Effect of Cytokinin and Anticytokinin on the Tillering of Barley*

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Abstract : Cytokinin, N-[2-isopentenyl] adenine (2iP), increased and anticytokinin, 4-chloro-2-cyclobutylamino-6-ethylamino-s-triazine (CCET), retards tillering of barley. Tillering was also shown to be retarded by auxin, α -naphthalene acetic acid (NAA) and promoted by antiauxin, 2, 3, 5-triiodobenzoic acid (TIBA). TIBA and 2iP treatment promoted tillering by altering the parallelism of leaf emergence between main stem and tiller ; and the tillering itself, including secondary or tertiary tillers, was hastened which resulted in more tillers being produced at high leaf positions where the untreated control plants had not produced any tillers. Conversely anticytokinin CCET and auxin NAA inhibited or delayed the release of tiller buds even when corresponding leaves on the main stem had already developed to the point where tiller buds normally occur.

Key words : Antiauxin, Anticytokinin, Auxin, Barley, Cytokinin, Tillering.

オオムギの分げつ発生に及ぼすサイトカイニン及びアンチ・サイトカイニンの影響：菅 洋（東北大学遺伝生態研究センター）・岩村 徹（京都大学農学部）

要 旨：サイトカイニン（2iP）はオオムギの分げつ発生を促進し、アンチ・サイトカイニン（CCET）は分げつ発生を抑制した。同様に、オーキシン（NAA）は分げつ発生を抑制し、アンチ・オーキシン（TIBA）は分げつ発生を促進した。TIBA や 2iP による分げつ発生促進は、主稈葉の展開と同調している、各分げつの発生の平行関係が変わり、第2次及び第3次分げつを含めて、分げつ発生それ自身が促進される結果、無処理の標準区でまだ発生していないような高次の分げつが、発生することによっている。一方、アンチ・サイトカイニンの CCET とオーキシンの NAA は、無処理標準の植物の分げつ発生から考えて、理論的に発生が予測される、分げつの発生が抑制され、あるいは遅延した結果、分げつ数が減少した。

キーワード：アンチ・オーキシン、アンチ・サイトカイニン、オーキシン、オオムギ、サイトカイニン、分げつ。

A first experiment attempting to control the tillering in grasses by auxin application was undertaken by Leopold⁶⁾ with the idea that tillering in grasses would be under the regulation of auxin just as branching in dicotyledonous plant. Evidence supporting this idea has been reported with wheat¹²⁾, rice¹⁴⁾ and oat¹⁾, in which application of TIBA, an antiauxin, promoted tillering.

Later, the knowledge that cytokinins release lateral buds from correlative inhibition in a wide range of species led to the view that these compounds may be limiting for lateral bud growth³⁾.

Exogenously applied kinetin is known to increase tiller growth and the percentage of tiller-bud release in wheat⁵⁾ and oat¹⁾.

However, it is necessary to control the endogenous cytokinin level to know the role of

endogenous cytokinins in the regulation of lateral bud or tiller-bud growth. However, no attempts have been made to control tiller-bud release by the use of anticytokinins probably due to the inavailability of useful anticytokinins in the tillering of grasses.

One of the present authors developed chemicals showing anticytokinin activity^{2,10)} and they proved to act as anticytokinins in the tobacco callus assay system. In this report we present evidence showing anticytokinin inhibits lateral bud (tillering) release in barley plants. And for comparison, the effects of auxin and antiauxin, as well as those of cytokinin on the tillering were also examined. The present report may be the first indication that anticytokinin works well in the experimental system of intact plants.

As geostimulation is known to affect tillering¹⁾, a mutant line that shows an abnormal response to gravity¹³⁾ was also compared with a normal wild-type line.

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Materials and Methods

Seeds of barley, *Hordeum vulgare* L, cv Chikurin Ibaragi No. 1 and its ^{60}Co induced mutant which showed an abnormal response to gravity, were germinated and sown on Oct. 26, 1992 in plastic pots (9.0×8.0 cm) filled with a commercial soil mixture containing 0.5 g N, 1.5 g P and 0.5 g K per kg of soil, Kureha Engei Baido, Kureha Chemicals, Tokyo, in a greenhouse under a natural photoperiod and supplementary illumination by fluorescent lights providing a 24h photoperiod. The greenhouse was heated not lower than 14°C .

