

GENETIC STUDIES OF THE MAIZE STATURE MUTANT, NANA₁

by

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ABSTRACT

Genetic Studies of the Maize Stature Mutant, $Nana_1$

by Alan L. Hodgdon

This research was undertaken to elucidate the dwarfing mechanism of the maize dwarf $Nana_1$. A large part of the experimentation was testing the hypothesis of excess IAA oxidation in $Nana_1$.

It was shown by growth studies that caffeic acid and ferulic acid, inhibitors of IAA oxidase, had no effect in overcoming the dwarfism in $Nana_1$. Electrophoretic separation of general protein extracts from $Nana_1$ and normal maize showed no differences in general proteins or in enzymes which would mediate in oxidation of IAA. A spectrophotometric assay system showed that there was very little difference in peroxidase activity between the $Nana_1$ and normal protein extracts. Peroxidases are responsible for the oxidation of IAA. Maize coleoptile section elongation tests indicated that $Nana_1$ responded to added IAA, but the normal did not. Avena elongation tests employing general extracts showed that $Nana_1$ extracts did not inhibit elongation of Avena coleoptile sections more than the normal extract.

The research suggested that the dwarfism of Nana₁ was not due to enzymatic destruction or other type of inactivation of IAA. The results indicated that the cause of dwarfism might be a lack of an optimum amount of the IAA.

INTRODUCTION

There are a number of genetic dwarfs of Zea mays which are characterized by a lack of normal cell elongation. Van Overbeek (1935) working on the dwarf Nana₁ discovered that the lack of cell elongation was caused by a sub-optimal amount of the growth hormone known as auxin and later identified as indole-3-acetic acid (IAA). Other maize dwarfs were soon shown to be deficient in auxin (van Overbeek 1938).

The sub-optimal amount of IAA present in dwarfs could be caused by lower IAA production or by destruction of IAA mediated by peroxidases or other agents, or dwarfism could also be due to lack of ability to utilize IAA. Van Overbeek (1938) showed that IAA production in Dwarf₁ was significantly less than in the normal sib, but Harris in 1953 found that the reason for dwarfism was actually the inability to use IAA. In the Nana dwarfs IAA inactivation seemed to be the dwarfing mechanism.

In the cases of Nana₁ van Overbeek (1935) showed by Avena curvature tests that Nana coleoptiles produced less auxin than the normal. He also showed by coleoptile curvature that Nana responded less to auxin. Next he demonstrated

that coleoptile and mesocotyl sections of the dwarf destroyed more auxin in agar blocks than those of the normal. He theorized that this auxin destruction could be responsible for the lack of auxin necessary for normal cell elongation. He thought peroxidases could be responsible for the auxin destruction, and an experiment showed what seemed to be a higher peroxidase content in $Nana_1$. Similar experiments by al-Salih (1958) also indicated that there was enzymatic destruction of IAA.

Van Overbeek in 1938 using five day old plants showed that the amount of diffusible auxin from $Nana_1$ coleoptile tips was nearly the same as that of the normal, but was 87% less than that of the normal below the coleoptile node. This gave further evidence of a high rate of auxin destruction by the dwarf.

The objective of this thesis research was further study of the auxin destruction and other possible dwarfing mechanisms in $Nana_1$ by employing different and, in some cases, more modern and precise techniques.

Much can be learned about dwarfing mechanisms by trying to overcome the dwarfness. Phinney (1957) and al-Salih (1958) had tested the response of $Nana_1$ to gibberellic acid with negative results. Much of my research was attempting

to overcome the dwarfness in Nana₁ using IAA and other compounds.

If there were large differences in peroxidases, protein studies would show this. The analysis of protein differences between Nana₁ and normal became a large part of my research. I attempted to analyze differences in numbers of peroxidases with disc electrophoresis. Peroxidase activity differences were tested by a spectrophotometric assay system.

MATERIALS AND METHODS

Source of the mutant

Seed segregating about 5:1 normal to Nana₁ was furnished from U.C.L.A. The seed was from parental stock which was field pollinated. Nana was first described by Li (1933).

Source of the Avena

The Avena used in elongation tests was a genetically pure hulless variety from the H. Wilder Tomlin Company.

Maize and Avena culturing methods

For most experiments seed segregating for normal and Nana₁ were grown five or seven days from planting in tap water. This was done using either Speed-Pak Growth Pouches, plastic bags with paper towelling to support the seeds, or water cultures in plastic trays. Growth pouches were filled with 25 ml of water and five seeds were put into each pouch. The seeds in water cultures were supported at the water level by cheese cloth on hardware cloth coated with wax. Fifty seeds were planted in each tray containing 1 liter of water.

