Effects of Indole-3-Butyric Acid on the Germination and Growth of *Glycine Max* (L.) Merr.

by

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ABSTRACT

Effects of Indole-3-Butyric Acid on the Germination and Growth of *Glycine max* (L.) Merr.

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The impact of indole-3-butyric acid (IBA) on the germination of pella '86' soybean (*Glycine max* (L.) Merr.) was studied. Indole-3-butyric acid treatment had an inhibitory or stimulatory effect on germination depending on the concentration of the solution used. It was found that with no pretreatment (soaking), IBA could stimulate germination at a range of concentrations of about 0.001% - 0.0001%. On the other hand, treatment at concentrations above this range will inhibit germination; for example, using a 1% solution totally inhibited germination. Treatment of the seeds with hormex

or lowering the concentration of indole-3-butyric acid below the optimum range had no (statistically significant) effect on germination. Tests showed that the inhibitory effects of 0.01% - 1% indole-3-butyric acid could be reversed if a non-indole-3-butyric acid solution with the same concentrations was used.

Soaking time had an inhibitory effect on germination, and percentage germination was inversely proportional to the soaking hours. Soaking the seeds in a .0001% solution of indole-3-butyric acid for 1, 2, 4, 12, and 24 hours reduced percentage germination from 62.5 (no soaking) to 28.1, 18.75, 15.62 and 4.7 respectively.

Indole-3-butyric acid treatment at a concentration of 0.0001% resulted in a few roots which rapidly became long and established fibrous root systems. Treatments at higher concentrations (.01% and 0.001%) on the other hand, produced a very dense, thick and bushy root system. However, as the concentration of IBA in the solution was reduced, the root system gradually changed to that developed under distilled water (control) treatment.

The impact of treatment with Hormex on germination was found to be statistically insignificant. However, only tests using Hormex provided information on amount of indole-3-butyric acid absorbed by the root system. Tests show that indole-3-butyric acid absorbed by the roots ranged from 21.68

to 2.83×10^{-3} mg of IBA per one gm of wet plant tissue.

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CHAPTER I

INTRODUCTION

Seed Germination:

While a seed is said to have germinated when the radical bursts through the outer coverings, at about the same time that cell division and elongation begins, these events are considered as the terminal period of germination (Toole, et al, 1956). However, this initial phase represents only part of what happens in seed germination. To define germination more completely, it is natural to include the processes which take place to insure the establishment of the seedling. A second phase of germination concerns the development of the seedling. These two phases can be separated physiologically because during phase one, a seed can still be desiccated and survive, but if dehydration occurs in phase two, it is killed (Berrie and Drennan, 1971; Sen and Osborne, 1974). In this study a seed is considered germinated when the cotyledons are completely open.

Germination can be considered complete when all the seed's available food reserve has been consumed and the seedling is capable of independent existence (Bradbeer, 1988).

It is the number of seeds which complete germination successfully that is of greatest importance to both plant scientists and those concerned with seed technology.

Germination inhibitors:

A very large number of substances are known to inhibit germination. All those compounds which are toxic to living organism will also, at toxic concentrations, prevent germination, simply by killing the seed. Some substances can prevent germination without affecting the seeds irreversibly. The simplest of cases, and one perhaps most frequently met within nature, is that of osmotic inhibition. It is possible to prevent the germination of seeds simply by placing them in a solution of high osmotic pressure. When the seeds are removed from such an environment and placed in water they can then germinate (Mayer and Poljakoff-Mayber, 1982).

Another type of inhibition is that caused by substances which are known to interfere with certain metabolic pathways. As germination is closely associated with active metabolism, all compounds which interfere with normal metabolism are likely to inhibit germination. An example of this type of interference is provided by various respiratory inhibitors. Compounds such as cyanide, dinitrophenol, azide fluoride, hydroxylamine, and others could inhibit germination. In some

cases these compounds may increase the light requirement of some seeds.

Herbicides of various kinds inhibit germination to a greater or lesser extent. Many of the commonly used substances such as 2,4-Dichlorophenoxyacetic Acid (2,4D) affect germination at comparatively low concentrations. These herbicides have been frequently used in order to prevent germination of weed seeds in agricultural crops.

Phenolic compounds of various kinds inhibit germination. Some of the selective herbicides, such as the substituted phenols and cresols, inhibit germination due to their general phytotoxicity. All compounds mentioned so far are inhibitory and cannot be regarded as being dormancy inducing.

Another group of inhibitors is concerned with natural compounds present in the seed. These compounds are often the cause of dormancy and usually act by blocking some process essential to germination. These inhibitors, however, do not reduce the viability of the seed nor do they produce any growth abnormalities in the seedling after germination. Natural inhibitors may be found in any part of a seed, including the structure covering the seed (e.g., the glumes of oat grain contain an inhibitor). This type of inhibitor has been found in pulp or juice of the fruit containing the seed, in the seed coat, in the endosperm and in the embryo. Evenari (1949) found their presence common and widespread among

plants. Some of the natural germination inhibitors that have been identified are: coumarin, parascorbic acid, ammonia, phthalids, ferulic acid and abscisic acid (ABA). There are also numerous inhibitors that include cyanide releasing, ammonia releasing substances, phenolic compounds, alkaloids, organic acids and oils. The most important natural inhibitor is abscisic acid. At very low concentration, between five and ten parts per million-ABA will completely inhibit the germination of lettuce seeds. Sankhla and Sankhla (1968) demonstrated that ABA inhibition of lettuce seed germination can be entirely counteracted by concentrations of kinetin as low as one part per million. However, in the same study, counteraction by gibberlic acid of the abscisic acid effect could not be demonstrated. Both kinetin and gibberlic acid are well known for their stimulatory effect on lettuce seed germination.

Germination Stimulators:

A number of chemical substances can stimulate germination. These substances are varied in chemical nature. Some of them are simple compounds such as potassium nitrate and thiourea, while others are complicated molecules such as hormones, gibberlins and cytokinins. Potassium nitrate promotes germination of a number of seeds, e.g. *lepidium virginicum* (pepper grass seeds). Its effect is dependent on

its concentration. As with light or sunlight, potassium nitrate stimulation (at low concentration) shows interaction with temperature. The germination of many seeds is stimulated by thiourea in the absence of light. Similar to potassium nitrate, the degree of stimulation depends on the concentration of the chemical and temperature, e.g. for lettuce seeds, the effective concentration is of the order 10^{-2} to 10^{-3} M. These concentrations apply if the seeds are actually germinated in the solution of thiourea. A frequently adapted procedure is soaking the seeds in a concentrated solution of thiourea (0.6-4 x 10^{-2} M) for a short period of time and then transferring them into water. The effect of thiourea on the germination of lettuce was first described by Thompson and Kosar (1939) and its interaction with light was studied by Evenari (1954) both in lettuce and endive. Thiourea abolished the inhibitory effect of temperature; in lettuce it also abolished the light requirement of the seed. In addition to stimulating germination, thiourea inhibits growth. Prolonged treatment of seeds with high concentrations of thiourea is therefore liable to cause an apparent inhibition of germination, because the emergence of the root is prevented (Poljakoff-Mayber, 1958). Villiers and Wareing (1960) found that thiourea also substitutes for the natural germination stimulator which develops in *Fraxinus* (ash tree) seeds during chilling.

Hormones:

The term hormone is used here in the widest sense of the word, including various growth substances and natural compounds having a regulatory function in the plant. Kahn (1956) was among the first to show that gibberlic acid stimulates the germination of *lactuca sativa* (lettuce seeds) in the dark. In these cases gibberlic acid substituted for light. However, in a number of seeds whose germination is either promoted or inhibited by light, treatment with gibberlic acid was not effective in promoting germination.

