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Peroxidase activity in genotypes and genotrophs of *Linum**

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With 3 figures

Introduction

Varieties of flax, *Linum usitatissimum* L., differ considerably in the number and size of the basal branches produced from the axils of the cotyledons. Examples are shown in Fig. 1. These differences are particularly

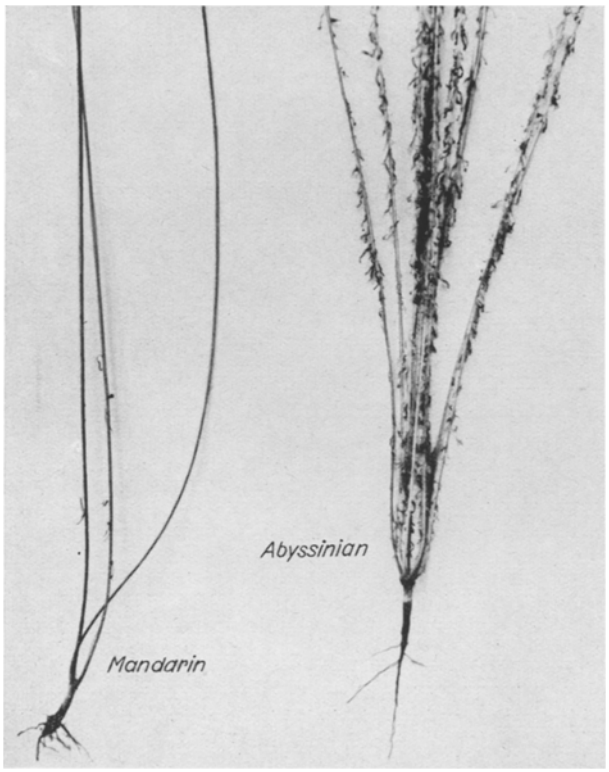

Fig. 1. Comparison of basal branching in two genotypes of *Linum*. Plants grown singly in pots. On the left, Mandarin, a fibre type, used in these studies; on the right, Abyssinian, an uncultivated type with profuse basal branching.

noticeable when the plants are widely spaced, 25 to 30 cms. apart. It was shown by THIMANN and SKOOG (1934) that indole-3-acetic acid (IAA) could suppress the growth of lateral buds when supplied to the apex of a stem in *Vicia faba* from which the terminal bud had been removed. One might suggest that the variation in basal branching noted among flax varieties might be mediated by IAA or by an enzyme system inactivating IAA. As a starting point in investigating the control of branching in flax, peroxidase activity was measured. This enzyme has been described as part of an IAA oxidase system by GALSTON, BONNER and BAKER (1953). Two flax varieties, or genotypes, differing widely in their amount of basal branching, were used, together with a third variety which had been conditioned with fertilizers by DURRANT (1962). The effects of all combinations of N, P and K on the parents and progeny generations of this third variety have been fully described by DURRANT (1962). Two of the most contrasting types of progeny, or genotrophs, produced by DURRANT from this third variety, were used in these studies, since the difference in their branching mimicked that between the two genotypes. A comparison was thus made of peroxidase activity in two flax genotypes produced by selection, and in two flax genotrophs produced by fertilizer treatment; these will be referred to collectively as the four types. A preliminary study was also made of F_1 hybrids between some of these four types. The possibility that peroxidase inhibitors might be present in the four types of material was investigated by measuring activity not only in the raw extracts, but in extracts after dialysis.

Materials and Methods

The flax genotypes used were Royal and Mandarin. Royal, an oil seed flax, branched profusely from the axils of the cotyledons, while Mandarin, a fibre flax, exhibited much less branching. The two flax genotrophs used were third generation progeny from plants of the variety Stormont Cirrus which had been treated with NPK or NK. NPK induced profuse branching in the treated plants and their progeny, while NK, in contrast, induced very little branching. NPK and NK induced changes, then, that caused the progeny to resemble Royal and Mandarin so far as branching was concerned.

One plant each of Royal, Mandarin, NPK and NK, were grown together in 17.5 cm. diameter pots in the greenhouse under a natural daylength of 15 hours. Ten pots were harvested 39 days after sowing; each plant was cut at soil level and the fresh weight of its basal branches and of the main stem obtained. The plants were stored at −10 °C until assayed for peroxidase. A further sample of 10 pots was harvested when the plants were all entering the early flowering phase 62 days after sowing.

