Studies on Branching Habits in Crop Plants

VII. On auxin concentration, indoleacetic acid oxidase activity and comparative amount of its inhibitor in stem tissue in relation to the tillering character in two varieties of corn*

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INTRODUCTION

Branching is a very important character for crop production, as it affects the yield and the quality of crop products. It is known that the growth of branch is controlled by auxin since the work of Thimann and Skoog on bud development of *Vicia faba*,²⁵⁾ although nutrient substances, of course, are necessary for the growth, and other kinds of growth substances such as gibberellin and cytokinin have something to do with the growth.^{19,26)}

As to the relationship between the growth of lateral buds and auxin, it has been said that auxin produced at apical part of a stem inhibits the growth, though the mechanism how auxin inhibits the growth remains still obscure. As another important factor which controls the auxin concentration in plant tissue than the synthesis and translocation, the destruction of indoleacetic acid (IAA), the most important auxin in higher plants, by an enzyme system "indoleacetic acid oxidase" is known in many kinds of plants.¹⁸⁾ Furthermore, the existence of some kinds of naturally occurring or synthetic substances which inhibit or promote the action of IAA oxidase have been found^{10,15,16,18,20,24)}; kaempferol-3-triglucoside (KG), its *p*-coumaric acid ester (KGC), quercetin-3-triglucoside (QG) and its *p*-coumaric acid ester (QGC) in pea plants,^{7,8,17)} and gossypol in cotton plants.^{16,23)} The IAA oxidase and its inhibitor present in the tissue seem to control the growth and other phenomena

which are known to be controlled by auxin.

The present paper deals with auxin concentration, activity of partly-purified IAA oxidase and comparative amount of inhibitor with reference to the varietal characteristics about tillering in corn plants.

MATERIALS AND METHODS

Two varieties of corn (*Zea mays* L.), Golden Cross Bantam (GC) as a good-tillering variety and White Dent Corn (WD) as a poor-tillering one, were used as the material plants, whose seeds were obtained from Yamato Seeds and Implements Co. Ltd., Tokyo. The experiments were carried out at Komaba, Tokyo in 1967. The corn plants were grown twice a season in the field under natural condition. The first crop (Expt. I) were sown on May 13 and the second crop (Expt. II) on July 10. The plants were harvested at leaf age 5*. After leaves were taken off as completely as possible, the young stem upper than the middle of internode between the 2nd node and the 3rd were collected and divided into 3 parts, the 3rd node as the lower, the 4th and 5th nodes as the middle and the upper part above the 6th node as the tip respectively, and then frozen with dry ice immediately. After freezing, the tissue was lyophilized and kept at -20°C in a deep freezer until use.

Auxin was extracted from the tissue with ether. After centrifugation of the brei, the supernatant was evaporated to dryness at 40°C under lower pressure, and the residue was redissolved in a mixture of acetonitrile and *n*-hexane. With hexane layer discarded, the acetonitrile layer was evaporated to dryness, redissolved in 1 ml ether, and separated by paperchromatography with isopropanol: ammonia:

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water (8:1:1). Amount of auxin was estimated by Avena curvature test.

For estimating the activity of IAA oxidase, crude enzyme solution was prepared as follows; the stem tissue was ground in 1/50 M phosphate buffer pH 5.6, 2 ml for each mg dry weight of tissue, with a Potter-Elvehjem homogenizer in an ice bath, extracted at 0°C for 30 min and centrifuged at $3,000\times g$ for 10 min. The supernatant liquid was used as crude enzyme preparation. Since a great amount of IAA oxidase inhibitor was found in stem of corn,¹³⁾ the enzyme solution was treated with cold acetone in order to remove the inhibitor by the technique reported previously¹³⁾; that is, 2.5 ml cold acetone was added into each ml crude enzyme preparation, the mixture was centrifuged at $3,000\times g$ for 3 min, the precipitate was redissolved in the same volume of the same buffer as the original, and kept at 0°C overnight and centrifuged again at $3,000\times g$ for 5 min. The supernatant liquid was used as purified enzyme preparation.