Although the effect of gibberlic acid seems to be similar to that of light, gibberlic acid is far more effective than red light in reversing the high temperature inhibition of germination in lettuce. Several experiments have demonstrated that gibberlic acid and red light act partially in the same way but that their mode of action is not identical.

Origin of the gibberlic acid is not the same in different seeds, e.g. in peas, the gibberlins are released from a bound form in the cotyledons and move to the embryonic axis. On the other hand in barely gibberlic acid like substances are formed in the scutellum and then move out of the embryo into the aleuron layer and the endosperm. The level of gibberlin changes during seed development and shows fluctuations so that it is difficult to relate development directly to a given level of gibberlic acid.

Another plant hormone, cytokinin, also affects germination of seeds. Miller (1958) showed that kinetin promotes the germination of the lettuce seeds. The cytokinins are actively metabolized in germinating seeds. A large number of derivatives of kinetin also stimulate germination, e.g. benzyladenine. Weiss (1960) showed that, contrary to the original idea that kinetin substitutes for red light in germination, seeds are in fact sensitized by kinetin so that a smaller dose of light would induce their germination. In addition to their interaction with light, cytokinins also interact with other exogenously applied compounds, such as abscisic acid and gibberlic acid, and also with temperature. As in the case of abscisic acid and gibberlic acid, the correlation between the breaking of dormancy and the rise of level of cytokinins is not always clear (Thomas 1977).

The rise in cytokinins sometimes (not always) coincides with the increase in the ability of seeds to germinate after, e.g. chilling. Like gibberlic acid, cytokinins can interact with both light and/or temperature. The interaction of hormones with light, temperature and also with osmotic stress have not allowed investigators to answer the important questions: "Just when do they act and what is their function in dormancy/germination of seeds?"

The effect of hormones of the indolylacetic acid (IAA) type on germination has long been in dispute. Numerous

studies (Radley, 1979; Tillberg, 1977; Soeding 1975) on the effect of IAA and similar substances on the germination of a variety of seeds have produced conflicting results - stimulation or inhibition - depending on the concentration of IAA and the type of seeds used. However, the most general effect is an absence of response of the seeds to physiological concentration of IAA. (Mayer and Poljakoff-Mayber, 1982).

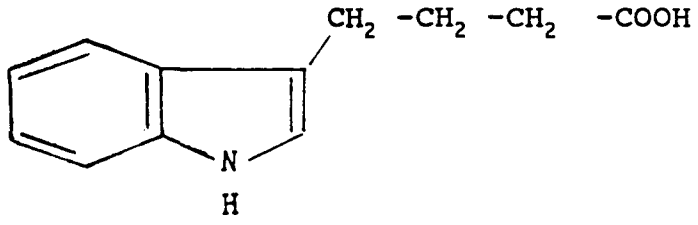
Ethylene is another plant hormone which can induce germination in some types of seeds. This stimulatory effect is more general, especially in seeds showing some degree of dormancy (Ketring, 1977). Ethylene is effective when applied exogenously at low concentration. Ethylene can be applied to seeds as 2-chloroethane phosphoric acid which liberates ethylene in the presence of water above pH 4.0. Ethylene chlorhydrin can in some cases also stimulate germination in a manner similar to thiourea, but it is less effective than thiourea. It is found that ethylene can affect the level of other hormones and its production can be affected by them (Lieberman, 1979).

It is clear that germination of seeds is controlled by a variety of external and internal factors. These factors include, in addition to simple environmental conditions, the presence of external and internal germination-inhibiting and stimulating substances. One of the controlling factors is the balance between stimulatory and inhibitory concentration of

compounds at their site of action.

Indole-3-butyric acid (IBA):

Auxin is a generic term for compounds characterized by their capacity to induce elongation in shoot cells. Auxins resemble indole-3-acetic acid (IAA) in physiological action. They may, and generally do, affect processes besides elongation, but elongation is considered critical. They are generally acids with an unsaturated cyclic nucleus or derivatives of such acids (Devlin, et al, 1983). The subject of the effect of auxins in the stimulating adventitious root formation has been studied by a number of researchers and results have been well documented. Indole-3 butyric acid, with chemical formula, is the most widely used auxin for



Chemical structure of IBA

rooting of plant cuttings. It is believed that the high rooting ability of IBA results from its high stability in the plant (Wiesman, et al, 1989). Wiesman, et al, (1989) demonstrated that IBA is metabolized very rapidly during rooting of mung bean cuttings, at a rate similar to indole-3

acetic acid (IAA) which has a low rooting capability. But only a few studies have investigated the IBA metabolism in plants. Andreae and Good (1957) reported that plants treated with IBA accumulated substances which were identified as indolbutyramide and indole-3 acetylasparatic acid (IAASP). These substances formed following application of IBA, and play an important role in rooting by slowly releasing free IBA. The uptake and metabolism of IBA (and IAA) have been studied in suspension cell culture of some plants (Epstein, et al, 1993). Ethylene treatment can speed up transport and metabolism of IBA in some plants. Epstein and Sagee (1992) studied the effect of ethylene treatment on transport and metabolism of IBA in citrus leaf midribs and found that exogenous IBA was metabolized by the midribs to a polar compound and probably an ester conjugate. Ethylene pretreatment of the midribs reduced their capacity to metabolize IBA by 70% as compared to air pretreatment.

Soybean (*Glycine max* (L.) Merr.):

The soybean (*Glycine max* (L.) Merr.) is believed to be among the oldest plants cultivated by man. Soybeans were mentioned in Chinese literature as early as 2838 B.C. It is believed they were grown extensively in China and Manchuria centuries earlier. Prior to World War II, China was the

leading grower of soybean. However, by 1946, the United States of America was producing more than any other country in the world. Today the United States is the world's largest producer and exporter of soybeans.

Glycine max (L.) Merr. is an upright, branching, and herbaceous annual that can range in height from one to six feet. There are many types of soybeans with different seed sizes and colors. Seed colors range from white yellow through olive green to black, with the cotyledons either green or yellow. Edible types have large seeds, light in color with good flavor. Stems are normally round, often hairy, and varying in color depending on type of soybean. Leaves are variable in shape, hairy in some varieties, and normally trifoliate. The most common color is dark green. Flowers are small and are borne in clusters in the axile of the leaves. They are self-pollinating and purple or white in color. Fruit is normally a short, hairy pod which can vary from 2-10 cm in length and 2-4 cm in width. The number of seeds per pod ranges from one to five with two to three the usual number. The root system is extensive, with a tap-root which may exceed 1.5 m in length, giving rise to many lateral branches.

Soybeans are grown in rotation with other crops. Depending on location and climate conditions, the rotation period varies from one to four years. The effect of soybeans

on the other crops in the rotation is significant.

With the proliferation of breeding programs directed to soybean, and consequent production of cultivars adapted to various environments, the range of soybean is now virtually world-wide. The plant is tolerant of a wide range of soil conditions, with the highest yield obtained on well-drained fertile loams. Highly compacted soils usually produce short plants with restricted root development.

Glycine max (L.) Merr. is highly photoperiodic (day length), and this is a major constraint on selection, since a variation of 15 minutes in day-length may be sufficient to inhibit flowering in a specific variety. Temperature is another important factor in soybean growth. However, plants appear to be generally unaffected by a fairly wide variation in day temperatures prior to flowering.

Pre-planting cultivation which is necessary for cotton, maize or beans is also suitable for soybean. However, on heavy clay soils and those with hardpan, deeper cultivation is necessary. Good quality seed is uniform in size and weight. Seeds should be sown 3-5 cm deep, and generally as much as 7.5 cm deep. A seed bed temperature of 25-33° C produces optimum emergence, with temperature below 15° C and above 37° C adversely affecting it. The seed bed should be prepared to allow even depth and spacing.