Before assay, while the material was held at −10 °C, the leaves were separated from the stems, and then leaves and stems were macerated. Two samples of 10 g. were weighed out from leaf and stem material of each of the four types harvested at 62 days, and one 10 g. sample from the 39 day harvest. Each 10 g. sample was broken up in a Serval Omni mixer in 200 ml. of distilled water, and the extract then centrifuged at 3000 R.P.M. for 6 minutes to remove debris. The supernatant was used for peroxidase estimation.

Peroxidase activity was measured with a modified form of the guaiacol technique described by MAEHLY and CHANCE (1954). Four aliquots of 0.5 ml. each were taken from the extract derived from each 10 g. sample, and these aliquots were placed in spectrophotometer tubes. Three ml. of 10 mM phosphate buffer at pH 7.0 plus 3 ml. of 20 mM guaiacol were added to each tube. The percentage transmittance at a wavelength of 470 μm was then read on a Bausch

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and Lomb spectronic 20 and the reaction started by adding 1 ml. of 10 mM hydrogen peroxide. Five readings of percentage transmittance were made at 60 second intervals after the start of the reaction. The 6 readings were converted to optical density values; the regression of optical density on time was linear. The 62 day harvest supplied two 10 g. samples of leaves and of stems of each of the four plant types. There were, therefore, 8 sets of optical density/time data for leaf or for stem material of any one type. From these 8 sets of data, the joint regression of optical density on time was calculated, and the peroxidase activity could then be expressed as the rate of increase in optical density per minute. Comparisons among the four types were made by using the standard errors of the regression coefficients and a '*t*' test. Samples for extraction and measurement were drawn from cold storage at random, and room temperature during measurement was kept constant. The 39 day harvest provided only one 10 g. sample of leaf or of stem material for each plant type, and the regression coefficients in this case were each obtained from only four sets of data.

Table 1. *Fresh weights (g) and percentage basal branching*[1] *of genotypes and genotrophs of Linum at two stages of growth.* Each figure is the mean of 10 plants.

Days after sowing	Genotypes: Royal: Plant fresh weight	Royal: % basal branches	Mandarin: Plant fresh weight	Mandarin: % basal branches	Genotrophs: NPK: Plant fresh weight	NPK: % basal branches	NK: Plant fresh weight	NK: % basal branches
39	27.45	36.9	22.79	25.9	23.62	51.8	20.03	18.4
62	96.87	59.6	75.82	38.5	80.32	64.2	53.41	25.2

[1] Percentage basal branching calculated as: $\frac{100 \times \text{weight of basal branches}}{\text{Total plant weight}}$

The residual supernatant from each sample was placed in a cylindrical dialysis membrane and dialyzed against running tap water for 24 hours. The peroxidase activity was remeasured using the technique described above.

In addition to the measurements made above, peroxidase activity was measured in Royal, Mandarin NPK, and NK, and in F_1's from crosses of Royal by Mandarin, NPK by NK, Royal by NPK, and Royal by NK. One plant each of the two parents and the two F_1's (reciprocals) of a given cross was grown per 17.5 cm. diameter pot in the greenhouse under a natural daylength of 18 hours. Two pots per cross were assayed. The plants were cut at soil level after 20 days growth, weighed fresh, and each whole plant individually assayed for peroxidase immediately after cutting. The extracts were prepared in the same way as described above, but the volume of the aliquot added to the spectrophotometer tube was adjusted so that equal fresh weights of plant material of the four plants in one pot were being tested. The estimation of peroxidase here was based on a technique described by GUTHRIE (1931) in which a buffer at pH 4.5 was employed together with a p-phenylene diamine hydrochloride and α-naphthol reagent to track the release of oxygen from hydrogen peroxide by the enzyme. Optical density readings were obtained at a wavelength of 540 μm over a 10 minute period, and from each set of 10 readings the regression of optical density on time was calculated. Peroxidase activity was thus expressed, as before, in terms of increase in optical density per minute, and activities were compared by using a '*t*' test. This second technique gave comparable results to that using guaiacol.