Four seeds were sown in a pot. Before chemical application, at the 1.5 leaf stage, two plants were removed and the remaining two plants were grown in pots. Five pots were used for one plot then 10 plants were used for the measurements. Eleven days after the sowing, at the 1.5 leaf stage, chemicals 2, 3, 5-triiodobenzoic acid (TIBA, antiauxin) 1.7×10^{-3} M, α -naphthalene acetic acid (NAA, auxin) 5.4×10^{-3} M, 6-(γ , γ -dimethylallylamino)-purine (N-[2-isopentenyl]adenine, 2iP, cytokinin) 5×10^{-3} M, and 4-chloro-2-cyclobutylamino-6-ethylamino-*s*-triazine (CCET, anticytokinin) 5×10^{-3} M, were sprayed to run off, 1.5 ml per plant, using a 200 ml glass sprayer for chromatography connected to a diafram vacuum pump. Chemicals used in this experiment were commercially supplied from Sigma Chemical Co., St. Louis, Mo., U.S.A. except for antiauxin TIBA and anticytokinin, CCET. TIBA was purchased from Aldrich Chemical Co., Milwaukee, WI., U.S.A.

Tiller release and successive leaf emergence on main stem were recorded periodically for about one month. Tiller nomenclature is almost the same as that used by Katayama⁴⁾ in which the first leaf of tiller carried in the axil of the coleoptile is designated 1/P and the first leaf of tillers carried in the axil of the first, second and third leaf of the main stem are designated as 1/1, 1/2 and 1/3, respectively. Nomenclature of the secondary tillers is also determined and recorded using this system. Thus, for example, the first leaf developed from profile of the first leaf tiller and from first leaf of the first leaf tiller are 1/1P and 1/11, respectively. Tertiary tiller is also recorded according to this system. For example, first

leaf developed from the first leaf of secondary first tiller developed from the first leaf of main culm is recorded as 1/111.

Results

Application of cytokinin (2iP) and antiauxin (TIBA) resulted in the promoted tillering, though the recording of the tiller was made for 35 days after the treatment (Figs. 1, 2 and 3), whereas that of anticytokinin (CCET) and auxin (NAA) inhibited the release of tiller buds compared with untreated control plants (Figs. 1 and 2).

Release of each tiller was recorded periodically, thus percent of plants bearing the indicated tiller was plotted against the number of days after the chemical application as indicated in Fig. 4 and 5. It was evident that application of antiauxin, TIBA and that of cytokinin 2iP promoted special tillers as 1/P. Under the conditions of the present experiment, tiller from the profile node did not appear without chemical application. However TIBA induced appearance of this tiller, 1/P, from all plants, although only 20% of plants bear this tiller in 2iP treated plants.

In addition to this, the emergence of tillers from the leaf position, at which those of the control plants have not occurred, was seen and increased number of secondary and tertiary tillers observed in TIBA and 2iP-treated plants might be due to the hastened tiller production not being linked to the leaf emergence on the main stem (Fig. 4 and 5).

More secondary and tertiary tillers such as 1/4P, 1/P1, 1/11P, 1/3P, 1/1PP, 1/31, 1/41, 1/P2, 1/P1P and 1/111, which can not be seen in untreated control plants, were developed on TIBA or 2iP treated plants. On the other hand, application of auxin, NAA, and that of anticytokinin, CCET, inhibited or delayed the appearance of tiller buds. In plants treated with NAA, 40% of plants did not bear first tiller from the first leaf of main stem, 1/1, and this is greatly delayed in plants treated with anticytokinin, CCET. In plants treated with auxin, NAA, and anticytokinin, CCET, tillers from the fifth leaf of the main stem, 1/5, did not appear within the period of this experiment, until 35 days after the chemical application. Thus the number of tillers per plant was decreased in auxin- and anticytokinin-treated plants as shown in Figs 1 and 2.

Fig. 1. Effect of auxin (NAA), antiauxin (TIBA), cytokinin (2iP) and anticytokinin (CCET) on tillering of barley seedling, Chikurin Ibaragi No. 1.

Fig. 2. Effect of auxin (NAA), antiauxin (TIBA), cytokinin (2iP) and anticytokinin (CCET) on tillering of barley mutant (serpentina) from Chikurin Ibaragi No. 1.

Almost the same tendency with the normal wild-line in the release of tiller buds was observed in not only cytokinin and anticytokinin treated plants but also auxin and antiauxin treated plants in the mutant line showing abnormal response to gravity (Figs. 2 and 5).

Adenylate anticytokinin, 4-cyclobutylamino-2-methylpyrrolo [2, 3-*d*] pyrimidine²⁾, at the concentration of 5×10^{-3} M, was also tested in this experiment, however this agent has almost no inhibitory effect on the tiller-bud release in not only the normal Chikurin Ibaragi No. 1 but also in the induced mutant line. In addition to this, another cytokinin, 6-(furfurylamino) purine (kinetin), was tested but no promotive effect was detected in the concentration tested, 4.6×10^{-3} M (data not shown).