Soybean Varieties:

Plant breeding, the genetic improvement of crops, is one of the reasons for the soybean's successful introduction into North America from its homeland in Eastern Asia. The first soybean breeders in the United States and Canada developed varieties that were adapted to North American soils, climates and production systems. Soybean breeding continues to be a major focus of research which results in the introduction of new varieties into the market. Today a grower can select from many varieties suitable for particular region, soil, climate and production system. A knowledge of characteristics of different types of soybean and the effect of various cultural practices and environmental factors have on production make it possible to select the best varieties for particular production systems and sets of growing conditions. The following factors should be considered when selecting soybean varieties:

- Proper maturity
- Resistance and/or tolerance to phytophthora root rot
- Yield potential
- Lodging score
- Plant type
- Seed size
- Height of pod set

In Ohio these variety characteristics are measured annually for more than 200 varieties, blends and brands and results are reported in the "Ohio Soybean Performance Trails" Agronomy Department each winter (Beuerlein, et al, 1987).

Proper maturity is the most important factor to consider in variety selection. When planted at a normal time, soybean varieties adapted to Ohio will develop six to ten trifoliolate leaves before flowering begins. Most varieties are indeterminate types, plants continue to develop new leaves and grow in height for several weeks after flowering begins, and the stem terminates with a vegetative bud. Flowering progresses from lower to upper nodes. A few adapted varieties are determinate types. Growth in height is essentially complete when flowering begins, and flowering is initiated at all nodes at about the same time. The stem terminates with a flowering shoot called a raceme. Determinates are usually 1/2 to 2/3 the height of indeterminates, hence the name "semidwarfs". Semideterminate types also occur; they are intermediate in height.

Flower induction is influenced by photoperiod, temperature and size of plant. The soybean is a short-day plant, flowering is hastened by shorter photoperiods and delayed by longer photoperiods. For any photoperiod, flowering will be hastened by higher temperature and larger plant size. A few genotypes are insensitive to photoperiod

but are influenced by plant size and temperature. Because daylengths are shorter toward the equator in late spring and early summer, a variety will flower and complete its life cycle in fewer days as it is moved toward lower latitudes. Also, its life cycle tends to be hastened by the decreasing daylengths after June 21. Thus, differences in maturity dates tend to be less than differences in planting dates. For this reason, days to maturity is not a reliable predictor of maturity unless planting date is also specified.

To get around this problem, soybean breeders developed a scheme to group varieties according to their maturities relative to a standard adapted variety in each group. Maturity Groups (MG) range from MG 00, adapted to Canada, to MG 10, varieties adapted to Central America and northern South America. In the United States, maturity groups range from MG 0 to MG 8, and in Ohio from MG 1 to MG 4. Most commercial varieties of MG 00 to MG 4 are indeterminate types, whereas those of MG 5 to MG 8 are determinate types. Limitations to this system are that the standard variety in each group changes frequently and the absolute maturity date varies with growing conditions.

Most varieties grown in Ohio are in MG 2 and MG 3, although some MG 1 varieties are grown in northern Ohio and some MG 4 are grown in southern Ohio. The varieties of a maturity group will all mature during a period of eight to ten

days if planted on the same date.

Appendices A and B compare a number of popular varieties of soybean used in Ohio on the basis of relative maturity, lodging score, height, seed quality, seed size and yield. This data, along with other published information (which compares varieties used in Ohio on many bases including resistance and/or tolerance to phytophthora root rot), indicates that with the exception of yield, Pella brands are generally rated inferior to many other brands (Beuerlein, et al, 1987). These factors may explain Pella brand's relative unpopularity with soybean growers.

This study investigated the impact of a specific type of Auxin (indole-3-butyric acid) on the germination and growth of a special variety of soybean pella '86' seeds. This research's primary objectives were to determine the range of concentrations at which IBA might influence germination, the effect of each level of concentration on the root system developed, and the amount of IBA being absorbed by the root system.

In addition, several hypotheses were to be tested concerning the impact of IBA treatment and soaking on growth rate, germination time, and percentage germination of the seeds.

CHAPTER II

MATERIALS AND METHODS

The determination of germination characteristics for a plant species requires the performance of controlled tests in which sample batches of seeds are germinated under different environmental conditions. In this research four distinct sets of experiments were conducted to study the impact of the auxin, IBA on germination. Each experiment involved studying the germination of 64 seeds under various treatment conditions. Treatment groups were as follows: IBA solutions (1%, 0.01%, 0.001%, 0.0001%, 0.00001%, 0.000001%), Hormex concentrate, and soaking seed in IBA 0.0001% (1, 2, 4, 12, and 24 hrs.) prior to treatment. (Table 1)

Materials:

Soybean seed: Pella '86', bought from Gries Seed Farms, Inc., Fremont, Ohio.

IBA solutions: IBA solutions used in these experiments and the protocols for dissolving IBA at treatment concentrations in water are listed in Appendix C.

Hormex concentrate: Bought from Brooker Chemical

Table 1
List of Germination Tests

Set	Exp. number	Soaking time (hrs)	Treatment
A	1A	24	0.0001 % IBA
	2A	12	0.0001 % IBA
	3A	4	0.0001 % IBA
	4A	0	Dist. Water
	5A	0	0.01 % IBA
	6A	0	1.0 % IBA
	7A	0	0.0001 % IBA
B	1B	4	0.0001 % IBA
	2B	2	0.0001 % IBA
	3B	1	0.0001 % IBA
	4B	0	Dist. Water
	5B	0	0.01 % non-IBA
	6B	0	1.0 % non-IBA
	7B	0	100 % IBA
C	1C	0	Dist. Water
	2C	0	0.01 % IBA
	3C	0	0.001 % IBA
	4C	0	0.0001 % IBA
	5C	0	Hormex solution

Table 1
(continued)

Set	Exp. number	Soaking time (hrs)	Treatments
D	1D	0	Dist. Water
	2D	0	0.00001 % IBA
	3D	0	0.000001 % IBA
	4D	0	Hormex solution

Corporation, North Hollywood, California. The chemical composition of Hormex includes:

Active Ingredients:	
1-Napthaleneacetic acid	0.240 %
Indole-3-Butyric acid	0.013 %
Inert Ingredients:	99.747 %
Vitamin B-1 (Thiamin hydrochloride)	0.250 %

Instruction for dissolving Hormex concentrate in water are to add 5 ml of hormex concentrate to 5 liters of deionized water. Make sure the concentrate is dissolved in deionized water completely and a homogenous mixture is obtained.

Method:

Each treatment consisted of a black plastic planting flat containing 64 (1.5" x 2") compartments. Soybean seeds were selected at random, weighed, numbered, and planted at a depth of approximately 5 mm in regular planting vermiculite. Treatments were then covered in clear plastic bags to reduce evaporation by greenhouse air currents. For treatment groups subjected to soaking, before planting each of the 64 seeds were placed at the bottom of a compartment. The flat was then placed in a tray containing the chemical solution. The chemical would seep through the small opening at the bottom of each compartment and immerse the seed. Each treatment was placed at random on greenhouse tables in chamber two of YSU's

greenhouse. The vermiculite was kept moist for the duration of the experiment by adding the chemical solution to the trays as needed. Germination data were collected/recorded on a daily basis. At the conclusion of each set of tests, plants were taken out of their compartments, washed, towel-dried, weighed individually, and stored in plastic bags in a freezer. Roots of these plants were later used for analysis and determination of amount of IBA absorbed by each treatment group during germination.