Seeds were also grown in plastic trays using vermiculite as a supporting medium.

Seeds were soaked for 3 to 6 hours in tap water. Distilled water was also used, with less success. When fungi became a problem, seeds were rinsed in chlorox diluted 3:1 in water for ten minutes, and this was followed by normal soaking. Seeds were incubated in the Avena Room at about 90% relative humidity and 25°C or in an incubator at 25°C. Growth was in the dark, and in certain cases one hour of red light per day was used to prevent excessive mesocotyl elongation.

Avena for elongation studies were grown and sensitized according to the procedures of Nitsch and Nitsch (1956). Avena were grown in deep petri dishes in distilled water on filter paper supported by stacking watch glasses. The Avena were planted groove down and embryo out around the edge of the filter paper.

Water cultures with supplements

Water cultures were set up as outlined above in plastic trays with one liter of solution.

A stock solution of IAA was prepared using 10 mg IAA dissolved in 5 ml 95% ethyl alcohol. This was diluted to 1

liter with glass distilled or demineralized water. Fifty seeds per tray were grown in concentrations of 1 mg and 0.1 mg IAA per liter diluted from the stock solution. Control trays were grown in water. Gibberellic acid solutions were prepared in the same concentrations; however, no alcohol was needed to dissolve this chemical.

Caffeic acid and ferulic acid stock solutions were prepared at concentrations of 10 and 0.1 mg per liter in distilled water. For the growth studies these were diluted to concentrations of 5×10^{-2} and 5×10^{-4} mg per liter, (see Thimann, Tomaszewski, and Porter 1962).

These liquid cultures were incubated seven days in the Avena Room and then the dwarf and tall coleoptiles and mesocotyls were measured to determine response.

Extract studies using Avena elongation tests

In order to test possible IAA inactivation by the Nana_1 and normal maize, extracts were made from the coleoptiles of each, and these were used in conjunction with the Avena section test (Nitsch and Nitsch 1956) to determine their effect on IAA. Maize segregating for normal and Nana_1 were grown in water cultures for seven days. Coleoptiles of the dwarf and normal were excised just above the coleoptile

node. Equal numbers or equal fresh weight of the normal and dwarf coleoptiles (depending on the experiment) were ground using a mortar and pestle or other grinding device in equal volumes of citric acid buffer at pH 5.0 with 2% sucrose. An amount of buffer-sucrose solution four times the weight of the coleoptiles was used. The extract was centrifuged 15 minutes at 22,000 x g, and the supernatant was used with the section tests.

Control and IAA solutions for the Avena elongation tests were prepared according to Nitsch and Nitsch (1956). The standard IAA solution was in the concentration of 0.1 mg per liter. Avena which had been growing about 60 hours and had attained the height of approximately 25 mm were sectioned. From each plant selected one 5 mm section was taken from the coleoptile 3 mm below the tip. These were then sensitized according to the procedures of Nitsch and Nitsch (1956).

Test solutions were the control and IAA solutions and also the dwarf and normal coleoptile extracts added to various concentrations of IAA. For each solution tested, ten coleoptile sections were placed in a glass stoppered pyrex elongation tube with 2 ml of the test solution. When the extracts were mixed with the IAA solutions, 1 ml of each was used. The coleoptile sections were then incubated for twenty

hours in the dark on a Scientific Industries multi-purpose rotator. After the incubation each section was measured.

In certain experiments the buffer for the extract and the IAA and control solutions was phosphate buffer, pH 7.3, (Gortner and Gortner 1949) in order to approximate more closely physiological pH.

Maize coleoptile elongation studies

In another attempt to study the response of $Nana_1$ to IAA, coleoptile elongation tests were used.

Maize segregating for normal and $Nana_1$ were grown in water tray cultures or in vermiculite for 5 days after planting. Equal numbers of dwarf and normal seedlings were selected, and 5 mm sections were cut from the coleoptiles 3 mm below the tips. These were then put into elongation tubes and incubated for 24 hours in control and IAA solutions identical to those used for the Avena elongation tests. Again 2 ml of solution were used for each tube. Seven to 10 sections were incubated in each test. Incubation was in the dark, employing the same rotator used for the Avena. IAA solutions were 0 (control), 0.1, 0.2, 0.4, 1.0, and 10.0 mg per liter concentration. As controls in elongation experiments, some dwarf and normal seedlings were kept intact and

5 mm sections were marked 3 mm below the tips with India ink. As in the *Avena* elongation experiment both citric acid buffer, pH 5.0, and phosphate buffer, pH 7.3, were used.

Disc electrophoresis

The disc electrophoresis apparatus was a modification of Davis' apparatus (Davis 1964) constructed by R. M. Harris and staff at the Botany Department.

