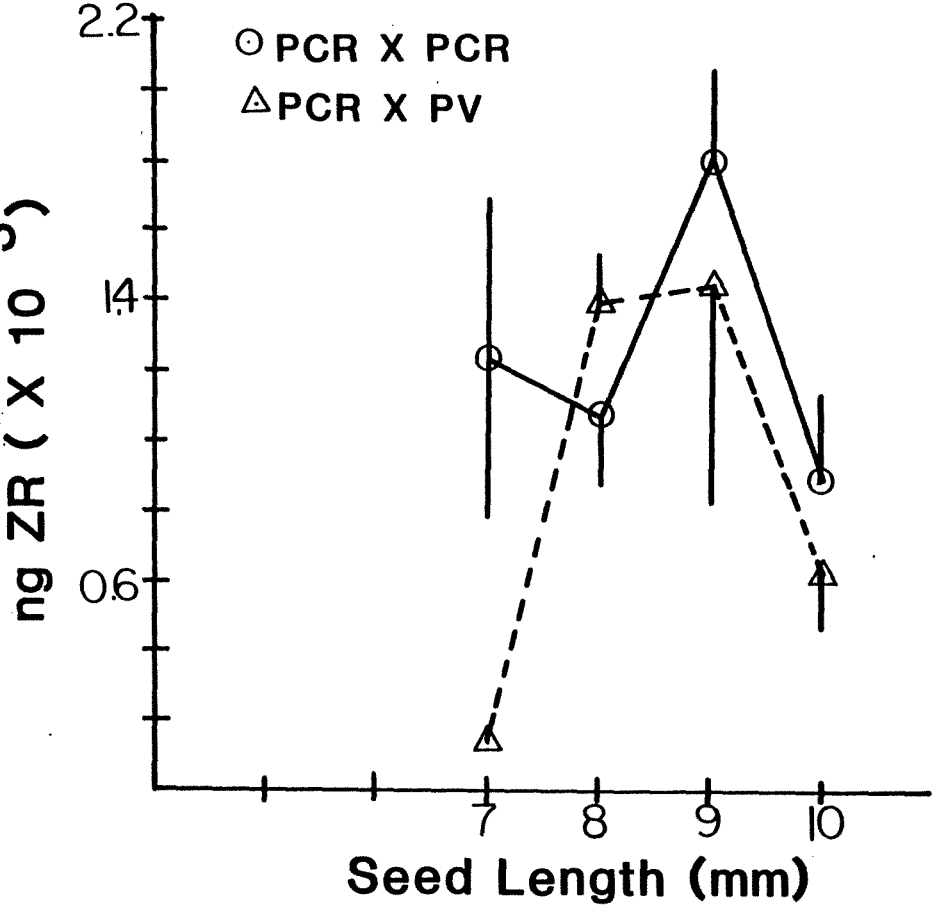


Fig. 8

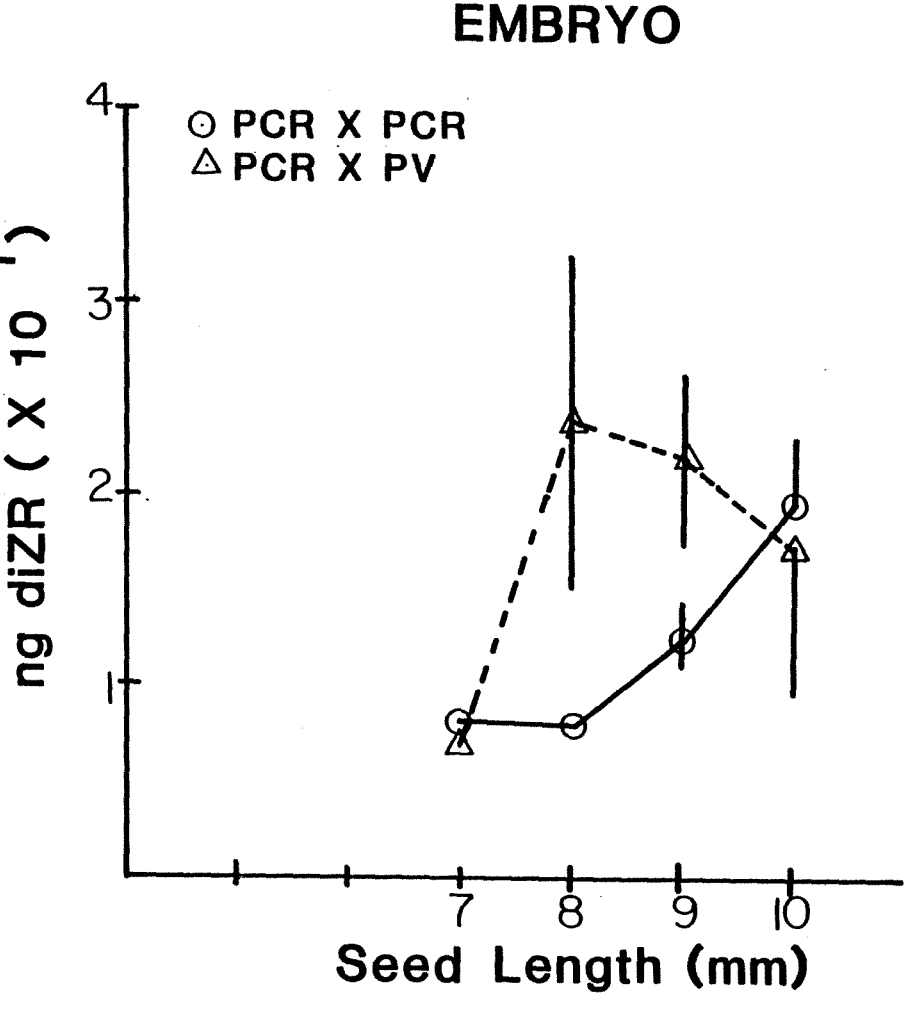

EMBRYO
PCR X PCR
PCR X PV
ng diZR ( X 10 1 )
4
3
2
1
7
8
9
10
Seed Length (mm)