In order to determine the amount of IBA absorbed by plant roots, it was necessary to examine possible auxin extraction procedures used in previous studies (Morgan, et al, 1983). A restriction in the choice of procedures was to employ one which could effectively utilize the equipment available in the YSU analytical laboratory. The final procedure used, which was recommended by the laboratory coordinator, was that of Hill, 1980.

Data Analysis:

The mathematical analysis of data collected from the treatments involved using a number of standard statistical procedures designed to make inferences about certain hypotheses concerning the impact of IBA on germination and growth rate of the seeds. In order to determine whether there is significant difference in growth rates resulting from

different IBA treatments, nine distinct treatment levels were selected for analysis.

The experimental design for this study was a completely randomized block design, using each treatment as an independent variable. The dependent variable was growth rate. Growth rates were calculated from the formula "growth rate= (dW)/(dT)" for each individual observation. A similar statistical method (single factor analysis of variance) was used to determine if there is a significant difference in the growth rates resulting from different soaking times. In another analysis six distinct soaking times constituted the different treatment levels used for this analysis.

A convenient way of determining whether the differences between treatments in germination tests are statistically significant was developed by Roberts (1963). It involves the calculation of the limits of X^2 (chi square) insignificance at 5% probability level for a single germination test. This means that if the value for percentage germination of another treatment lies outside these limits, the difference between it and the first test is significant. The limits of insignificance were calculated as follows: Assuming 64 seeds are used in each treatment in the germination tests, and with the X^2 value of 3.840 (the value appropriate for 1 degree of freedom at the 5% probability level), the following quadratic equation was derived,

$$Y^2 + 1.06X^2 - 4.841X - 2YX + Y + 0.25 = 0$$

where X and Y are the average number of seeds germinated in the first and second test respectively after applying Yate's correction (Zar, 1984). For a given value of X, the alternative solutions for Y give the minimum higher and maximum lower value which achieve significance.

CHAPTER III

RESULTS

The analysis of the experimental data in the context of objectives pursued in this study provided the following results.

Effect of IBA on growth rate:

A single factor analysis of variance (ANOVA) was used to test the hypothesis that there is no difference in growth rate resulting from different treatments. Growth rate as used in this analysis was defined as the ratio (dW)/(dT) where dW is the net gain in weight from the time the seed was planted to the time the plant was taken out of the compartment, washed, weighed and stored. Although growth rate is more accurately calculated as dW (dry weight)/dT (Evans, 1972), the fact that in this analysis only relative values of growth rates were important, the use of wet plant weight instead of dry weight would not alter the results of the analysis. The data set used for this analysis and the corresponding results are shown in Tables 2 and 3, respectively. As Table 3 indicates, the null hypothesis of

equal mean growth rate for different treatments should be rejected ($\alpha = 0.05$). The calculated F value of 4.898 was compared to the critical value from the F Tables ($F_{.05,8,567} = 1.94$) which resulted in the rejection of the null hypothesis. The analysis indicates that the mean growth rate resulting from at least one of the treatments is different from the others.

Table 2

Single factor Analysis Of Variance.
Growth Rate with Different IBA Treatments.
(growth rates [(dW)/(dT)] are all multiplied by 1000)

Treat-ments	Observations										
	1	2	3	4	5	6	7	8	9	10	11
Dist. Water	128	166	188	179	151	202	110	164	168	190	193
.01 % IBA	1850	1370	287	415	410	1470	185	238	0	0	0
.0001 % IBA	204	206	318	264	275	100	231	195	210	263	223
.01 % non-IBA*	467	550	425	432	485	610	380	515	402	440	865
1 % non-IBA*	605	670	675	251	810	765	865	1410	665	665	1250
.001 % IBA	332	179	156	2030	230	154	228	236	167	396	214
Hormex **	1590	185	174	148	308	298	172	255	240	308	278
.00001% IBA	297	288	300	235	232	242	251	480	283	315	254
.000001% IBA	284	265	282	311	241	201	324	211	241	343	200

* Non-Indole-3-Butyric Acid

** Hormex contains 0.013 % IBA

Table 2
(continued)

Treat-ments	Observations										
	12	13	14	15	16	17	18	19	20	21	22
Dist. Water	162	136	145	305	155	210	165	198	187	121	168
.01 % IBA	0	0	0	0	0	0	0	0	0	0	0
.0001 % IBA	101	191	198	347	111	170	072	103	174	124	085
.01 % non-IBA	680	625	392	640	322	605	535	1050	317	300	450
1 % non-IBA	615	665	1220	590	760	420	302	965	960	860	107
.001 % IBA	341	115	141	116	234	285	234	233	094	106	110
Hormex	300	241	248	310	216	260	174	565	198	257	253
.00001% IBA	266	372	183	135	147	201	214	330	220	196	160
.000001% IBA	265	401	226	220	185	290	306	241	190	201	200

Table 2
(continued)

Treat-ments	Observations										
	23	24	25	26	27	28	29	30	31	32	33
Dist. Water	217	132	200	151	127	150	133	700	133	147	097
.01 % IBA	0	0	0	0	0	0	0	0	0	0	0
.0001 % IBA	221	077	224	083	074	089	138	145	093	133	201
.01 % non-IBA	300	335	765	575	745	630	382	615	750	490	485
1 % non-IBA	980	630	0	0	0	0	0	0	0	0	0
.001 % IBA	172	216	144	085	101	352	116	103	159	213	111
Hormex	221	183	211	146	253	261	315	335	200	095	119
.00001% IBA	295	231	194	287	252	340	270	254	213	277	220
.000001% IBA	210	0	0	0	0	0	0	0	0	0	0

Table 2
(continued)

Treat-ments	Observations								
	34	35	36	37	38	39	40	41	42, 43,... 64
Dist. Water	163	078	0	0	0	0	0	0	0, 0.... 0
.01 % IBA	0	0	0	0	0	0	0	0	0, 0,... 0
.0001 % IBA	098	167	1650	0	0	0	0	0	0, 0,... 0
.01 % non-IBA	0	0	0	0	0	0	0	0	0, 0,... 0
1 % non-IBA	0	0	0	0	0	0	0	0	0, 0,... 0
.001 % IBA	089	121	155	143	119	110	900	0	0, 0,... 0
Hormex	173	200	0	0	0	0	0	0	0, 0,... 0
.00001% IBA	665	234	188	296	0	0	0	0	0, 0,... 0
.000001% IBA	0	0	0	0	0	0	0	0	0, 0,... 0

Table 3
Summary of Analysis

Analysis Of Variance Table

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F
Treatments	8	2.726	0.341	4.898**
Error	567	39.443	0.070	
Total	575	42.169		

** reject null hypothesis since 4.898 > $F_{0.05,8,567}$ =1.94

Summary of Population Statistics

Treatment	Observation	Mean	Std. Dev.
1	64	0.097	0.014
2	64	0.097	0.043
3	64	0.118	0.027
4	64	0.265	0.037
5	64	0.290	0.053
6	64	0.152	0.035
7	64	0.168	0.033
8	64	0.153	0.019
9	64	0.091	0.016
Total	576	0.159	

Effect of soaking time on growth rate:

The application of the single factor ANOVA resulted in the rejection (at the 5% probability level) of the null hypothesis that there is no difference in growth rates resulting from different soaking times. This study indicates that at least two treatments (soaking time) differ substantially in their effect on mean growth rate. Table 4 provides the data used in this analysis, and Table 5 presents a summary of the single factor analysis of variance.

As Table 5 indicates, the null hypothesis of equal mean growth rate for different soaking times should be rejected (α = 0.05). The calculated F value of 8.343 was compared to the critical value from the F Tables ($F_{.05,5,378}$ = 2.21) which resulted in the rejection of the null hypothesis.