In order to estimate the IAA oxidase activity, the concentration of IAA in the reaction mixture was measured colorimetrically with Salkowski reagent at 530 m μ at definite interval.¹³⁾ The composition of the reaction mixture was the same as described in the previous paper.¹³⁾ The reaction mixture in a test tube was kept at 26°C in a water bath. The reaction by purified enzyme proceeded under diffusing light.

The inhibitor was extracted with 10 ml methanol, which was found appropriate for extraction by a preliminary experiment described later, for 5 mg dry weight of tissue, and was made into 50 ml solution with 1/50 M buffer pH 5.6 after methanol was evaporated away. One ml of the inhibitor solution and 4 ml buffer were added into reaction mixture instead of 5 ml buffer. The destruction of IAA was followed and compared with that of control without inhibitor) and the "degree of inhibition" of IAA destruction was used as an index of comparative amount of the inhibitor. The standard purified enzyme was prepared from the stem of GC. The reaction for the inhibitor assay proceeded in complete darkness.⁹⁾

The data presented in this paper are averages of 3 replication unless any special description is given.

RESULTS

1. The growth of tillers

In a preliminary experiment conducted in 1965, it was found that the 2nd and 3rd tillers of GC were bigger than those of WD, but there were a slight or no difference in the tillers upper than the 4th node between the two varieties at leaf age 5, although the upper tillers of GC were bigger than those of WD at the later age. The length of tillers of the two varieties in this experiment is shown in table 1. In the table, big varietal difference was observed in the 3rd tiller at leaf age 5, and in the 4th to 6th tillers at leaf age 6 or 8. So, the harvest time (leaf age 5) in the experiment corresponded to the stage when the varietal differences in the 4th and the 5th tillers were about to be seen.

2. Concentration of auxin in each part of stem of the two varieties

The histograms of auxin extracted from each part of stems are presented in fig. 1. The result of Expt. I is average of 4 replication. The amount of auxin is expressed as IAA. The region of Rf 0.3 to 0.5 corresponds to IAA, with referring the result

Table 1. Length of tiller on each node at leaf age 5*, 6 and 8.

Expt. No.	Node	Variety	Length of tiller in mm at leaf age		
			5	6	8
I	3	GC	87.3	177.7	
		WD	7.1	7.7	
	4	GC	5.6	39.0	
		WD	4.6	7.5	
	5	GC	2.5	6.8	34.0
		WD	2.7	7.4	17.9
II	6	GC		3.4	19.2
		WD		4.3	15.8
	3	GC	67.4	151.6	560.7
		WD	8.2	12.2	8.8
	4	GC	4.8	19.5	39.9
		WD	4.4	7.6	9.5
	5	GC	2.0	5.4	36.5
		WD	2.0	4.1	9.1
	6	GC		2.5	56.4
		WD		2.5	14.3

* At leaf age 5, the plants were harvested for assay of auxin, IAA oxidase and its inhibitor.

Fig. 1. Histogram of auxin from stem tissue of two varieties of corn.

of assay, with synthetic IAA, conducted in parallel. The concentration of auxin is presented in fig. 2. The whole length of column represents the total amount of auxin, the lower part of the column represents IAA and the upper part the other auxins. Fig 2 shows that WD contained higher level of auxin than GC in each part, especially in tip part, of the stem, and the auxin content in the stem diminished from upper part to lower part.

Fig. 2. Varietal difference of auxin content in stem tissue of two varieties of corn.

3. The time course of IAA destruction by purified IAA oxidase and its difference between WD and GC.

The time course of IAA destruction by purified IAA oxidase from each part is presented in fig 3. It shows that the reaction is first order, and the activity of IAA oxidase can be represented by a first order reaction velocity constant, k , as follows;¹³⁾

$$k = \frac{2.303}{t} \log_{10} \frac{a_0}{a_t}$$

Signs a_0 and a_t are the concentrations of IAA at time 0 and t respectively.

IAA oxidase activities of the two varieties are shown in fig 4. Higher activity was obtained from GC than from WD especially in tip part of the stem.