The technique followed was that of Davis (1964) and Clarke (1964) for use with protein extracts which do not gel, such as plant proteins. The disc electrophoresis was used for determining differences in general proteins and in peroxidases.

For a general protein extract equal numbers of normal and Nana₁ coleoptiles were excised just above the coleoptile node and were ground in four times their weight of phosphate buffer containing 0.5 M sucrose. The extract was then centrifuged 15 min. at 22,000 x g. The precipitate was discarded. The procedure was adapted from Wildman and Jagendorf (1952) and Keller and Block (1960).

After each run the gels were stained overnight in amido black stain (Davis 1964). After the destaining the bands were counted and relative positions were noted.

The extracts for peroxidases were prepared according to the procedures of Farkas and Stahmann (1966) with the exceptions that equal numbers of coleoptiles (five days old) were ground by a mortar and pestle and centrifuged 15 min. at 22,000 x g.

The peroxidase electrophoresis was also carried out on starch gel using the techniques of Smithies (1955) with slight apparatus modifications. The buffer system utilized was described by Ockerse, Siegel, and Galston (1966).

Spectrophotometry

The extracts for the peroxidase assay system were made by the methods of Farkas and Stahmann (1966) with modifications. The spectrophotometry was performed on a Bausch and Lomb Spectronic 20 at 420 m μ for five minutes. Readings were taken at the start of the reaction, at 15, 30, 60 sec., and at 1.5, 2, 3, 4, and 5 min. as percent of the blank's transmittance. The instrument was calibrated with the blank at 80 % transmittance of 420 m μ .

EXPERIMENTAL RESULTS

Liquid Cultures

Gibberellic acid and IAA

In attempts to overcome the dwarfness of Nana₁, seeds were grown in the various solutions for seven days.

Under the prescribed growing conditions dwarfs of this age can be differentiated easily from normal seedlings. Dwarfs have shorter and thicker coleoptiles and have very little elongation of the mesocotyl. After the growing period, the coleoptiles of the seedlings were measured and elongation of the mesocotyl was noted. Seedlings were classified Nana₁ or normal on the basis of mesocotyl elongation. All means are tabulated as coleoptile length plus or minus two times the standard error of the mean.

Experiment 1 tested the response to gibberellic acid and IAA in concentrations of 0.1 and 1 mg per liter. This experiment was done in growth pouches with 20 ml of liquid in each. Water was used as the control. The results are shown in Table 1. In no case did the Nana₁ or normal maize respond significantly beyond the control.

Experiment 2 was a repeat of experiment 1 except that tray water cultures were used rather than the bag cultures. The results were the same; neither Nana₁ nor normal responded significantly to gibberellic acid or IAA beyond the control. The results are shown in Table 2.

TABLE 1

RESPONSE OF NORMAL AND NANA₁ TO GIBBERELIC ACID AND IAA
(bag cultures)

coleoptile length in cm

mg/liter:	IAA			GA	
	0	0.1	1.0	0.1	1.0
Nana ₁	1.82 _± .24 ^a	1.88 _± .25	2.13 _± .18	1.94 _± .21	2.08 _± .19
normal	3.38 _± .19	3.43 _± .19	3.60 _± .14	3.19 _± .16	3.22 _± .17

^a two times the standard error of the mean

TABLE 2

RESPONSE OF NORMAL AND NANA₁ TO GIBBERELIC ACID AND IAA
(tray cultures)

coleoptile length in cm

mg/liter:	IAA			GA	
	0	0.1	1.0	0.1	1.0
Nana ₁	1.83 _± .28 ^a	1.70 _± .20	1.82 _± .27	1.64 _± .22	1.98 _± .17
normal	2.67 _± .20	2.97 _± .22	2.99 _± .20	2.97 _± .24	2.75 _± .32

^a two times the standard error of the mean

In the tests of response of the maize to gibberellic acid and IAA in water cultures, there was no significant response beyond the control. Probably more significant was the fact that the dwarf growth habit of Nana₁ was not overcome. In all cases any plants which had the coleoptile characteristics of the dwarf also had the extremely short mesocotyls. An extreme response by Nana₁ should have caused elongation of the mesocotyl to the length of the normal, and this was not observed.

Caffeic and ferulic acids

According to the hypothesis of van Overbeek (1935) Nana₁ should have higher IAA oxidase activity than the normal maize.

Furuya, Galston, and Stowe (1962) noted that natural inhibitors of IAA oxidase had been isolated. Among these were ferulic acid and caffeic acid.

The maize was grown in liquid cultures of caffeic and ferulic acids in attempts to overcome the dwarfness of Nana₁. This experiment was performed twice and each time extremely high fungal contamination of the cultures made the seedling number low. Table 3 shows the results of one of these experiments.