Table 4

Single factor Analysis Of Variance.
Growth Rate for Different Soaking Times.
(growth rates [(dW)/(dT)] are all multiplied by 1000)

** Treat-ments	Observations										
	1	2	3	4	5	6	7	8	9	0	11
24 hrs soak	197	160	145	0	0	0	0	0	0	0	0
12 hrs soak	206	105	104	091	203	133	170	0	0	0	0
4 hrs soak	177	197	165	166	121	094	042	151	140	113	152
2 hrs soak	360	410	320	900	680	370	432	375	720	132	317
1 hr soak	740	355	357	392	272	535	382	372	710	487	580
no soak	204	206	318	264	275	100	231	195	210	263	223

** IBA concentration for all treatments = 0.0001%

Table 4
(continued)

Treat-ments	Observations										
	12	13	14	15	16	17	18	19	20	21	22
24 hrs soak	0	0	0	0	0	0	0	0	0	0	0
12 hrs soak	0	0	0	0	0	0	0	0	0	0	0
4 hrs soak	141	0	0	0	0	0	0	0	0	0	0
2 hrs soak	0	0	0	0	0	0	0	0	0	0	0
1 hr soak	765	397	297	325	440	575	0	0	0	0	0
no soak	101	191	198	347	111	170	072	103	174	124	085

Table 4
(continued)

Treat-ments	Observations										
	23	24	25	26	27	28	29	30	31	32	33
24 hrs soak	0	0	0	0	0	0	0	0	0	0	0
12 hrs soak	0	0	0	0	0	0	0	0	0	0	0
4 hrs soak	0	0	0	0	0	0	0	0	0	0	0
2 hrs soak	0	0	0	0	0	0	0	0	0	0	0
1 hr soak	0	0	0	0	0	0	0	0	0	0	0
no soak	220	077	224	083	890	074	138	145	093	133	201

Table 4
(continued)

Treat-ments	Observations			
	34	35	36	37, 38, 39 , ... 64
24 hrs soak	0	0	0	0, 0, 0 , ... 0
12 hrs soak	0	0	0	0, 0, 0 , ... 0
4 hrs soak	0	0	0	0, 0, 0 , ... 0
2 hrs soak	0	0	0	0, 0, 0 , ... 0
1 hr soak	0	0	0	0, 0, 0 , ... 0
no soak	098	167	0	0, 0, 0 , ... 0

Table 5
Summary of Analysis

Analysis Of Variance Table

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Treatments	5	0.798	0.160	8.343 **
Error	378	7.233	0.019	
Total	383	8.031		

** Reject null hypothesis since 8.343 > $F_{0.05,5,378}$ =2.21

Summary of Population Statistics

Treatment	Observations	Mean	Std. Dev.
1	64	0.008	0.004
2	64	0.016	0.006
3	64	0.026	0.007
4	64	0.078	0.024
5	64	0.125	0.028
6	64	0.106	0.018
Total	384	0.060	

Effect of different treatments on percent germination:

The results of the germination tests are presented as graphs where curves show percentage germination plotted against time (Figs. 1 and 2). From these curves it can be seen that IBA treatment had a stimulatory effect on germination at a concentration of 0.001% - 0.0001%, while soaking had an inhibitory effect on germination. The stimulatory effect of IBA treatment or the inhibitory effect of soaking time was a function of the concentration of the IBA solution used or the duration of soaking. Table 6 summarizes the results of X^2 test applied to all treatment data. These results confirmed the above analysis. In addition, Table 6 shows that:

1) IBA treatment at concentrations in the range of 0.001% - 0.0001% significantly stimulates germination.
2) IBA treatment at concentrations above this range significantly inhibits germination, while IBA treatment at concentrations below this range has no effect on germination.
3) Soaking has an inhibitory effect on germination. The inhibitory effect increases as the soaking period increases.

Fig. 1

The effect of treatment concentration on percentage germination

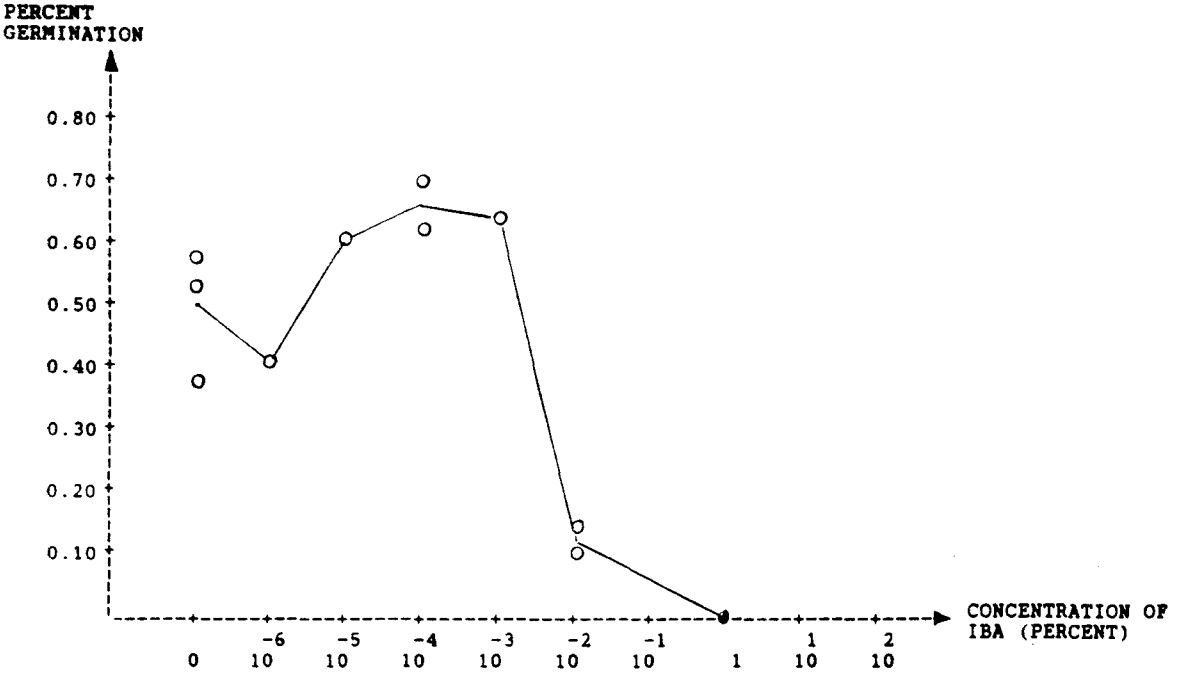

PERCENT
GERMINATION
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0
10^-6
10^-5
10^-4
10^-3
10^-2
10^-1
1
10^1
10^2
CONCENTRATION OF
IBA (PERCENT)

Fig. 2

The effect of soaking on percentage germination. Treatment concentration =0.0001% IBA