Fig. 9

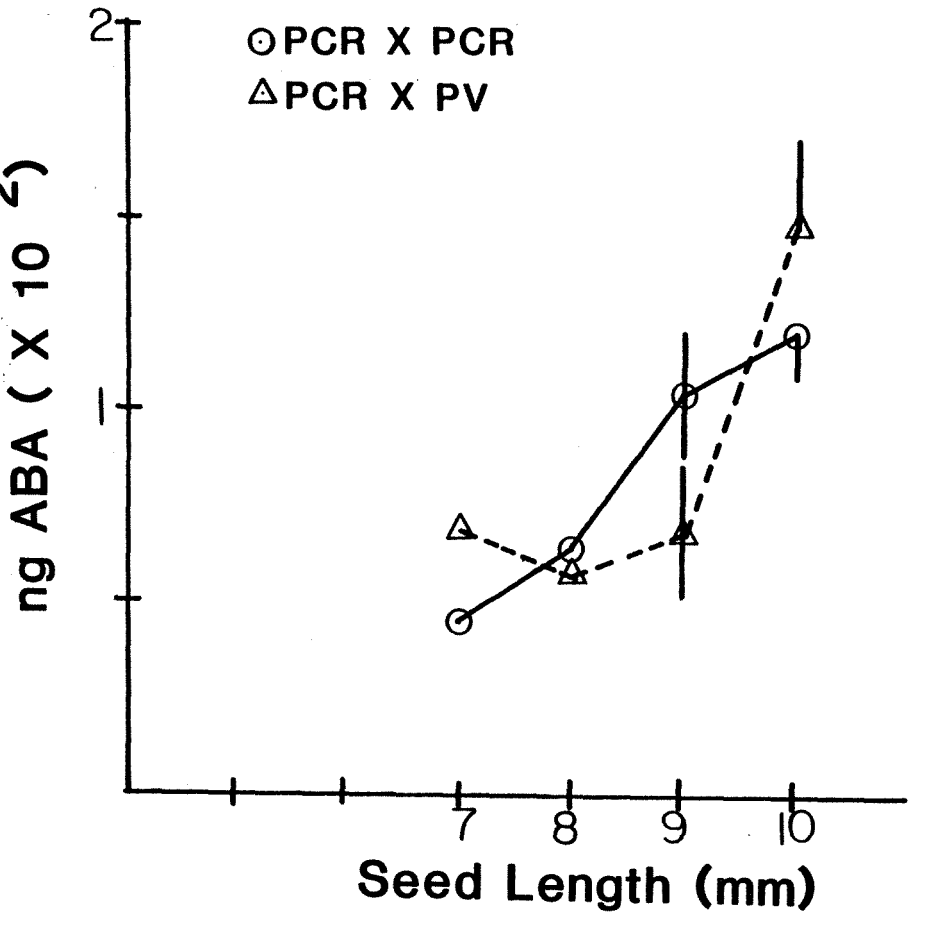


Fig. 10

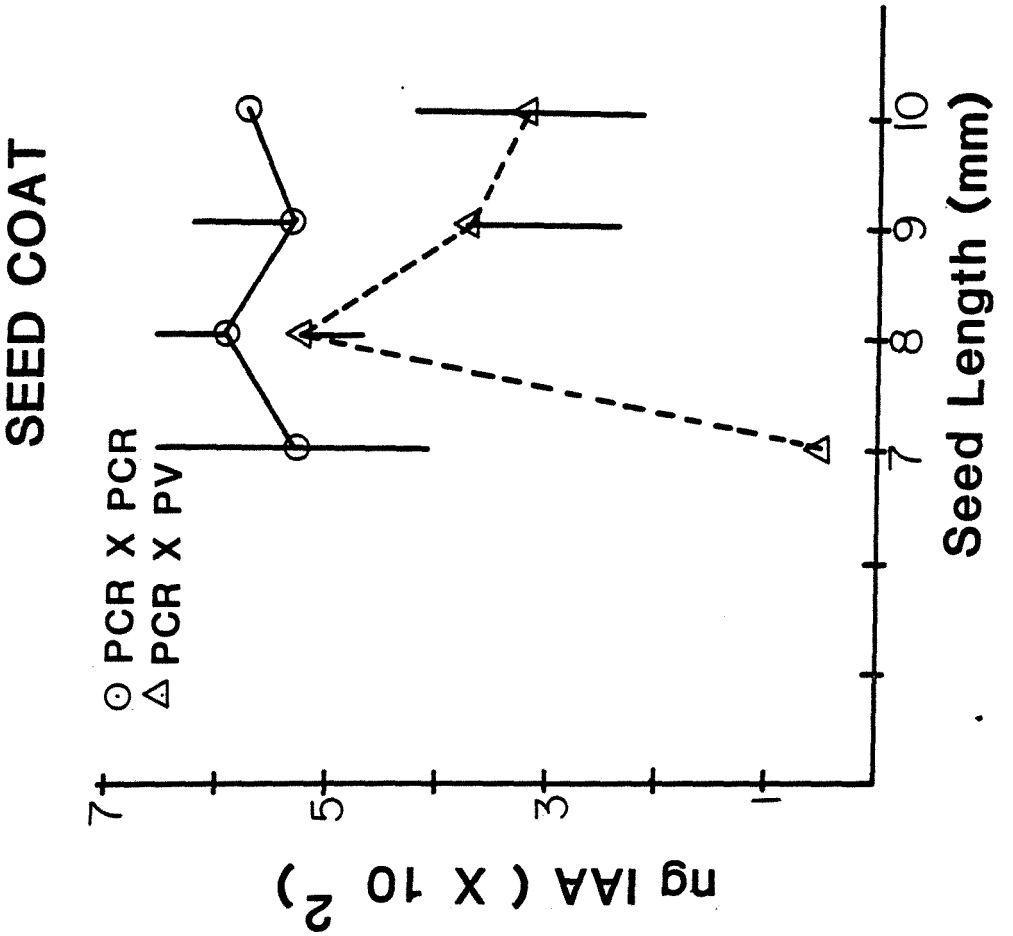

SEED COAT
⊙ PCR X PCR
△ PCR X PV
ng IAA ( X 10 2)
7
5
3
1
7
8
9
10
Seed Length (mm)