TABLE 3

CAFFEIC AND FERULIC ACIDS

(tray cultures)

coleoptile length in cm

mg/liter:	0	5×10^{-4}	5×10^{-2}	5×10^{-4}	5×10^{-2}
Nana ₁	2.20 _± .18 ^a	1.78 _± .69	2.87 _± .29	1.70 _± .80	1.46 _± .41
normal	3.08 _± .51	2.87 _± .54	2.85 _± .48	2.92 _± .96	2.97 _± .27

^a two times the standard error of the mean

Table 3 shows a significantly higher mean for Nana₁ coleoptile length using caffeic acid (5×10^{-2} mg per liter) over the control (distilled water). This could be due to sampling error caused by the small number of seedlings. In some cases only 2 or 3 dwarf seedlings were measured per tray. Again in all treatments in both experiments Nana₁ seedlings had extremely short mesocotyls. This probably demonstrates a lack of response of the Nana₁ seedlings in the liquid cultures.

Maize extract studies using Avena elongation tests

In testing van Overbeek's (1935) hypothesis of the

higher IAA oxidase activity in Nana_1 , general protein extracts were made from both normal and Nana_1 . These were used in Avena elongation tests (Nitsch and Nitsch 1956) to determine the inhibitory effects of Nana_1 on Avena elongation. Presumably any inhibition could be caused by inactivation or destruction of IAA. This type of experiment was repeated eight times, using eight different extracts with similar results. Tables 4 and 5 show typical results.

TABLE 4

AVENA ELONGATION TEST FOLLOWING MAIZE EXTRACTS I^b

Avena coleoptile length in mm

IAA solution mg/l	extracts		
	Nana_1	normal	no extract
CONTROL (0 IAA)	5.91 \pm .07 ^a	5.84 \pm .23	7.15 \pm .19
0.05	5.91 \pm .30	5.94 \pm .33	-
0.1	5.99 \pm .20	6.08 \pm .18	8.03 \pm .32
0.2	6.02 \pm .13	5.99 \pm .20	-
0.4	6.12 \pm .23	5.97 \pm .32	-

^a two times the standard error of the mean

^b 1 ml extract + 1 ml IAA solution with citric acid buffer, pH 5.0

TABLE 5

AVENA ELONGATION TEST EMPLOYING MAIZE EXTRACTS II^bAvena coleoptile length in mm

IAA solution mg/l	extracts		
	Nana ₁	normal	no extract
control (0 IAA)	6.43±.10 ^a	6.64±.15	7.08±.30
0.05	6.73±.14	6.74±.13	-
0.1	6.63±.17	6.78±.12	8.54±.40
0.2	6.63±.14	6.61±.20	-
0.4	6.82±.21	6.89±.16	-

^a two times the standard error of the mean

^b 1 ml extract + 1 ml IAA solution with phosphate buffer, pH 7.3

Table 4 shows that both Nana₁ and normal extracts inhibited the elongation of the coleoptiles in the Avena tests. At each concentration of IAA there was no significant difference in the inhibition of Avena elongation by the normal or Nana₁ extract. Without using extracts there was significant elongation of the Avena coleoptiles over the control when a concentration of 0.1 mg per liter IAA was added to the coleoptiles.

Table 5 shows the same type of experiment with a

change of buffers from citric acid, pH 5.0, to phosphate, pH 7.3. The table shows essentially the same results. The dwarf and normal extracts were not significantly different in their degree of inhibition of elongation. In the series of two experiments using phosphate buffer and the dwarf extract, there was significant elongation over the control with 0.4 mg per liter IAA. This was not the case using the extract from the normal seedlings. Such results show that the dwarf extract may inhibit the elongation of Avena less than the normal.

Maize coleoptile response to IAA

Phinney (1957) and al-Salih (1958) showed that Nana₁ responds only slightly to gibberellic acid. The question of response to auxin was partially answered by van Overbeek (1935) using maize coleoptile curvature.

My method was to test the response of straight coleoptile sections over a wide range of IAA concentrations. The concentrations of IAA used varied from 0 (control) up to 10 mg per liter. The elongation test was modified from that of van Overbeek (1966). Instead of floating the coleoptile sections, the sections were incubated in elongation tubes on a rotator. This experiment was done twice with the same

results. Also in these experiments normal and Nana₁ seedlings were kept intact as controls. Five mm sections of the coleoptiles were marked with India ink. The marked sections were measured after 24 hours growth.

Table 6 shows the results of elongation tests using sections of seven-day old coleoptiles when the primary leaf had just broken through.