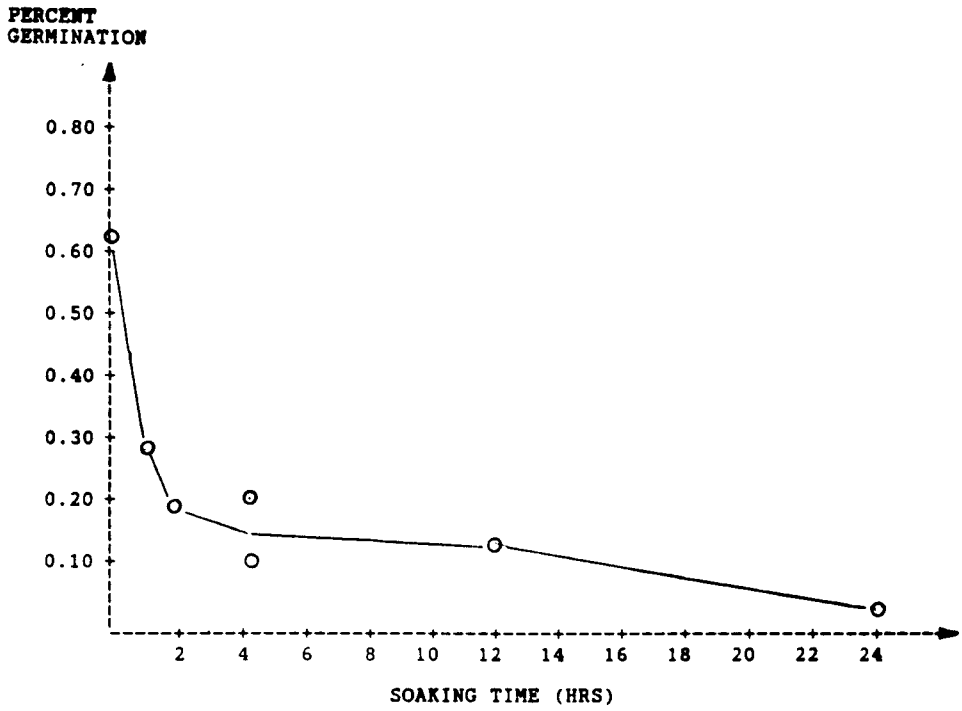

PERCENT
GERMINATION
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
2
4
6
8
10
12
14
16
18
20
22
24
SOAKING TIME (HRS)

Table 6

Results of X^2 Test Comparing Treatment Groups to Dist. Water (control). a = 0.05

Experiment no.	Soaking time (hrs)	Treatment	No. of seeds germinated	N= Accept Ho Y= Reject Ho *
1A	24	0.0001 % IBA	3	Y (1)
2A	12	0.0001 % IBA	8	Y (1)
3A	4	0.0001 % IBA	14	Y (1)
4A	0	Dist. Water	36	
5A	0	0.01 % IBA	4	Y (1)
6A	0	1.0 % IBA	0	Y (1)
7A	0	0.0001 % IBA	40	Y (2)
1B	4	0.0001 % IBA	6	Y (1)
2B	2	0.0001 % IBA	12	Y (1)
3B	1	0.0001 % IBA	18	Y (1)
4B	0	Dist. Water	34	
5B	0	0.01% non-IBA	36	N -
6B	0	1.0 % non-IBA	27	N -
7B	0	100 % IBA	0	Y (1)
1C	0	Dist. Water	24	
2C	0	0.01 % IBA	9	Y (1)
3C	0	0.001 % IBA	41	Y (2)

Table 6
(continued)

Experiment no.	Soaking time (hrs)	Treatment	No. of seeds germinated	N= Accept Ho * Y= Reject Ho
4C	0	0.0001 % IBA	45	Y (2)
5C	0	Hormex sol.	38	N -
1D	0	Dist. Water	36	
2D	0	0.00001 % IBA	39	N -
3D	0	0.000001% IBA	25	N -
4D	0	Hormex sol.	28	N -
**	0	Dist. Water	32.5	

* Ho: There is no significant difference in % germination between dist. water and the treatment.
** : Average value for tests 4A, 4B, 1C, and 1 D (all using dist. water)
(1) : Inhibits germination
(2) : Stimulates germination

Relationships between seed weight and germination time:

The research considered the effect of the seed size (weight) on germination time (Table 7). A bivariate regression analysis of seed weight versus germination time showed relatively small (negative) correlation between the two factors (Fig. 3). The statistical analysis points to the fact that the relationship between these two factors could be practically ignored (Table 8).

Table 7
Germination Time - Seed weight data
for Dist. Water (control) Treatments

Observation no.	Seed weight (0.01 gm)	Germination time (days)
1	18	11
2	22	11
3	25	11
4	24	11
5	16	11
6	27	11
7	26	9
8	22	11
9	21	12
10	23	11
11	22	11
12	20	12
13	20	11
14	18	12
15	28	8
16	19	11
17	27	11
18	20	11
19	22	9
20	21	9
21	21	9
22	20	11
23	24	9
24	14	9
25	26	11
26	23	9
27	21	11
28	22	11
29	21	9
30	18	11
31	18	12
32	22	9
33	19	12

Table 7
(continued)

Observation no.	Seed weight (0.01 gm)	Germination time (days)
34	24	9
35	25	9
36	19	11
37	18	9
38	19	9
39	22	7
40	18	7
41	21	9
42	25	7
43	18	13
44	23	8
45	18	7
46	29	7
47	18	9
48	17	11
49	20	7
50	18	7
51	20	7
52	21	8
53	17	7
54	26	8
55	18	8
56	26	9
57	19	9
58	21	7
59	14	7
60	21	7
61	17	9
62	23	9
63	25	8
64	30	8
65	21	7
66	26	9
67	22	8

Table 7
(continued)

Observation no.	Seed weight (0.01 gm)	Germination time (days)
68	21	7
69	19	7
70	19	9
71	27	9
72	19	11
73	18	9
74	19	9
75	22	7
76	18	7
77	21	9
78	25	7
79	18	13
80	23	8
81	18	7
82	29	7
83	18	9
84	17	11
85	20	7
86	18	7
87	20	7
88	21	8
89	17	7
90	26	8
91	18	8
92	26	9
93	19	9
94	21	7
95	14	7
96	21	7
97	17	9
98	23	9
99	25	8
100	30	8

Fig. 3

Relationship between
germination time and seed weight

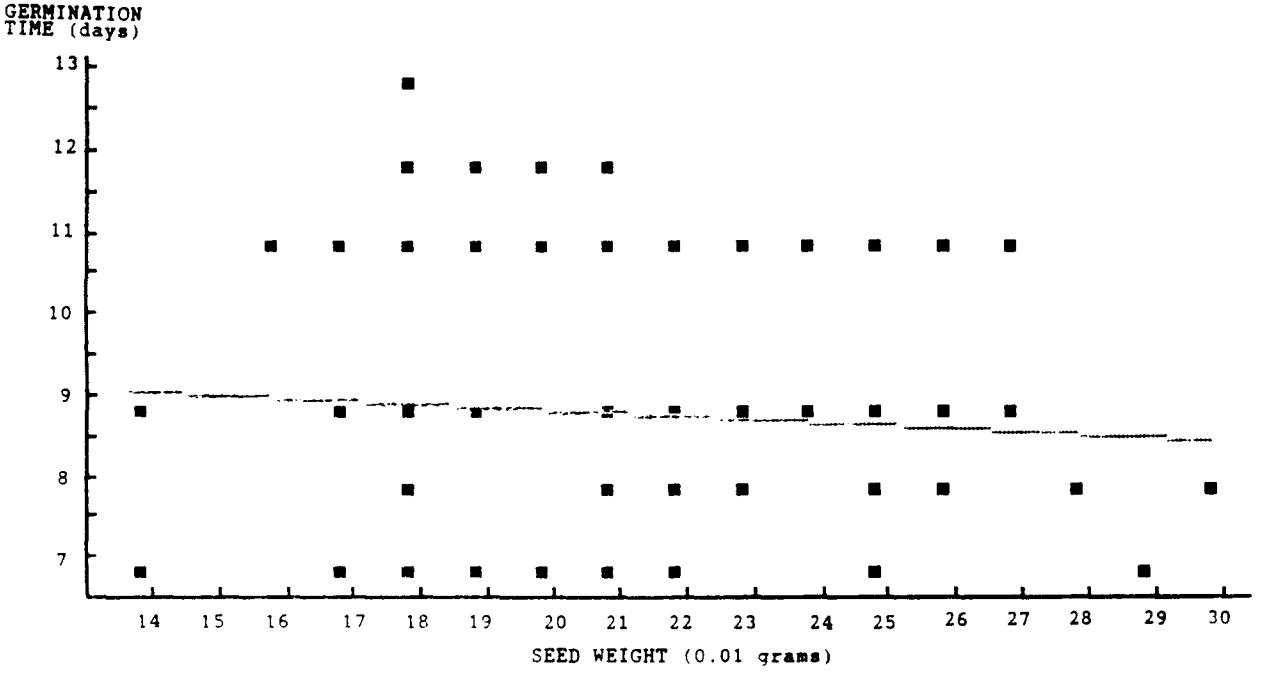

GERMINATION
TIME (days)
13
12
11
10
9
8
7
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
SEED WEIGHT (0.01 grams)