Experimental Results

Significant differences (P 0.05–0.01) occurred among the four types for total plant weight and weight of basal branches at both samplings. Royal produced more basal branches than Mandarin, and NPK more than NK. The total plant weights and percentage basal branches are shown in Table 1.

Peroxidase activities at the two growth stages of the four types are shown in Table 2, and as an histogram in Fig. 2. The differences between Royal and

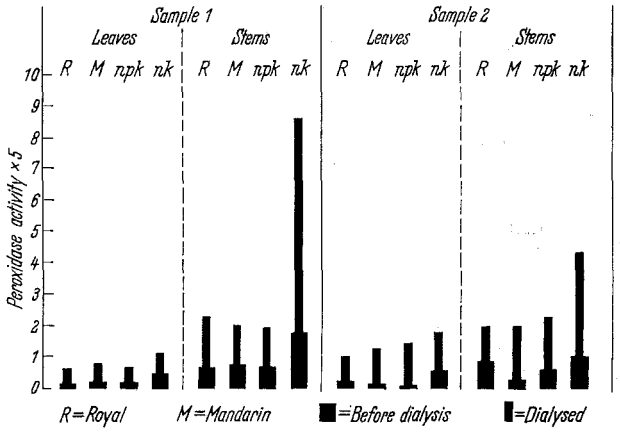

Fig. 2. Peroxidase activity in genotypes and genotrophs of *Linum* measured at two growth stages. Sample 1 consisted of plants harvested after 39 days growth; sample 2 of plants harvested after 62 days growth.

Mandarin, and between NPK and NK were all significant at P 0.05–0.01, with the exception of the Royal and Mandarin dialyzed stem extracts at the first and second sampling. It can be seen from Table 2 and Fig. 2 that, prior to dialysis, leaves and stems of Mandarin had a higher activity than Royal at the 39 day stage, whereas at the 62 day stage the reverse occurred. After dialysis, Mandarin leaves alone contained more activity than Royal at the two

Table 2. *Peroxidase activity per unit of fresh weight in genotypes and genotrophs of Linum.* Activity measured before and after dialysis of extracts, and expressed as rate of increase in optical density per minute at 470 μm

Harvested	Part	Genotypes: Royal	Genotypes: Mandarin	Genotrophs: NPK	Genotrophs: NK
Before dialysis					
39 days	Leaf	.030	.039	.043	.094
39 days	Stem	.144	.159	.153	.364
62 days	Leaf	.046	.030	.016	.109
62 days	Stem	.169	.045	.116	.192
Dialyzed					
39 days	Leaf	.125	.161	.142	.228
39 days	Stem	.467[1]	.419[1]	.398	1.720
62 days	Leaf	.199	.254	.282	.348
62 days	Stem	.390[2]	.393[2]	.439	.853

All comparisons of Royal with Mandarin and NPK with NK were significant except those marked 1 and 2.

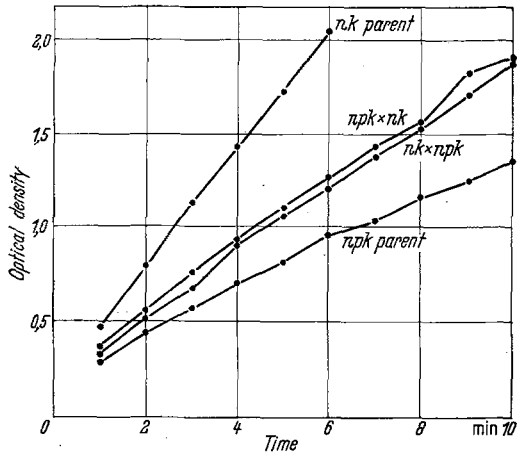

Fig. 3. Peroxidase activity in two genotrophs of *Linum*. Optical density readings plotted directly against time.

growth stages. NK, both before and after dialysis, at both growth stages, and in both leaf and stem, showed more peroxidase activity than NPK.

Dialysis of the extracts produced a very large rise in peroxidase activity, suggesting the removal of an inhibitor. This was confirmed by concentrating the dialysate and adding it to a dialyzed extract; activity was reduced. The nature of the inhibitor(s) removed by dialysis is being investigated.