Fig. 11

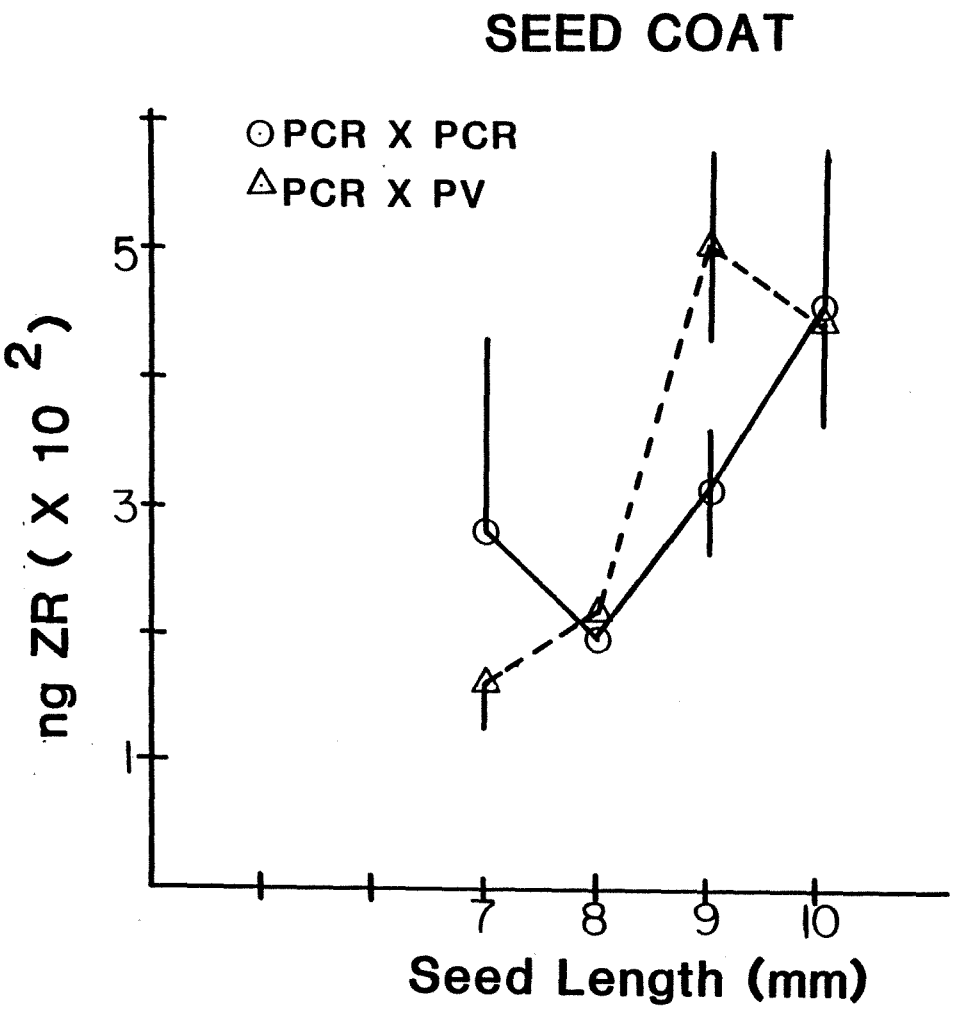


Fig. 12

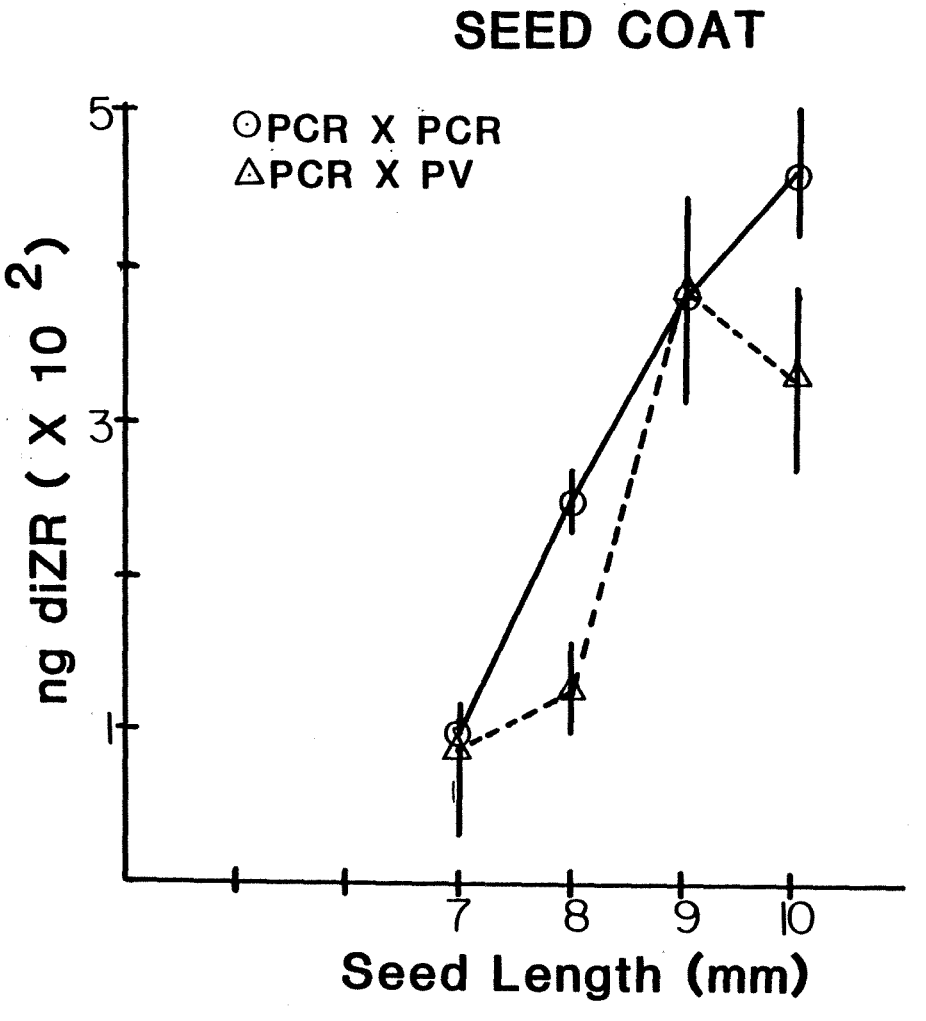

SEED COAT
⊙PCR X PCR
△PCR X PV
ng diZR ( X 10 2)
5
3
1
7
8
9
10
Seed Length (mm)