TABLE 6

MAIZE COLEOPTILE SECTION ELONGATION

length in mm

<u>solutions</u>	<u>Nana₁</u>	<u>normal</u>
control	6.50 ₋ .27 ^a	6.64 ₋ .225
0.4 mg IAA/l	6.21 ₊ .167	6.54 ₊ .232

^a two times the standard error of the mean

At the age of seven days neither the Nana₁ nor the normal coleoptiles responded to IAA. Therefore the experiments were performed using sections from five-day old seedlings. Figures 1 and 2 show the results of these experiments. Both the Nana₁ and the normal coleoptile sections elongated significantly more than the control as the concentration of

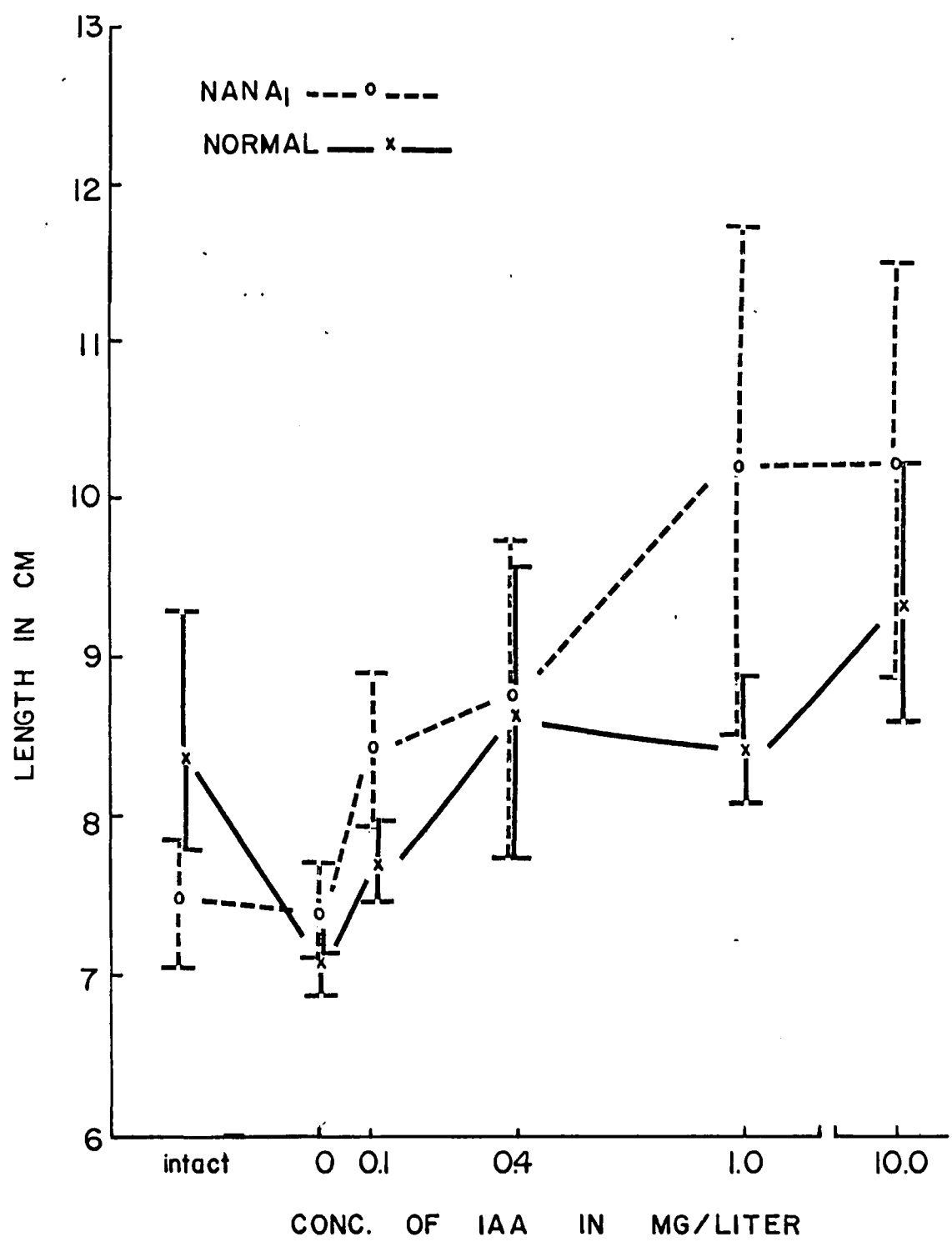


Figure 1. Maize coleoptile section elongation, Exp. I
a. 5 day old coleoptiles

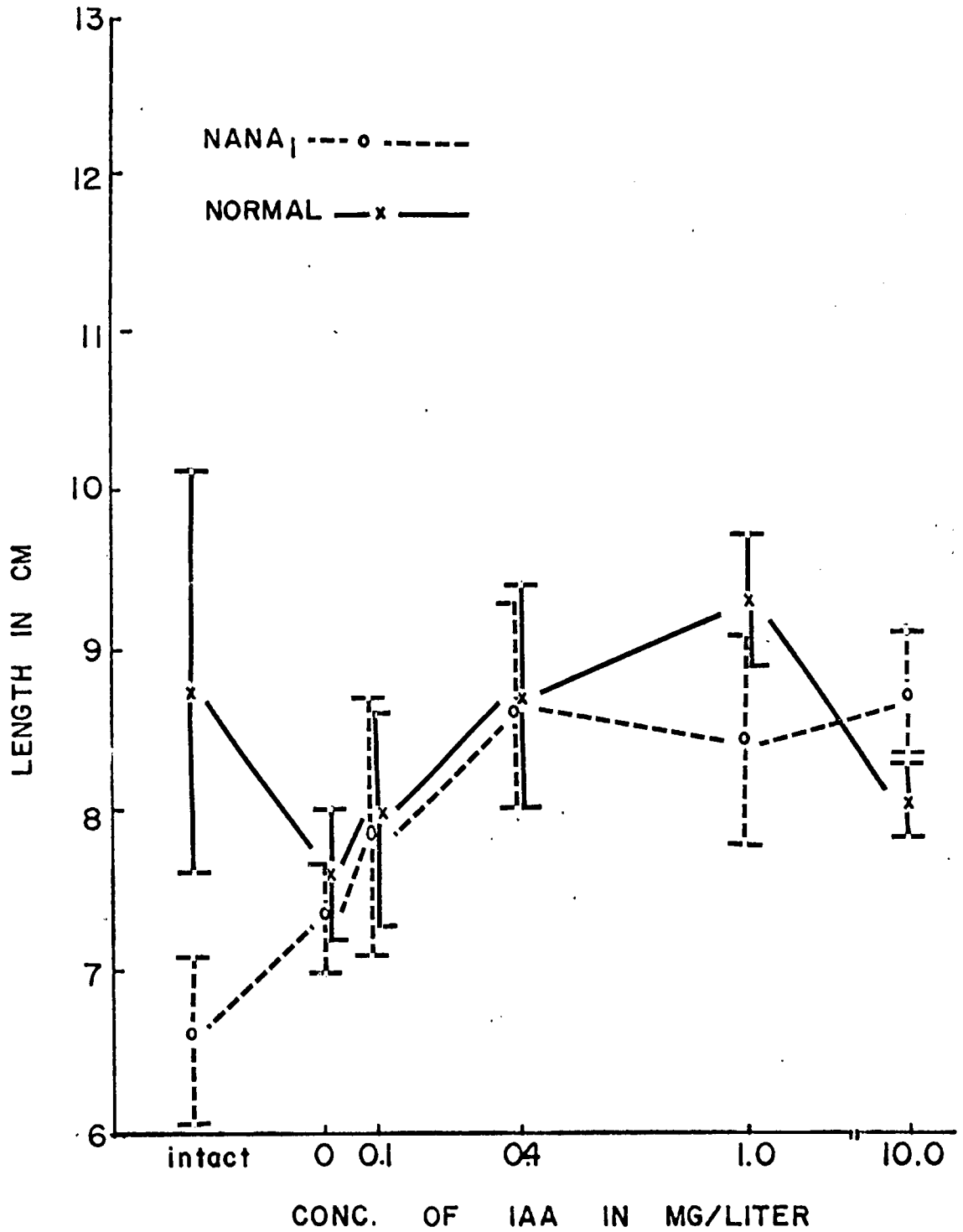


Figure 2. Maize coleoptile section elongation, Exp. II
 a. 5 day old coleoptiles

IAA increased. The dwarf coleoptiles elongated significantly beyond the intact seedlings, but the normals did not. This shows a definite response to IAA in the dwarf sections as compared to normal sections.

Disc electrophoresis

Since disc electrophoresis should give very high resolution of proteins, it was thought that a protein difference between normal and Nana₁ extracts might be found using this process. The protein extracts were made according to the procedure outlined above and were run on the same day they were made at 16 milliamps current for 1 hour. Two sets of extracts were made and each extract was separated in a total of four gels. There were no differences found in band number or intensity. However, the best resolution was only five bands.

