

## Endogenous Factors Controlling Stem Growth Habit of Soybean Plants

### I. Comparison of abscisic acid levels in determinate and indeterminate types

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**Abstract** : Soybean plants show diversity in stem growth habit ranging from determinate type to indeterminate type. Stem growth of determinate type plants abruptly terminates near flowering, while that of indeterminate type plants continues after flowering. In order to elucidate endogenous factors controlling such growth habit of soybean plants, ABA levels in leaves and pods of determinate and indeterminate isolines were compared. No differences were found in ABA levels, which suggests that ABA is not involved in stem termination of soybean plants. Some unknown factors seem to cause the stem termination.

**Key words** : Abscisic acid, *Glycine max*, Stem termination.

**ダイズの伸育型を決定する内生要因** 第1報 有限型と無限型のアブシジン酸含量の比較：幸田泰則・由田宏一・後藤寛治・岡澤養三（北海道大学農学部）

**要旨**：ダイズには、開花後まもなく茎の伸長が停止する有限伸育型と、開花後も伸長を続ける無限伸育型がある。伸育型のみが異なる同質遺伝子系統が分離されていることから、開花と茎の伸長停止の両現象を誘起する内生要因はそれぞれ別のものであると考えられる。茎の伸長停止を引き起こす内生要因を究明するため、有限型と無限型の同質遺伝子系統を用いて葉及び莢のアブシジン酸含量の経時的変動を比較した。アブシジン酸含量は両型共ほぼ等しく推移し、差異は認められなかった。したがってダイズの伸育型の決定にアブシジン酸は関与していないと考えられた。

**キーワード**：アブシジン酸、伸育型、ダイズ。

Soybean plants show diversity in the stem growth habit<sup>4)</sup>. Most varieties can be classified into three categories ; indeterminate type, semi-determinate type and determinate type<sup>1)</sup>. Indeterminate plants continue growth in stem length and leaf production after commencement of flowering, while determinate plants abruptly terminate stem growth near flowering. Usually the stem termination is brought about by cessation of growth at the apical meristem.

The stem termination and the flowering are induced by short days. Bernard<sup>1)</sup> reported that inheritance of the determinate and indeterminate types is under monogenic control, and he developed isolines of determinate and indeterminate types. This indicates that the endogenous factor controlling the stem termination is different from that of flowering. The stem termination may be induced by some growth inhibitor(s) and the flowering by unknown "flowering hormone".

Quebedeaux et al<sup>3)</sup>, reported a large amount of abscisic acid (ABA) in immature soybean seeds. This suggests the possibility that ABA produced by immature seeds causes

the termination of stem growth. The present study was carried out to see whether or not ABA is implicated in the termination of the stem growth of soybean, using a pair of determinate and indeterminate isolines developed by Bernard<sup>1)</sup>.

#### Materials and Methods

A pair of determinate (dt<sub>1</sub>) and indeterminate (Dt<sub>1</sub>) isolines of soybean [*Glycine max* (L.) Merrill cv. Harosoy] was planted in an experimental field on 20 May, 1986 and raised in the usual manner. Late in July, first flowering was observed at the lowest node. The leaves were harvested four times from five randomly selected plants each and subjected to extraction of ABA. The soil in the field at each sampling time was sufficiently wetted. Immature pods were also harvested twice and subjected to extraction of ABA.

The materials were homogenized immediately with a sufficient amount of ethanol to give a final 70% ethanol extracts, kept overnight at 4 °C and then filtered. The ethanol extract was concentrated *in vacuo* and the resulting aqueous residue was acidified to pH

3.0 and extracted three times with ethyl acetate. The ethyl acetate layer was extracted three times with 1 M sodium bicarbonate. The aqueous layer was adjusted to pH 3.0 and extracted again with ethyl acetate. The ethyl acetate layer was then dried with anhydrous sodium sulfate and evaporated to dryness. The acidic ethyl acetate fraction was then subjected to silicagel TLC developed with toluene : ethyl acetate : acetic acid (50 : 30 : 4 v/v). The silicagel present at zone identical to authentic cis-ABA was scraped off from the chromatogram, and extracted with water-saturated ethyl acetate followed by methanol. The extract was evaporated to dryness and resulting residue was analyzed by high performance liquid chromatography (column, Novapak C<sub>18</sub> : solvent, 60% methanol + 2% acetic acid). The amount of cis-ABA in a sample was calculated from a standard curve constructed from peak area measurements obtained from known amounts of cis-ABA. The recovery of ABA by this isolation procedure was around 60%. The authenticity of ABA was confirmed by EI mass spectrum of collected ABA fractions. The spectrum showed a molecular ion peak at  $m/z$  264.