Fig. 13

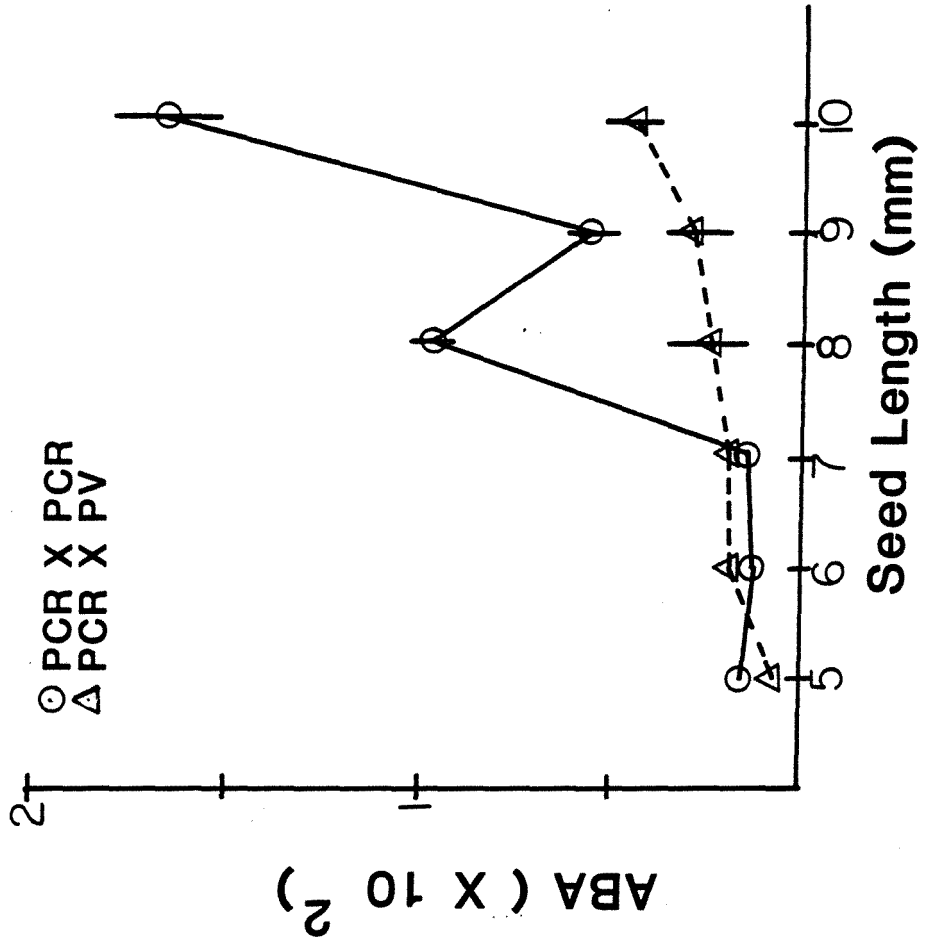

LIQUID ENDOSPERM
⊙ PCR X PCR
△ PCR X PV
ABA ( X 10 2)
Seed Length (mm)
2
5
6
7
8
9
10