The techniques of disc electrophoresis were also applied to the problem of determining differences in peroxidases which mediate in the oxidation of IAA (Ockerse et al. 1966). Two general protein extracts were prepared and each was run on the same day it was made, for 1 hour at 16 milliamps current. The gels were incubated in 0.25% guaiacol and stained in 0.3% hydrogen peroxide. The band pattern is

diagrammed in Figure 3. In each run equal numbers, intensities, and positions of bands were noted. One extract set used for spectrophotometry was also used for disc electrophoresis. These extracts showed the same electrophoretic pattern as the above mentioned extracts prepared for disc electrophoresis.

The first set of protein extracts were also run on starch gel, producing a total of three bands for both the normal and Nana₁ extracts. These bands were similar in all respects.

The electrophoresis results indicated that there was no difference in general proteins. The results of the peroxidase electrophoresis, with good resolution of up to eight bands, suggested strongly that there was no difference in peroxidases between the Nana₁ and normal protein extracts.

Spectrophotometry

For an additional test of peroxidase activity of the Nana₁ and normal maize, a spectrophotometric assay system was used (Farkas and Stahmann 1966). As in the electrophoretic techniques, guaiacol was used as the peroxidase substrate and it was stained by hydrogen peroxide.

Two different extracts were made, both giving similar

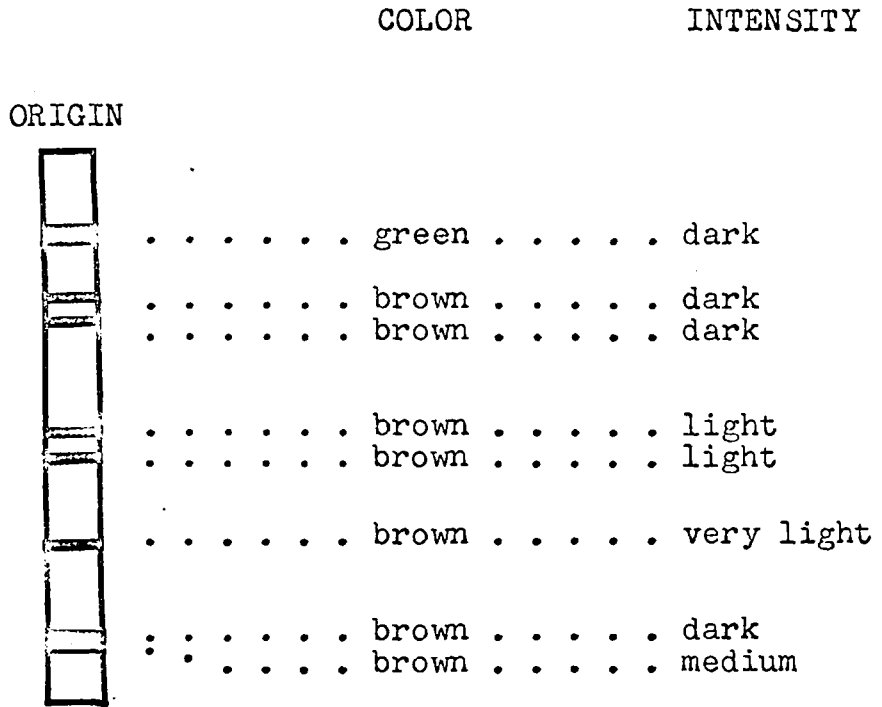


Figure 3. Diagram of Disc Electrophoretic Peroxidase Band Pattern of both Normal and Nana₁ Maize

- a. Run 1 hour at 16 m. Amp on polyacrylamide gel
- b. Stained with guaicol and peroxide

results. The readings were recorded as percent transmittance. Tables 7 and 8 show results using the first extract, Table 8 being the results when the extract was diluted to half strength.

TABLE 7SPECTROPHOTOMETRIC PEROXIDASE ASSAY I^{a,b}

extract	time									
	sec:			min:						
	0	30	45	1	1.5	2	2.5	3	4	5
Nana ₁	85	86	64	58	55	51	44	42	39	41
normal	87	88	53	46	43	42	45	44	40	39

^a readings were % transmittance at 420 m μ

^b Spectronic 20 set at 80% transmittance with blank before each reading

TABLE 8SPECTROPHOTOMETRIC PEROXIDASE ASSAY II^{a,b,c}

extract	time									
	sec:		min:							
	15	30	1	1.5	2	2.5	3	4	5	6
Nana ₁	76	74	72	69	70	69	69	69	66	65
Nana ₁	73	69	67	67	67	67	65	64	64	62
normal	76	72	73	70	70	69	69	69	68	67
normal	71	68	67	66	66	66	65	65	64	62

^a readings were % transmittance at 420 m μ

^b Spectronic 20 set at 80% transmittance with blank before each reading

^c extracts diluted to half strength and each extract run twice

These results show that there is very little difference in intensity of peroxidase activity between the Nana₁ and normal extracts. Table 9, which shows essentially the same results, was from data using the second extract set. Both extracts were run twice.