Peroxidase activity was higher in the stems than in the leaves. The stems contained more inhibitor(s) than the leaves at both samplings on the basis of the differences between dialyzed and non-dialyzed extract activities. There appeared to be a rise in the inhibitor(s) level in the leaves in the second sample.

The data on peroxidase activity measured after 20 days growth in the four plant types and in some of their F_1's are shown in Table 3. In the NPK and NK cross, NK was significantly higher than NPK in activity, but there was no significant difference between the reciprocals; the graph of optical density against time (Fig. 3) shows this. In the Royal by Mandarin cross, Royal was significantly higher in activity, and in one case a significant reciprocal difference occurred. The Royal by NPK cross showed a significant difference between the parents, Royal being higher in activity, but no reciprocal differences. The reciprocals lay closer to NPK than Royal. The final Royal by NK cross showed no significant parental difference but in one case a significant reciprocal difference.

Table 3. *Peroxidase activity per unit of fresh weight in genotypes and genotrophs of Linum, 20 days after sowing.* Activity measured on individual plants and expressed as rate of increase in optical density per minute at 540 μm

Parents and F_2's	Test 1			Test 2		
NPK	0.13			0.13		
NPK × NK	0.18	N.S.	**	0.16	N.S.	**
NK × NPK	0.18			0.16		
NK	0.32			0.23		
Royal (R)	0.29			0.30		
R × M	0.17	**	**	0.23	N.S.	**
M × R	0.21			0.22		
Mandarin (M)	0.14			0.16		
Royal	0.79			0.43		
R × NPK	0.46	N.S.	**	0.29	N.S.	**
NPK × R	0.45			0.30		
NPK	0.23			0.24		
Royal	0.28			0.22		
R × NK	0.29	N.S.	N.S.	0.20	*	N.S.
NK × R	0.28			0.23		
NK	0.28			0.22		

N.S. = Not significant
* = Significant at P 0.05
** = Significant at P 0.01

{ Smaller brackets indicate comparison of reciprocals.
} Larger brackets indicate comparison of parents.

Discussion

The results obtained from peroxidase assay with 20 day old seedlings showed Royal and NK to be higher in activity than Mandarin and NPK respectively. Although the number of plants involved in these particular comparisons was extremely small, additional assays at this stage with these four types were made, and were completely consistent, supporting the results above. The data pertaining to the F_1's in table 3 can only be regarded as suggestive. One point in connection with the NPK × NK cross may be made. In similar crosses made by DURRANT (1962), it was found that when total fresh weights of the plants were measured at maturity no reciprocal differences occurred. This was essentially the same as the finding here, which may be taken then as an additional support to the hypothesis that fertilizer induced changes are nuclear.

The more extensive assays of Royal, Mandarin, NPK and NK at two growth stages (Table 2 and Fig. 2) introduced some complications. A reversal in the relative levels of Royal and Mandarin peroxidase activity (undialyzed extracts) occurred between the first and second samples, and only the second (62 day or early flowering phase) showed agreement with the data from 20 day old seedlings (Table 3) discussed earlier. The essential differences in environment for the two sets of experiments were daylength and temperature; the 20 day old seedlings were grown in a longer daylength and lower temperature. SIEGEL and GALSTON (1955) have demonstrated enhancement of IAA oxidase activity by visible light, and the difference in daylength in the experiments reported here might have resulted in different rates of development of peroxidase activity. This discrepancy between the experimental results emphasizes the need to examine the effects of light and temperature, as well as different growth stages.

In examining the data from dialyzed stem extracts (Table 2 and Fig. 2) it was noteworthy that in the 62 day stage, the sparsely branched NK and Mandarin appeared to contain more inhibitor(s), (dialyzed-undialyzed levels in Fig. 2) than the profusely branched Royal and NPK. This was the only common element in the four types which might conceivably link peroxidase activity via IAA concentration to the amount of basal branching, assuming, for example, that low peroxidase resulted in high IAA concentration and thus inhibition of basal buds. However, there were several confusing factors in the data which made it difficult to accept this. Not the least of these were the degree of branching in Royal at 39 days, the Royal and Mandarin stem inhibitor(s) levels, and the very small difference in their leaf inhibitor(s) levels (see Fig. 2).