Fig. 14

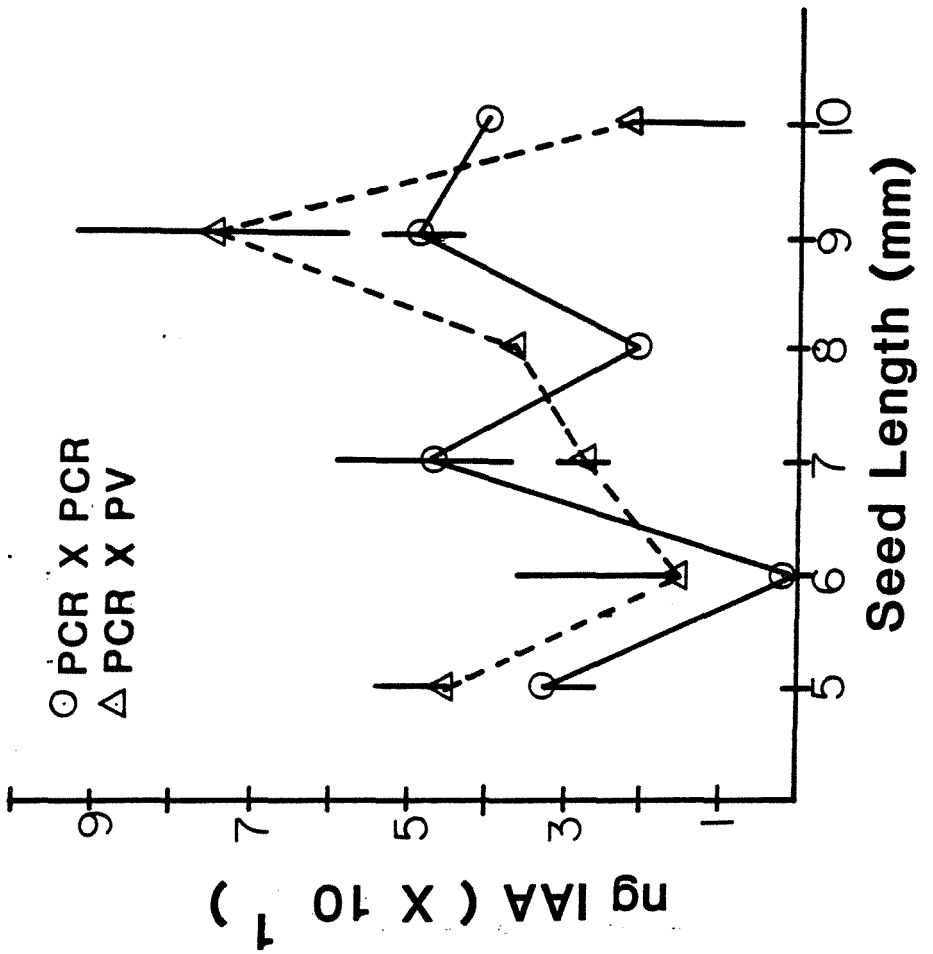

LIQUID ENDOSPERM
⊙ PCR X PCR
△ PCR X PV
ng IAA ( X 10 1 )
1
3
5
7
9
5
6
7
8
9
10
Seed Length (mm)

