Studies on Nitrogen Metabolism in Crop Plants

XI. The changes of abscisic acid and cytokinin-like activity accompanying with growth and senescence in the crop plants*

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INTRODUCTION

Studies of endogenous growth promoters and inhibitors provide an important basis for the understanding of hormonal control of nitrogen metabolism in the crop plants.

In our previous reports¹⁰⁾, it was shown that in the experiments using detached leaf the effect of abscisic acid (ABA) on accelerating senescence was mediated through its inhibitory action on RNA synthesis, which was evidently reversed partially or entirely by kinetin. In the intact plant, however, roots normally supply several cytokinins to the aerial shoot, where they play an important role in the regulation of nitrogen metabolism^{5,10,14)}. However, this ability of roots was nearly lost at the fruiting stage¹⁵⁾ and plant senescence occurred, although this condition was partially reversed if the fruit was removed^{7,16)}.

It has recently been reported that ABA was also found in ears of several species of the *Gramineae* and played a significant role in bringing about plant senescence¹²⁾.

The results so far obtained suggest that there may be present an endogenous ABA-cytokinin relation in regulating growth and senescence of the plant.

The present investigation thus attempts to elucidate the changes in the levels of ABA- and cytokinin-like activity in relation to plant growth and senescence.

MATERIALS AND METHODS

The crop plants used were rice plant (*Oryza sativa* L. var. *Honenwase*) and sweet potato

(*Ipomoea batatas* LAM. var. *Kokei* No. 14).

The rice plants were grown in the water culture with one-half strength of Kasugai's solution in the greenhouse. The plants were collected at the two contrasting growth stages. The first sampling was made at the fourth leaf stage, when the plants were growing vigorously. The second was made at the flowering stage, when the first sign of senescence appeared from the lower leaves. The collected samples were separated into leaves, leaf sheath with culms and roots respectively, and stored in a deep freezer.

Furthermore, in rice plant, it was attempted to study the relationship between the changes in the levels of endogenous growth regulators in ear and plant senescence. For this purpose, the ear was removed at the four growth stages ranging from heading to full ripening, and the effect of ear removal on plant senescence was investigated. The removed ears were collected and stored in a deep freezer.

Tuberous roots of sweet potato were harvested from the field in December and incubated in the growth cabinet at around 30°C. After fifteen days buds began to grow vigorously. At this time tuberous roots were collected and separated into buds, cortex and central cylinder respectively. These samples were cut into pieces and stored in a deep freezer.

Fifty or one hundred grams of frozen materials were extracted by maceration with 80 % methanol. After filtration methanol was evaporated. The aqueous solution was acidified to pH 2.0 and extracted three times with ethyl acetate. Combined ethyl acetate extract was further pu-

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Fig. 1 The changes of abscisic acid-like activity in rice plant at the fourth leaf stage (top) and flowering stage (bottom). Equivalent weight of tissue is 50 g fresh weight. A: leaves, B: leaf sheath with culms, C: roots. Solid line: length of second leaf sheath, Dotted line: length of root.

rified according to the method of the previous report¹²⁾. Paper chromatography of the acidic fraction obtained from the ethyl acetate extract was done on Toyo No. 51 filter paper using the solvent system of isopropanol-ammonium hydroxide (sp. gr. 0.88)-water (10:1:1). The chromatogram was cut into 10 strips and each strip was extracted with 5 ml of water at 3°C in a refrigerator. These aqueous extracts were bioassayed by means of rice seedling growth test^{6,12)}. On the other hand, the remained acidic aqueous fraction after ethyl acetate extraction was passed through a Dowex 50 W-X 4 column and eluted out with 3 N ammonium hydroxide. Paper chromatography of the ammoniacal eluate was done on Toyo No. 51 filter paper using the solvent system of n-butanol-acetic acid-water (12:3:5). The chromatogram was cut into 10 strips, each strip was treated with water as described above and its water extract was bioassayed by means of chlorophyll retention assay¹⁰⁾.

RESULTS

1. Changes of the ABA- and cytokinin-like activity accompanying with senescence of organs such as leaves, leaf sheath with culms and roots of rice plant.

The changes of the ABA-like activity in rice plants at the two con-

Fig. 2 The changes of the cytokinin-like activity in rice plant at the fourth leaf stage (top) and flowering stage (bottom). Equivalent weight of tissue is 50 g fresh weight. A: leaves, B: leaf sheath with culms, C: roots. Lines are the same as in Fig. 1

trasting growth stages are shown in fig. 1. In the histograms, which are based on equivalents to 50 g fresh weight of the three different organs, the ABA-like activity is present at Rf 0.6~0.8 which accords with Rf of the authentic ABA. However, the ABA-like activity of leaves

is higher than those of leaf sheath with culms and roots, and the activity does not so significantly change in leaves at the flowering stage as at the fourth leaf stage.