TABLE 9
SPECTROPHOTOMETRIC PEROXIDASE ASSAY III^{a, b}

extract	time							
	sec:		min:					
	15	30	1	1.5	2	3	4	5
Nana ₁	72	66	63	64	64	64	63	63
Nana ₁	72	68	66	67	66	65	67	65
normal	70	66	66	66	64	66	65	66
normal	71	66	65	66	65	66	64	65

^a readings were % transmittance at 420 m μ

^b Spectronic 20 set at 80% transmittance with blank before each reading

DISCUSSION

The liquid culture experiments using gibberellic acid and IAA seem to confirm the conclusions of Phinney (1957) that Nana₁ doesn't respond to gibberellic acid, and that of van Overbeek (1935) who showed that Nana₁ responded less to IAA than the normal maize. These results, especially with IAA, are open to question, since there is no assurance that the growth substances are absorbed by the plant through the root system. It has also become apparent that the concentration of IAA used might not have been great enough for such experiments. Unfortunately these experiments were concluded before the results of the maize coleoptile section tests were known.

Al-Salih (1958) found that extracts from Nana₁ coleoptiles had higher IAA inactivation than the normal. This was shown using chromatographic separation of the extract, followed by Avena curvature tests. My results using coleoptile extracts in conjunction with Avena elongation tests did not show the additional IAA inactivation by the Nana₁ extract. In fact, in two experiments the dwarf extract inhibited the elongation of the Avena less than the normal did.

A general extract such as used in my experiments should have liberated any inhibitors present in the cells, but possibly additional substances could have been released which masked the effect of the inhibitors. This could be used as a criticism of any extract study. The extract studies do indicate that there is little or no difference for IAA inactivation in the coleoptiles.

Next, experiments which would demonstrate the presence of an excess of IAA oxidases (peroxidases) in the *Nana*₁ as compared to the normal were performed. Caffeic and ferulic acids were known to be inhibitors of IAA oxidases (Thimann et al. 1962). These substances have growth promoting activity in certain dwarf peas. The growth of dwarf peas was stimulated to such an extent that the dwarf attained the growth habit of the normal. Caffeic and ferulic acids had no such effect on *Nana*₁ and this indicated that an excess of IAA oxidase was not the cause of the dwarfness.

Disc electrophoretic separation of general protein extracts followed by staining for peroxidases furnished strong indication that there was no difference in number or quantity of peroxidases in the coleoptiles. The resolution attained was as good as any reported for peroxidases.

The destruction of auxin seems to have been well dem-

onstrated in Nana₁ by van Overbeek (1935) and al-Salih (1958), but the idea of the destruction being enzymatic does not fit the results of the experimentation done for this thesis. A peroxidase assay system using spectrophotometer showed that the peroxidase activity was nearly the same for the normal and Nana₁ extracts. This, I believe, shows that the large difference in destruction of IAA could not be caused by any difference in peroxidase activity. Other types of inhibitors of IAA activity seem to be little understood at present.

Another experiment makes the situation more confusing. In the maize coleoptile elongation tests the Nana₁ coleoptiles not only responded more strongly to added IAA, but also to lower concentrations than the normal did. If inhibitors had been present in the dwarf, one would think that a higher concentration of IAA would have been required by the dwarf to equal the response of the normal. It should also be noted that no attempt was made to rid the sections of inhibitors. I cut the sections and placed them immediately in the elongation tubes with the test solutions. The results of this experiment indicated that the dwarf needed only more auxin to attain the normal's stature.

The maize coleoptile studies, in addition to the re-

sults of the peroxidase determinations appear to be in contradiction to the findings of van Overbeek (1935).

SUMMARY

1. Extracts made from $Nana_1$ coleoptiles inhibited the elongation of Avena coleoptiles no more than normal coleoptile extracts.

2. Using disc electrophoresis (polyacrylamide gel) and starch gel electrophoresis, extracts made from $Nana_1$ coleoptiles had the same number and intensities of peroxidase bands.

3. Spectrophotometric assays showed that normal and $Nana_1$ extracts had very nearly the same peroxidase activity.

4. Inhibitors of IAA oxidase failed to produce normal growth response in $Nana_1$ seedlings.

5. $Nana_1$ coleoptile sections responded at lower concentrations of IAA and elongated more than the normal coleoptile sections.

6. The experimentation for this thesis showed that excess IAA oxidation was probably not the cause of dwarfness in $Nana_1$. Results also indicated that there may be no extra inhibition of IAA activity in $Nana_1$. The results suggested that lack of an optimum amount of IAA caused the dwarf growth habit in $Nana_1$.

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