### Results and Discussion

Time course changes in node number on the main stem of determinate and indeterminate types were shown in Fig. 1. In both types, the flowering at the lowest node was observed 67 days after sowing. Stem growth of determinate type stopped abruptly around flowering. On the contrary indeterminate plants continued to grow after the flowering.

Although ABA levels in the leaves of determinate and indeterminate plants showed a slight fluctuation before and after the commencement of flowering (Fig. 2), no differences were found in ABA levels in them.

ABA levels in immature pods were high and increased sharply with plant growth (Fig. 3). However, the levels in determinate and indeterminate plants were almost equal. Immature pods harvested 81 days after sowing were divided into immature seeds and pod walls, and ABA levels were measured. ABA level in immature seeds was 2183 ng/g fresh weight and that in pod walls was 64 ng/g.

The results obtained here suggest that ABA is not involved in termination of stem growth.

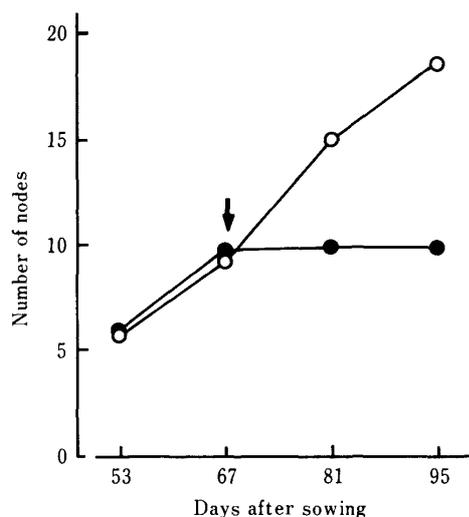


Fig. 1. Changes in node number on main stems of determinate (●) and indeterminate (○) types of soybean. The arrow indicate commencement of flowering at the lowest node.

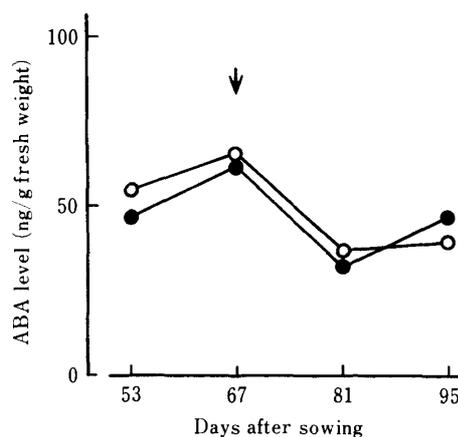


Fig. 2. Changes in ABA levels in soybean leaves of determinate (●) and indeterminate (○) types. The arrow means the same as in Fig. 1.

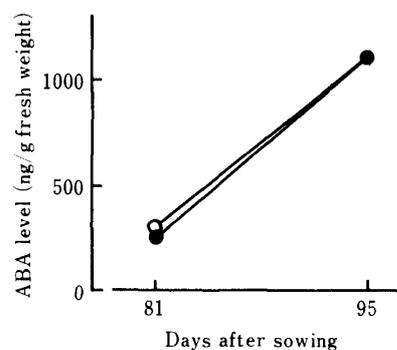


Fig. 3. Changes in ABA levels in soybean pods of determinate (●) and indeterminate (○) types.

As reported by Quebedeaux *et al*<sup>3)</sup>, soybean immature seeds contained extremely high amount of ABA. ABA is known to be a stimulant of soybean callus growth induced by cytokinin<sup>2)</sup>. ABA in immature seeds may play some regulatory role in soybean seed development.

The possibility is still remained that some unknown inhibitor(s) formed in the leaves under short days may cause the termination of stem growth. However, this is a topic of future study.

#### References

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