In contrast, the histograms in fig. 2 show changes of the cytokinin-like activity in rice

Table 1 The effect of ear removal of rice plant at the successive growth stages during ripening periods on development of new tillers.

Removed time of ear	New tillers* developed after heading stage	
	number	plant height(cm)
Heading stage (17/8)	21	55
Milk ripe stage (24/8)	19	53
Yellow ripe stage (31/8)	14	37
Full ripe stage (7/9)	2	10
Not removed	0	0

* Investigated time : 1/10

Fig. 3 The changes of the abscisic acid-like activity (top) and cytokinin-like activity (bottom) in ears during ripening periods. A: heading stage, B: milk ripe stage, C: yellow ripe stage, D: full ripe stage. Lines are the same as in Fig. 1

Fig. 4 The changes of the abscisic acid-like activity and root elongation activity in tuberos root of sweet potato during the budding. A-B: tuberos root at the harvest time, C-E: tuberos root at the budding stage. A and D: cortex, B and E: central cylinder, C: bud. Lines are the same as in Fig. 1

Fig. 5 The changes of cytokinin-like activity in tuberos root of sweet potato during the budding. Equivalent weight of tissue i.e. 500 g fresh weight of tuberos root and 100 g fresh weight of bud. A: tuberos root at the harvest time, B: tuberos root at the budding stage, C: bud at the budding stage.

plants at the two different growth stages. The chromatographic data exhibit the activity found at Rf 0.4~0.5 which corresponded to Rf value of zeatin ribotide as reported previously¹⁴⁾. A

marked decline in the cytokinin-like activity is detected in all three organs at the flowering stage as compared with the fourth leaf stage. Among the three organs, the cytokinin-like activity diminishes markedly in roots at the flowering stage.

It is now evident from these results that in comparison with the endogenous ABA-like activity a striking decline in the endogenous cytokinin-like activity resulted in advancing with organ senescence.

2. Changes of the ABA-and cytokinin-like activity accompanying with ear ripening of rice plant.

It is apparent from table 1 that the removal of ear of rice plant at the successive growth stages from flowering to full ripening delays effectively senescence of the plant. A remarkable retardation in advance of senescence is achieved by removing ear at the

heading and milk ripe stages, and new tillers develop after these stages. However, the removal effect of ear lasts until the full ripe stage and potency of producing new tillers still remains at this stage in spite of apparent yellowing of the plant. These results obtained agree with those of the previous studies^{7,16)} in which it was shown that flowering and fruiting brought about an intensification of sign for plant senescence, although we can not understand exactly what the physiological cause of plant senescence may be. Therefore, it is necessary to investigate further on endogenous growth regulators in relation to ear development.

The changes of the ABA- and cytokinin-like activities in ear during ripening periods are shown in fig. 3. In the histograms which are based on equivalents to 50 g fresh weight, the ABA-like activity inhibiting plant growth is found at Rf 0.6~0.8 and also the root elongation activity at Rf 0.0~0.3. The levels of these activities are very high at the early stage of ear development, and then gradually decreasing toward the full ripe stage.

It is obvious from the above results that the levels of the ABA- and cytokinin-like activities are much higher in ears at the heading and milk ripe stages than in those at the full ripe stage.

3. Changes of the ABA- and cytokinin-like activity accompanying with the budding of tuberous root of sweet potato.

The changes of the ABA-like and root elongation activities in tuberous root of sweet potato are shown in fig. 4. In the histograms, which are based on equivalents to 100 g fresh weight, the ABA-like activity is also found at Rf 0.6~0.8, whilst the root elongation activity is confined to Rf 0.0~0.2. The levels of the ABA-like activity is much higher in cortex and central cylinder of tuberous root at the harvest time than in those of tuberous root at the budding stage, whereas the levels of the root elongation activity show a reverse pattern.

On the other hand, the histograms in fig. 5

show the changes of the cytokinin-like activity in tuberous root of sweet potato at the budding stage. The chromatographic data exhibit the activity found at Rf 0.4~0.5 and 0.7~0.8. It has recently been reported¹³⁾ that an active substance at Rf 0.4~0.5 had the chemical nature as zeatin ribotide and the other probably consists of two substances as zeatin and zeatin riboside. The levels of the cytokinin-like activity are much higher in buds and tuberous root at the budding than in tuberous root at the harvest time.