4. Contents of inhibitor in stem tissue and its difference between the two varieties

An preliminary experiment was done in order to find out an appropriate solvent for extraction of inhibitor. Methanol, ethanol and *n*-butanol were tested. The supernatant liquid after precipitating enzyme from crude enzyme solution with acetone was also tested at the same time. The time course of IAA destruction with inhibitors extracted by thees

Fig. 3. Time course of IAA destruction by purified IAA oxidase from stem tissue of two varieties of corn.

solvents and without inhibitor as a control are shown in fig 5. From this result, methanol was found to

Fig. 4. Varietal difference of IAA-oxidase activity from stem tissue of two varieties of corn.

Fig. 5. Time course of IAA destruction in reaction mixture with inhibitor extracted from stem tissue of corn by various kinds of solvents.

be the best solvent. Methanol, therefore, was used for extraction in this experiment. The comparative amount of inhibitor was expressed with an index value "degree of inhibition" as seen in fig 6. Signs a_0 and a_t are IAA concentrations at the starting point and time t of the reaction respectively. Time t is an appropriate but definite time when the amount of IAA which was destroyed in the reaction mixture with inhibitor whose inhibiting action was the smallest of those 6 samples tested reached about 80% of the amount of IAA destroyed in the control mixture (without inhibitor). Sign a_T is IAA concentration in

Fig. 6. Calculation of "degree of inhibition" as an index of comparative amount of IAA-oxidase inhibitor from stem tissue of corn.

Fig. 7. Varietal difference of comparative amount of inhibitor expressed by "degree of inhibition" from stem tissue of two varieties of corn.

control at time t . The "degree of inhibition" is calculated by the formula,

$$(a_t - a_T) \times 100 / (a_0 - a_T) \%$$

The comparative amount of inhibitor is presented in fig 7. Value of the middle part in Expt. I is the average of 7 replication. It is clear that inhibitor activity diminishes from the lower to the upper and higher activity of inhibitor is contained in WD than in GC at the tip part.

DISCUSSION

From the present study, it was demonstrated that a corn variety GC with good-tillering ability contains less auxin, of which IAA is the majority, more active IAA oxidase and less IAA oxidase inhibitor than a variety WD with poor-tillering ability, at stage just before the varietal difference in tiller growth becomes evident. So, these biochemical characteristics may have a causal relationship with tillering character.

Auxin are known to play an important role in apical dominance.^{3,6,14,25,27} Cutting off the tip of stem promotes the growth of lateral buds, treatment of top with auxin inhibits the growth of buds, and auxin is produced at the tip of stem and translocated downwards. These facts seem to point out that higher auxin level in stem inhibits the growth of lateral buds. Some hypotheses have been proposed on the mechanism of auxin action to the growth of branches. Went, more than 30 years ago, explained that some elements necessary for the growth are drawn up to a site of auxin production.²⁶ Another explanation was that the optimal auxin level for the growth is different among organs; highest for shoots, medium for lateral buds and lowest for roots.² Sachs

and Thimann, however, obtained interesting results on this problem recently.²² A small amount of auxin or kinetin applied to lateral buds of light-grown pea seedlings induced a little but actual elongation of the lateral buds. So, they said that there was no evidence for growth inhibition due to auxin. Recently, Hanada studied the effect of auxin concentration on the growth of tiller buds and apical buds of rice and barley in vitro.¹² In that case, also, no tendency of lower optimal concentration of auxin for tiller bud growth was found. Taking into account these results, it seems likely that higher auxin level in stem inhibits the growth of lateral buds, just as in the case of abscission which is controlled by the auxin level gradient across the abscission layer.^{5,20}

There are some results which suggest the existence of relationship between action of IAA oxidase and some physiological phenomena such as growth or abscission. Bottomley et al⁴ and Goren and Galston¹¹ reported that irradiation of red light to dark-grown pea plants promoted the growth of plumule by about 1.5 times of dark control and increased the amount of IAA oxidase inhibitors, QG and QGC, in the same organ. This fact was confirmed later by Hanada. Another results about the similar phenomenon was reported by Russell and Galston.²¹ They observed that red light caused the suppression of elongation of stem internode and simultaneous increase of KG and KGC which are thought to promote IAA oxidase at the concentration occurring naturally in pea plants. Those changes of flavonoids and growth observed in plumule and internode were completely reversed by the following far red light irradiation.