It is clear that an essential corollary to these experiments is a study of actual IAA levels and IAA destroying activity in the four types. Furthermore,

it has been assumed that IAA has the single role of co-ordinating growth in various organs or parts of the flax plant. This may not be entirely true; for example, the work of Ross (1948) on *Epilobium* species crosses, in which he found a higher peroxidase activity in inhibited compared to uninhibited reciprocals of such crosses, suggests an association between low auxin and dwarfism. This is supported by VAN OVERBEEK's (1935) work with a dwarf and a normal variety of maize; the dwarf contained less auxin and destroyed more exogenously applied auxin than the normal variety. One might regard the NK form as an induced dwarf compared to NPK and thus separate the roles of IAA into one of possible co-ordination in Royal and Mandarin, and one of overall growth control in NPK and NK.

These studies have essentially raised more questions than they have answered. A much broader range of both genotypes and genotrophs will have to be examined, not only for peroxidase and peroxidase inhibitor(s) but IAA content, and response to exogenous IAA and IAA inhibitors, before clear conclusions on the control of branching in flax can be drawn.

Summary

Peroxidase activity was measured in two sets of experiments with the flax genotypes Royal and Mandarin, and the flax genotrophs NPK and NK produced by NPK and NK fertilizer treatments of Stormont Cirrus.

Royal and NPK produced more basal branches than Mandarin and NK. Royal was higher, except in one case, than Mandarin in peroxidase activity, while NK was higher than NPK.

Dialysis of the extracts caused a marked rise in peroxidase activity through removal of an inhibitor. Stems contained more peroxidase activity than leaves both before and after dialysis of the two samples.

F_1 hybrids between NPK and NK showed no reciprocal differences in peroxidase activity.

Zusammenfassung

Die beiden Leinsorten Royal und Mandarin unterscheiden sich wesentlich in der Intensität der basalen Verzweigung. Ähnliche Unterschiede weisen zwei „Genotrophe" der Leinsorte Stormont Cirrus auf; sie entstanden durch die über mehrere Generationen anhaltende Wirkung einer NPK- bzw. einer NK-Düngung. Um dem physiologischen Steuerungsmechanismus der unterschiedlichen basalen Verzweigung näherzukommen, wurde die Peroxydaseaktivität in zwei Versuchsreihen gemessen, und zwar an den beiden Genotypen bzw. Sorten Royal und Mandarin und an den beiden „Genotrophen" NPK und NK der Sorte Stormont Cirrus (Nachkommenschaften behandelter Pflanzen in der dritten Generation).

Royal und NPK hatten eine größere Anzahl basaler Zweige als Mandarin und NK. Die Peroxydaseaktivität war, mit einer Ausnahme, bei Royal höher als bei Mandarin, dagegen bei NK höher als bei NPK.

Dialyse der Extrakte verursachte ein beträchtliches Ansteigen der Peroxydaseaktivität infolge der Beseitigung eines Inhibitors. Die Stengel hatten eine höhere Peroxydaseaktivität als die Blätter, sowohl vor wie nach der Dialyse der beiden Proben.

Auch F_1-Hybriden verschiedener reziproker Kombinationen wurden auf ihre Peroxydaseaktivität hin untersucht. In einzelnen Fällen ergaben sich signifikante Reziprokenunterschiede, nicht jedoch bei „F_1-Hybriden" zwischen NPK und NK.

Intensivere Untersuchungen sind notwendig, bevor klare Schlüsse hinsichtlich der Ursache unterschiedlicher Verzweigung gezogen werden können.

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Untersuchungen zum Bestäubungsvorgang und der Samenentwicklung bei Birkenartkreuzungen

Von **IRMGARD EIFLER**

Mit 5 Abbildungen

Jahrelange, umfassende Kreuzungsarbeiten innerhalb der Gattung *Betula* führten zu grundlegenden Erkenntnissen über die Kombinationsmöglichkeiten zwischen verschiedenen Birkenarten, besonders zwischen *Betula pendula* und *Betula pubescens*, die an anderer Stelle bereits publiziert worden sind (EIFLER, 1956, 1958, 1960). Die in dieser Arbeit beschriebenen Überprüfungen des Bestäubungsvorganges und der Samenentwicklung bringen uns interessante Hinweise zur Erklärung bestimmter Unterschiede im