Fig. 15

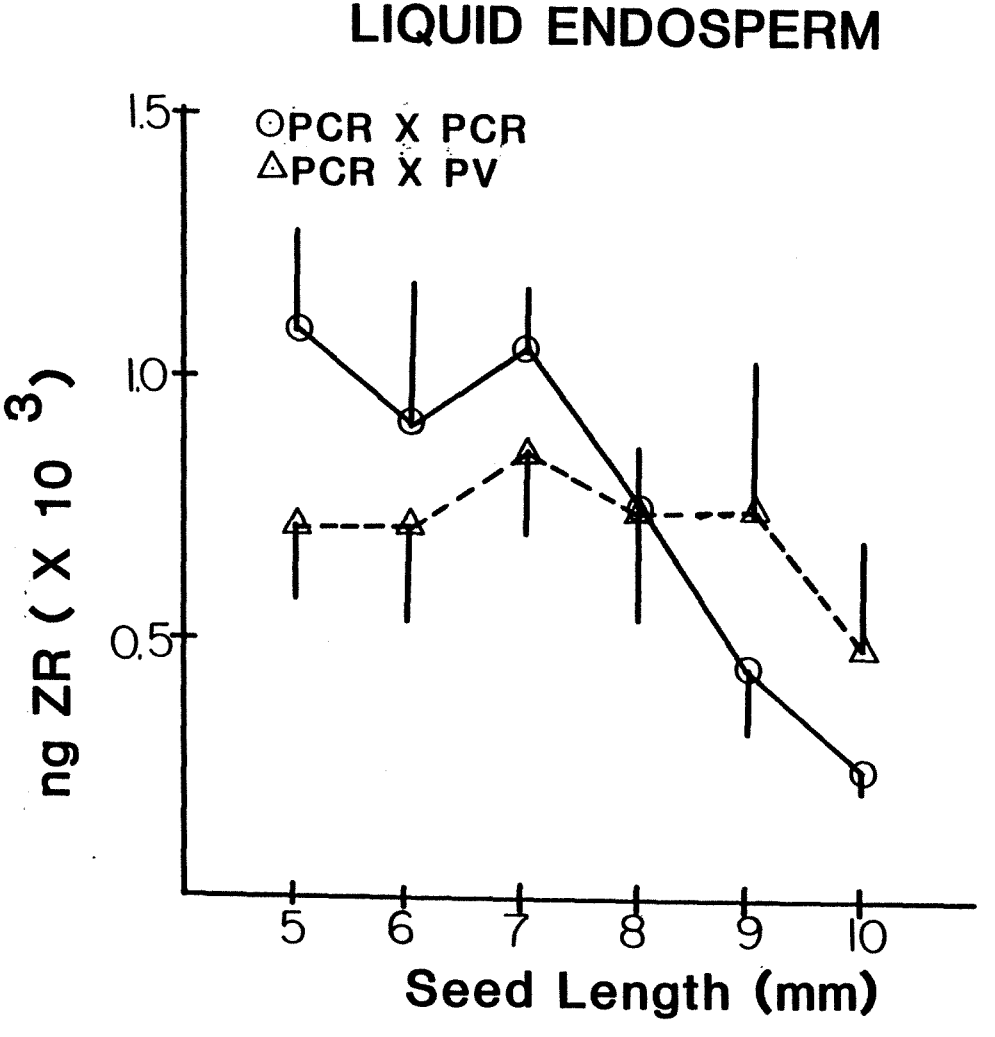
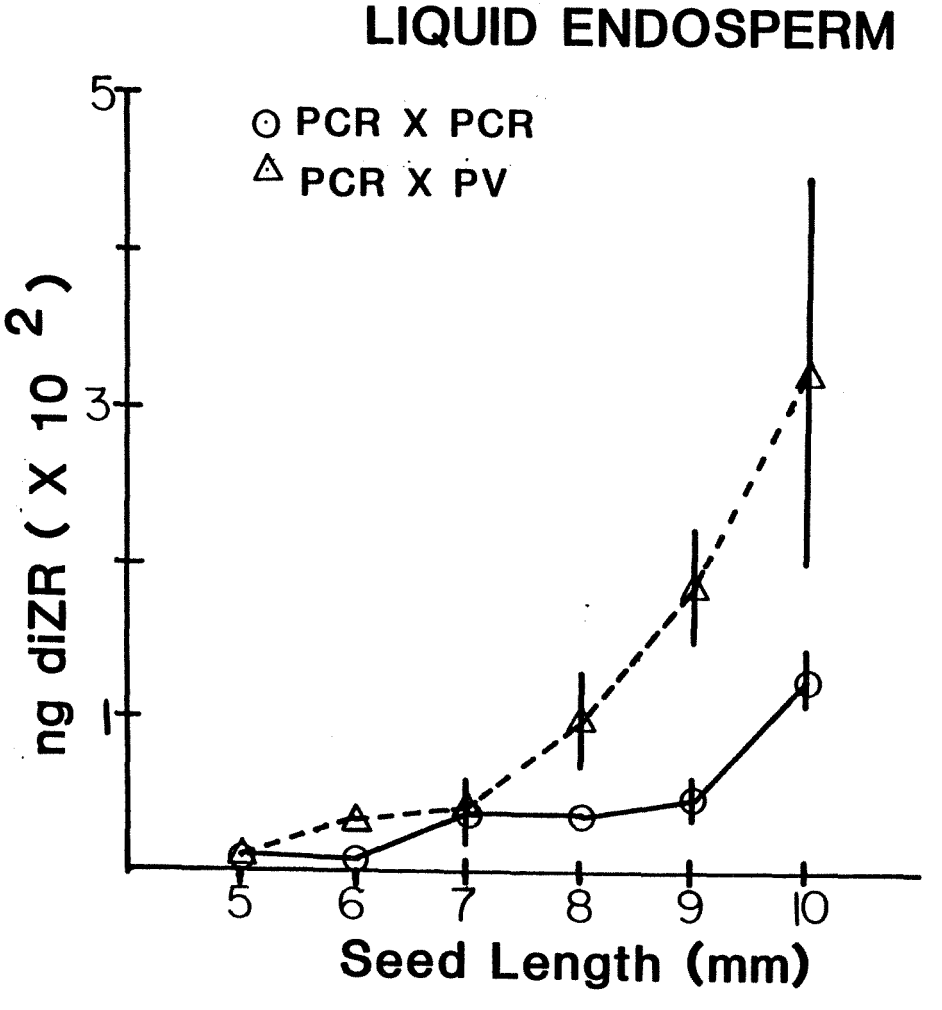

LIQUID ENDOSPERM
⊙PCR X PCR
△PCR X PV
1.5
1.0
0.5
ng ZR ( X 10 3)
5
6
7
8
9
10
Seed Length (mm)

Fig. 16


LIQUID ENDOSPERM
PCR X PCR
PCR X PV
ng diZR ( X 10 2 )
5
3
1
5
6
7
8
9
10
Seed Length (mm)