Another response of plant can be seen in the report of Schwertner and Morgan on abscission in cotton.²³ They applied IAA oxidase inhibitors or promoters to petioles. IAA oxidase promoters (2,4-dichlorophenol, manganese chloride, methyl umbelliferone and resorcinol) promoted the abscission, and IAA oxidase inhibitors (catechol, caffeic acid, quercitrin and gossypol) delayed the abscission. And, decarboxylation of ¹⁴C-IAA was promoted by 2,4-dichlorophenol and inhibited by catechol.

The present results seem to point out the responsibility of IAA oxidase and its inhibitor for the

difference in auxin concentration in stem, and in its turn, for the difference in the tillering ability between the varieties. But, whether any difference exists in the rate of auxin synthesis at tip part of the stem between the varieties still remains obscure.

SUMMARY

Relation of auxin concentration, IAA oxidase activity and comparative amount of IAA oxidase inhibitor in stem tissue to the tillering ability of corn was studied by using two varieties of corn, GC as a good-tillering variety and WD as a poor-tillering one.

The stem from light-grown corn seedlings at leaf age 5 was divided into 3 parts and used for experiment. As the results of experiment, it was demonstrated that GC contained lower amount of auxin, of which IAA was the majority, higher IAA oxidase activity and less IAA oxidase inhibitor in stem tissue than those of WD. These observations suggest the responsibility of IAA oxidase and its inhibitor for varietal difference in auxin concentration, and in its turn for varietal difference in tillering ability.

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〔和 文 摘 要〕

作物の分枝性に関する研究

第7報　とうもろこし品種の分げつ性と茎中のオーキシシン含量、IAA 酸化酵素活性およびその阻害物質含量との関係について

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とうもろこしについて、品種の分げつ性とオーキシシンとの関係を知る目的でこの研究を行なった。分げつ性の低いホワイトデント (WD) と分げつ性の高いゴールデンクロスバントム (GC) を戸外で栽培し、第5葉抽出完了期に採取して下位節として第3節、中位節としての第4、5節および先端部の第6節以上の3部分に分け、そのオーキシシン含量、IAA 酸化酵素活性および酵素阻害物質の量について測定比較した。なお、分げつの状態は、材料採取時には、第2、3節分げつでは品種間差異が明らか (GC>WD) であり、第4節以上の分げつ芽では採取当時は差がなく、その後差が現われた (GC>WD)。オーキシシン含量については、先端、中位節、下位節いずれの部分でもWDの方がGCよりも著しく高い値を示した。また、WDはGCよりも阻害物質を除いた純化 IAA 酸化酵素の活性が低く、また阻害物質の量が多かった。そしてこの差は先端部において著しかった。

従来、植物の分枝の発育にオーキシシンが関係する、すなわち頂芽で生産されて基部に向って転流するオーキシシンが側芽の生長を抑制すると考えられている。一方、植物体内のオーキシシン濃度を左右するものに IAA 酸化酵素と、その阻害物質および促進物質が存在することが知られている。実際に、IAA 酸化酵素の阻害物質あるいは促進物質が、えんどうでの頂芽および節間の生長、さらに棉での落葉に関係することを示す実験結果が報告されており、IAA 酸化酵素系がオーキシシン濃度を通じてこれらの現象に関与することを暗示している。この実験においても、とうもろこしの分枝性について、茎中のオーキシシン含量の高いWDがその低いGCよりも分げつ性が弱く、茎中の高濃度のオーキシシンが分げつの発育を抑えていること、さらにこのようなオーキシシン濃度の差を起こす有力な原因として茎中の IAA 酸化酵素活力ならびに阻害物質の量の差があることが考えられる。