Table 8

Summary of Regression Analysis.
Relationship between Seed Weight and Germination Time

Correlation Coefficient = -0.081

Minimum (X) = 14.000 Maximum (X) = 30.000
Minimum (Y) = 7.000 Maximum (Y) = 13.000

Mean of X = 21.160 Mean of Y = 8.990

F Statistic for Hypothesis Test of Beta 1 = 0

F = 0.65 with 1 and 98 Degrees of Freedom

This F Statistic would be significant at an Alpha level of 0.427

MODEL: Y = B0 + B1 * X

Sum of Squares for Regression (SSR) = 1.903
Sum of Squares for Error (SSE) = 285.09
Total Sum of Squares (SST) = 286.99

Unconditional Variance of 'Y' S(Y) = 2.959
Conditional Error Variance S(Y/X) = 2.910

Estimate of B0 = 9.814 Variance = 1.068
Estimate of B1 = -0.039 Variance = 0.002

Correlation between B0 and B1 = -0.986

95% Confidence Limits on B0 and B1:

Probability (9.81 < B0 < 9.81) = 95%
Probability (-0.04 < B1 < -0.04) = 95%

Absorption of IBA by the roots:

The chemical analysis of the auxin extracted from the roots indicates that a measurable amount of IBA was absorbed by the roots in germination tests where hormex was used (Table 9). However, chemical analysis of all other extracts did not indicate any measurable amount of IBA in roots collected from other treatments.

The absence of IBA in the root extracts from the other treatments could be attributed to the difference in chemical composition of hormex and IBA solutions. Hormex (0.013% IBA content) treatment resulted in about 50% germination. On the other hand the 0.01% IBA treatment, on average, resulted in about nine percent germination. The difference in percentage germination must be attributed to the difference in chemical composition of the two solutions. The inhibitory effect of IBA is neutralized by other chemical substances present in hormex. Since the IBA content of hormex is not totally dissolved in the solution, when applied exogenously, free IBA becomes available to be released, transported and accumulated in the site of root formation (Epstein and Ackerman, 1993).

It is suggested that free IBA and particularly its conjugates serves as the source of auxin during the later stages of root development (Wiesman, 1989; Jacobs, 1990). The difference in percentage germination between the 0.01% IBA treatment and hormex is also attributed to the above-

mentioned difference in chemical composition of the two treatments.

Table 9
Amount of IBA Absorbed by the Roots

Treatment (Exp. no.)	IBA in the extract (mg)	Weight of the wet plant tissue (gm)	Proportion of IBA
Hormex (5C)	374.1	17.25	$\frac{21.68 \text{ mg IBA}}{1 \text{ gm wet plant tissue}}$
Hormex (4D)	0.0573	220.42	$\frac{2.83 \times 10^{-3} \text{ mg IBA}}{1 \text{ gm wet plant tissue}}$

CHAPTER IV

DISCUSSION

Pella '86' brand soybean has a very high yield but it is a very slow germinator (Beuerlein, 1987). Yield potential and proper maturity are the most important factors considered in selecting soybean varieties. Therefore, in spite of a very high yield, pella '86' is not a popular brand because of low maturity. The rationale for this study was to investigate potential methods for improving this variety's maturity including using the germination stimulator IBA. The stimulatory impact of auxins on germination and root formation are well documented (Mayer and Poljakoff-Mayber, 1982; Nordstrom, 1991; Pluss, 1989).

A large number of studies have investigated the effects of IAA on a variety of seeds (Soeding and Wagner, 1955). However, considering the stimulatory effect of the auxins on germination, IAA is not considered the most suitable compound to be used in practice. IBA and alpha-naphthylene acetic acid (NAA) are found to be much superior because of their higher chemical stability and their low mobility in the plant. Low mobility in the plant means that the compound is retained near its point of application and relatively better growth responses are induced. For example, because of high mobility,

IAA may spread into buds and inhibit their early growth and development. Although chemical stability and low solubility are considered as important advantages for IBA and NAA compared to IAA, such advantages as are shown by NAA are offset by narrow range of concentrations over which NAA can be used safely. IBA shows a much greater flexibility in this respect (Audus, 1965).

There have been reports that amides of IBA and NAA are more effective for the rooting of some species than either free acids or their salts. Zimmermann and Hitchcock (1942) show that there are a number of other compounds (i.e., 2,4-Dichloro- and 2,4-Dibromo derivatives) which have either equal or better rooting activities compared to IBA or NAA. However, most of these compounds suffer from the disadvantage of having undesirable secondary properties which limit their particular applications. For example, they may produce thick stunted roots and have a toxicity limit very near the optimum rooting concentration.

Different compounds affect differently the quantity and the quality of the roots they induce. For example, IBA treatment at low concentrations normally results in a few roots which rapidly become long and establish a fibrous root system. Treatment at high concentration, on the other hand, produces a dense, thick and bushy root system. Significant progress has been achieved in regulating vigor and general

structure of the root system by using appropriate mixtures of these compounds, as such mixtures induce roots with intermediate characteristics. In many cases a greater number of roots results from mixture of IAA and NAA, or of IBA and NAA, in equal proportions, than from either compounds alone at the same total concentration (Nordstrom, 1991). IBA and NAA, separately or mixed, together with small amount of 2,4-trichlorophenoxyacetic acid (about 0.1 mg/ml of mixture) has proved to be a very effective combination (Nordstrom, 1991).

The results of this study suggest both a stimulatory and inhibitory effect of the auxin (IBA) on germination, and support previous findings that used similar compounds like IAA (Mayer and Poljakoff-Mayber, 1982). Although IBA treatment at a concentration of 0.0001% resulted in a substantial increase in percentage germination, its stimulatory effect was neutralized when seeds were soaked in the solution for a number of hours. The change in percentage germination due to soaking was inversely proportional to the number of soaking hours. For example, a one hour soaking time reduced percentage germination from 50% (0 soak) to about 27%, while 24 hours soaking time reduced the germination to only 5%. This indicates that soaking time has an inhibitory effect on germination. The percentage germination improved from about 50% for distilled water treatment (control) to about 69% for 0.0001% IBA treatment.

However, IBA treatment at these concentration levels (or other levels) did not have significant positive impact on germination time (Table 10). In fact, the average germination time seems to increase moderately by about one - two days with these treatments. This moderate increase in germination time may not be totally attributable to the treatment since a number of factors including sample size, greenhouse conditions, and time of planting, may have influenced the experimental results.