Speed of successive leaf emergence on main stem was recorded, however almost no difference was detected between non treated control and each chemical treatment.

Discussion

In 1967, Sachs and Thimann⁹⁾ indicated that when kinetin is applied directly to the lateral buds of pea seedlings, it releases them from inhibition by the growing apex. Pillay and Railton⁸⁾ also showed that benzyladenine

and zeatin dramatically enhance elongation of pea lateral buds for at least two weeks.

In a recent genetic-engineering experiment, cytokinin levels were increased in whole tobacco and *Arabidopsis thaliana* plants⁷⁾. The most pronounced morphological effort of high cytokinin levels was extensive development of lateral buds. But, a final approach to this problem may arise from the comparison between cytokinin-deficient and normal plants. However, adequate cytokinin-deficient plants are still not available.

In the tillering of monocotyledonous plants, stem segments containing a single node and quiescent lateral bud (tiller) were excised from the bases of oat shoots and used to study the effects of plant hormones on release of lateral buds by Harrison and Kaufman¹⁾. Kinetin stimulates development of tillers. However, this isolated segment was made free from the influence of the apical bud. Again a lateral bud of 3-mm length was isolated and inserted in test-tubes containing kinetin. Leaves and leaf sheaths were split off to expose a lateral bud. Kinetin promoted bud elongation⁵⁾. But in this case, the lateral bud was also made free from the dominance of the apical bud.

Thus, it is necessary to change the en-

Fig. 3. Tiller-bud release as affected by antiauxin (TIBA) and cytokinin (2iP) in barley seedlings of Chikurin Ibaragi No. 1 (from A to C) and of mutant serpentina from Chikurin Ibaragi No. 1 (from D to F). Tiller development as affected by TIBA (B and E) and those by 2iP (C and F) is indicated by arrows (1/1) and arrows with the letter P, which shows tillers from the profile (1/P). A and D are untreated control.

ogenous cytokinin level to know the role of endogenous cytokinin in the regulation of lateral-bud growth. Skoog and Abdul Ghani¹¹⁾ tested the relative activity of cytokinins and antagonists in relating lateral buds of *Pisum* from apical dominance compared with their relative activities in relation to growth of tobacco callus. They found that a range of cytokinins as well as compounds which show anticytokinin activity in bioassay are equally effective in promoting bud growth in the sys-

tem they tested. Therefore, it is remarkable because, in their case, both cytokinins and anticytokinins acted equally, to the same direction, in the promotion of bud growth. In many instances, however, anticytokinin acted as an agonist rather than antagonist. Many unsolved problems, such as the site of chemical binding within a plant for example, will be concerned with the effects of anticytokinins on plants and whether they act as antagonists or not. Kinetin has been reported as a promoter

Fig. 4. Effect of auxin (NAA), antiauxin (TIBA), cytokinin (2iP) and anticytokinin (CCET) on the production of tiller in barley seedlings, Chikurin Ibaragi No. 1. Tillers shown by the symbol FT (foot note) in the column of TIBA include 1/5, 1/41, 1/2P, 1/P1P, 1/1PP and 1/31 and those in the column of 2iP include 1/P1, 1/111, 1/22 and 1/12.

Fig. 5. Effect of auxin (NAA), antiauxin (TIBA), cytokinin (2iP) and anticytokinin (CCET) on the production of tillers in barley seedlings, mutant (serpentina) from Chikurin Ibaragi No. 1. Tillers shown by the symbol FT (foot note) in the column of TIBA include 1/4P, 1/5, 1/P1, 1/P11 and 1/PP.

of tiller-bud release^{1,5)} in oat and wheat. However no promotion was detected in this experiment.

In the present experiment, cytokinin 2iP promoted tillering and anticytokinin (CCET) inhibited tillering. This may be the first case that anticytokinin showed its original activity well in an experimental system using intact plants. Thus, the cytokinins offer a promising line of inquiry into the hormonal control of bud growth but a considerable amount of basic knowledge about endogenous cytokinin biosynthesis, metabolism and distribution is urgently needed³⁾. In intact oat plants, geostimulation was reported to induce tiller release¹⁾, however there is no sign that a mutant line, which does not respond to gravity, responds differently to chemicals applied in the release of tiller buds from that of a corresponding wild-line.

Use of adequate anticytokinin agents must

become the useful tool for studying correlative growth phenomena such like as apical dominance and the release of lateral bud (tiller).

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