It became evident that a decrease in the ABA-like activity and an increase in the cytokinin-like activity occurred accompanying with the budding of tuberous root.

DISCUSSION

It has been shown that the ABA-like activity is found in various organs of crop plants. The levels of the ABA-like activity does not significantly change in mature organs as compared with young organs, although the activity is higher in ear and lower in roots. Contrary to the above, the levels of the cytokinin-like activity are much higher in young organs than mature ones, the activity conspicuously decreasing in old roots.

From these results, it may be deduced that organ senescence of rice plant depends upon the levels of cytokinin rather than those of ABA by itself. However, the levels of the ABA- and cytokinin-like activities in ear during the ripening period are different from the other vegetative organs, indicating that the levels of both activities are very high at the early stage of ear development, decreasing toward the full ripe stage. These results well agree with Let- ham's finding⁹⁾ that the activity level of inhibitor was high when intensive cell division occurred in fruit, but low when cell division stopped, whereas the activity of neutral inhibitor in avocado fruit⁴⁾ increased with fruit development, correlating well with the decrease in the fruit growth rate.

It has also recently been reported that ABA can act as a synergist to gibberellins¹⁾ and kinetin²⁾. The similar results were found to exist between ABA and zeatin in bringing about the growth of carrot-root callus, and between ABA and gibberellin A₃ inducing rice internode elongation (Oritani et al., unpublished). The results of previous and present investigations suggest that endogenous ABA act not always as an inhibitor, but also a synergist to the action of the other endogenous growth substances such as gibberellins and cytokinins.

There are some reports^{3,8,16)}, however, that reproductive organs of the plant may be considered as not only a rich reservoir of growth regulators such as cytokinins, abscisic acid, auxin and gibberellins for developing fruit, but also a sink for drawing nutrients to fruit. These seem to be a reason why the developing ear plays an important role in advancing plant senescence as the most effective mobilizing center^{7,9)}.

Contrary to the behaviour of endogenous growth regulators in rice plant during growth and senescence, there is a reverse relation between the ABA- and cytokinin-like activities in tuberous root of sweet potato. In this plant, it is evident that the decrease of the ABA-like activity and the increase of the cytokinin-like activity occurred accompanying with the budding of tuberous root.

These results obtained from the above two crop plants may support a view that endogenous ABA participates in the endogenous regulation of growth and senescence in the plant as a synergist with endogenous cytokinins.

SUMMARY

Studies were made of the changes in the levels of both ABA- and cytokinin-like activities accompanying with growth and senescence in the crop plants, rice plant and sweet potato.

It was deduced that organ senescence of rice plant depends upon the levels of cytokinin rather than those of ABA alone, because in comparison

with ABA-like activity a striking decline in the endogenous cytokinin-like activity occurred in advancing with organ senescence. Contrary to those, the levels of both ABA- and cytokinin-like activities in ear were much higher at the early stage of ear development, gradually decreasing toward the full ripening stage.

However, in sweet potato, it was observed that there was a reverse relation between ABA- and cytokinin-like activities, indicating a decrease of the ABA-like activity and an increase of the cytokinin-like activity during the budding of tuberous root.

It should be noted that various patterns of the endogenous ABA-cytokinin relation were observed accompanying with growth and senescence in these two crop plants investigated.

These results may support a view that endogenous ABA participates in the regulation of growth and senescence of the plant as a synergist with endogenous cytokinins.

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〔和 文 摘 要〕

作物の窒素代謝に関する研究

第11報 作物体の生育に伴うアブサイシン酸とサイトカイニン様活性の変化について

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本実験では、水稻および甘藷などの作物の生育と老衰の過程で ABA-cytokinin 関係がどのように変化するかを調査した。その結果、

- 1) 水稻体各器官の ABA-cytokinin 活性をみると、若い器官と老衰を始めた器官との間には、ABA ではあまり差異が認められないが cytokinin 活性には大きな差異が認められる。
- 2) 水稻の穂の切除は個体の老衰に大きな影響を及ぼすが、この穂の発育に伴う ABA-cytokinin 関係をみると、乳熟期の穂では ABA と cytokinin とはともに高く、その後穂の発育に伴って著しく低下している。
- 3) 甘藷塊茎の萌芽期において ABA-cytokinin 活性をみると、ABA 活性は収穫期の組織では高く萌芽期では低下しているのに対して、cytokinin 及び根の生長促進物質の活性は萌芽期において著しく上昇している。

以上の結果から、作物体の内生的な ABA は内生的な cytokinin と相互に関連しながら、個体の生育および老衰の過程において重要な役割を果たすものと考えられる。