Table 10
Average Germination Time in Days
for Different Treatments

Treatment	no. of seeds germinated	Average germination time (days)
24 hrs soak, 0.0001 %IBA	3	7.67
12 hrs soak, 0.0001 %IBA	7	9.0
4 hrs soak, 0.0001 % IBA	12	10.58
Dist. Water	35	8.57
0.01 %IBA	4	18.00
0.0001 % IBA	35	10.57
4 hrs soak, 0.0001 % IBA	6	9.50
2 hrs soak, 0.0001 % IBA	12	8.66
1 hr soak, 0.0001 % IBA	17	9.21
Dist. Water	34	9.76
0.01 % non- IBA	36	9.41
1% non- IBA	27	10.45
Dist. Water	24	11.71
0.01 % IBA	9	17.13
0.001 % IBA	41	11.41
0.0001 % IBA	43	10.76

Table 10
(continued)

Treatment	No. of seeds germinated	Average germination time (days)
Hormex	37	13.83
Dist. Water	36	8.19
0.00001 % IBA	37	7.30
0.000001 % IBA	24	8.70
Hormex	27	10.40

CHAPTER V

CONCLUSION

Selecting soybean variety is one of the most important decisions a grower makes each winter. While the seed costs vary little among varieties, the yield potential for a given set of environmental conditions and cultural practices may vary by fifteen or more bushels per acre (Beuerlein, 1987). Selecting the best variety has become more difficult over the past two decades due to an increase in the number of varieties available. Considering the factors that must be taken into account, those brands with higher maturity and yield potential must be preferred over others. On that basis, it is evident that if it was possible to improve its maturity, Pella '86' could be rated as one of the best alternatives for certain environmental conditions, since it has the highest potential yield. As indicated in this study, it is possible to improve percentage germination of the seed substantially by IBA treatments. However, this may not be economically feasible in practice, since treatment materials are expensive. In addition, the treatment does not necessarily improve maturity. It was noted that average germination time for seeds planted

in early March was relatively shorter than those planted in early May, and early planting resulted in higher percentage germination than later planting. Therefore, it may be possible to improve maturity by a combination of timely planting and some IBA treatments. In one example, early planting and using 0.00001% IBA, the average germination time was reduced to about 7.2 days. It is reported that IBA is very effective in the promotion of rooting on a wide variety of plants and is being used commercially to root many plant species worldwide (Epstein, et al, 1989). A detailed study is necessary to investigate economic merits of using IBA treatment and consequently improving the soybean crop yield.

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Appendix A

Appendix A
Comparitive Growth and Quality for Different Soybean Varieties (in maturity order) from the Ohio Advanced Tests, 1982-86

Variety	[1] Relative Maturity	[2] Lodging Scores	Height (in)	Seed Quality score	Seed Size (gm/100)
Hardin	2	1.5	28	1.9	15
Vickery	2	2.5	34	-	-
BSR201	-	-	-	1.9	15
Elgin	2.3	1.7	29	1.9	17
Hack	2.6	1.6	31	1.8	17
Century 84	2.6	1.6	32	1.9	18
Century	2.7	1.7	33	1.8	18
Preston	2.7	-	-	2.2	18
Amcor	-	2.4	37	-	-
Gnome 85	3.1	1.5	22	1.9	15
Zane	3.0	1.7	33	1.9	18
Pella	3.0	1.6	34	2.0	19
Williams 82	-	1.9	38	-	-

1: Relative Maturity Scores are composed of whole digits maturity group followed by a decimal which relates to placement in maturity group. Higher score indicates later maturity.

2: Lodging scores are based on a 1.0 (100% erect) to 5.0 (100% prostrate) scale.

Appendix B

Appendix B
Comparative Yield (bu/a) for Different
Soybean Strains (in maturity order)
the Ohio Soybean Trials

Strain	Average Yield	
	1983-1985	1983-1986
Vickery	51	51
Beeson 80	53	53
Keller	51	53
AP 240	-	-
Elgin	-	-
Century 84	53	57
TS 222	-	-
Amcor	-	-
FFR 226	55	57
Zane	54	57
V 295	-	-
Pella	57	58

Appendix C

Appendix C
Protocol for dissolving IBA at specific concentrations in water

1% solutions:

1. To a four liter beaker add 1.97 g of sodium hydroxide for every liter of solution to be prepared.

2. Add approximately 100 ml/l of deionized water. Allow the sodium hydroxide to completely dissolve. Mix well on a stir plate with a magnetic stirring bar.

3. Add 10 g of 3-indole butyric acid for every liter of solution to be prepared.

4. Allow the IBA to dissolve completely.

5. Add deionized water to 3/4 of the final volume. Mix well. If the IBS is not completely dissolved add 1 M NaOH dropwise to the beaker until all of the IBA is in solution.

6. Using a pH meter adjust the pH to 7 with either 1 M sodium

hydroxide for 1 M hydrochloric acid.

7. Once the pH has stabilized add deionized water to bring the solution to its final volume and mix well. Check the pH to make sure it has not changed. If it has make adjustments as necessary.

0.01% Solutions:

1. Add 0.10 g of 3-indole butyric acid for every liter of solution to be prepared to a 50 ml beaker.

2. To the beaker add 0.5 ml of 1 M sodium hydroxide for every liter of solution to be prepared.

3. Allow the IBA to dissolve completely.

4. Add the resulting solution to a 4 L beaker. Add deionized water to 3/4 of the final volume. Mix well. If the IBS is not completely dissolved add 1 M NaOH dropwise to the beaker until all of the IBA is in solution.

5. Using a pH meter adjust the pH to 7 with either 1 M sodium hydroxide for 1 M hydrochloric acid.

6. Once the pH has stabilized add deionized water to bring the solution to its final volume and mix well. Check the pH to make sure it has not changed. If it has make adjustments as necessary.

0.001% Solutions:

1. Add 0.01 g of 3-indole butyric acid for every liter of solution to be prepared to a 50 ml beaker.

2. To the beaker add 0.05 ml of 1 M sodium hydroxide for every liter of solution to be prepared.

3. Allow the IBA to dissolve completely.

4. Add the resulting solution to a 4 L beaker. Add deionized water to 3/4 of the final volume. Mix well. If the IBS is not completely dissolved add 1 M NaOH dropwise to the beaker until all of the IBA is in solution.

5. Using a pH meter adjust the pH to 7 with either 1 M sodium hydroxide for 1 M hydrochloric acid.

6. Once the pH has stabilized add deionized water to bring the solution to its final volume and mix well. Check the pH to

make sure it has not changed. If it has make adjustments as necessary.

0.0001% Solutions:

1. Add 1 mg of 3-indole butyric acid for every liter of solution to be prepared to a 50 ml beaker.

2. To the beaker add 5 microliters of 1 M sodium hydroxide for every liter of solution to be prepared.

3. Allow the IBA to dissolve completely.

4. Add the resulting solution to a 4 L beaker. Add deionized water to 3/4 of the final volume. Mix well. If the IBS is not completely dissolved add 1 M NaOH dropwise to the beaker until all of the IBA is in solution.

5. Using a pH meter adjust the pH to 7 with either 1 M sodium hydroxide for 1 M hydrochloric acid.

6. Once the pH has stabilized add deionized water to bring the solution to its final volume and mix well. Check the pH to make sure it has not changed. If it has make adjustments as necessary.

0.00001% and 0.000001% IBA Solutions:

1. Weigh out 0.1 g of IBA in a 50 ml beaker.

2. Dissolve IBA with a few drops of 0.05 M NaOH. Add approximately 20 ml of deionized water.

3. Adjust the pH to 7 if necessary with 0.05 M HCl.

4. Pour solution into a 100 ml volummetric flask. Rinse beaker well and pour rinse into volummetric flask. Dilute to the mark with deionized water. Mix well. The concentration of the resulting solution is 1000 ppm.

5. To prepare the 0.00001% solution dilute 1 ml of 1000 ppm concentrate to 10 L with deionized water.

6. To prepare the 0.000001% solution dilute 0.1 ml of 1000 ppm concentrate to 10 L with deionized water.

For the preparation of non-indole butyric acid solutions (used in experiments 5B, 6B) we followed the same procedure as before except no IBA was added.