

## Growth Dependent Changes of Endogenous Ethylene Levels in Leaf Sheaths and Panicles in the Life Cycle of Rice Plants\*

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**Abstract** : We examined the changes of the endogenous ethylene levels in the leaf sheaths and the panicle in the life cycle of rice plants. The ethylene concentrations in the gas air squeezed from the leaf sheaths ranged from 12 nl/l to 22 nl/l in the active-tillering stage. Then, the ethylene was decreased to the concentrations of 6 nl/l-8 nl/l in the early panicle formation stage. A peak of ethylene concentration in the leaf sheath was observed at the heading and anthesis stage. At this stage the ethylene concentrations were 16 nl/l to 18 nl/l. Ethylene was hardly detected in the leaf sheaths 14 days after heading. The tendencies mentioned above were observed in the fluctuation profiles of the ethylene contents per leaf sheath and also in the fluctuations of the ethylene content per 1 g fresh weight of leaf sheath.

During vegetative growth stage, ethylene concentration was higher in the older leaf sheath (the fourth leaf sheath from the uppermost leaf unfolded) than in the younger one, although this tendency was not observed during the reproductive growth stage.

In the panicle, ethylene level was remarkably higher than that in the leaf sheaths. The ethylene concentration in the squeezed gas air from the panicle was 97 nl/l at the heading and anthesis stage. The concentration increased rapidly after anthesis and reached 220 nl/l fourteen days after heading. As the gas air content in a panicle gradually decreased after anthesis, the ethylene contents per panicle and 1 g fresh weight of panicle showed a peak at around the heading and anthesis stage.

**Key words** : Ethylene, Leaf sheath, Life cycle, *Oryza sativa* L., Panicle, Reproductive stage, Rice, Vegetative stage.

イネの葉鞘及び穂中における内生エチレンレベルの発育に伴う変化: 道山弘康・坂 齊\*\*\* (名城大学農学部・\*\*\*農林水産省北海道農業試験場地域基盤研究部)

**要 旨** : イネの葉鞘内及び穂中における内生エチレンレベルの発育に伴う変化について、押し潰し気体捕集法を用いて検討した。

葉鞘内ガスのエチレン濃度をみると、分けつ盛期にあたる田植え 29 日後 (9 葉期, 出穂前 59 日) においては、12 nl/l から 22 nl/l の範囲であった。その後は濃度が低下して、生殖生長初期の出穂前 22 日には 6 nl/l から 8 nl/l になった。その後エチレン濃度は上昇し、出穂開花期に 16 nl/l から 18 nl/l でピークを示した。出穂後 14 日 (乳熟期) になると、葉鞘内からほとんどエチレンが検出されなくなった。葉鞘一本当たりエチレン含有量や葉鞘 1 g 当たりエチレン含有量においても、以上に示したような発育に伴う葉鞘中のエチレン濃度の変化に見られた傾向と同様の傾向が見られた。葉鞘中のエチレン濃度の葉位間差は、栄養生長期においてのみ見られ、新しく展開した葉から数えて四番目にあたる古い葉のエチレン濃度がその上位葉より高かった。一方、穂においては、穂ばらみ期においてすでに葉鞘よりエチレンレベルが高いが、開花後著しく上昇するようになり、乳熟期頃にあたる開花後 14 日の実験終了まで上昇を続けた。開花期のエチレン濃度は 97 nl/l であり、乳熟期頃の開花後 14 日には 220 nl/l まで上昇した。しかし、穎花中のガス含有量が開花後減少するため、穂一本当たりエチレン含有量及び穂の生体重 1 g 当たりエチレン含有量は出穂開花期頃にピークを示すように変動した。

**キーワード** : イネ, 栄養生長期, 生活環, 生殖生長期, エチレン, 穂, 葉鞘。

Endogenous plant hormones in higher plants play an important role in the life cycle

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as growth regulating substances. So, it is important to know the changes of the endogenous levels of the plant hormones throughout the life cycle as the basic knowledge to make clear the process of the expression of physiological features and the fundamental growth characteristics, such as inter-

node elongation, ear development and senescence in plants. It is also important as the basic knowledge for their artificial chemical regulation.

Studies on the dramatic changes of the endogenous levels of gibberellins<sup>4,8)</sup>, abscisic acid<sup>8)</sup>, indoleacetic acid<sup>5)</sup> and cytokinins<sup>9,10)</sup> during life cycle have been reported in the rice plant. However, the changes of the endogenous ethylene levels throughout the life cycle in the rice plant have not been studied so far. In the previous paper<sup>6)</sup>, we reported the method for sampling gas air from leaf sheaths and panicles, and the possibility of its changes with growth in rice plant. We report herein the changes in the endogenous ethylene levels in the leaf sheaths and the panicle in the rice life cycle, especially at the transitional growth stage from vegetative stage to reproductive stage as well as the differences of ethylene levels in the leaf sheaths according to leaf position.

### Materials and Methods

Thirty-day old seedlings of rice (cv. Norin 8) were transplanted in a paddy field at the National Institute of Agrobiological Resources (NIAR) on 2nd June in 1987. Compound fertilizer (6 kg/10 a as the nitrogen), which contained 8% N, 8% P<sub>2</sub>O<sub>5</sub> and 8% K<sub>2</sub>O, was applied as a basal dressing. No more fertilizer was applied further. The rice plants were harvested 59 days (1st July), 45 days (15th July) and 36 days (24th July) before heading. The panicle was not yet differentiated during these stages. Furthermore, the rice plants were harvested after the panicle differentiation stage, namely 22 days (7th August), 16 days (13th August) and 8 days (21st August) before heading, at the heading and anthesis day (29th August), and 5 days (3rd September) and 14 days (12th September) after heading.

The gas air for ethylene determination was squeezed out of every leaf sheath of upper four leaves and the panicles on the main stems of six to fifteen plants, using the mechanical squeeze method<sup>6)</sup>. One milli-liter of one gas air sample was used for the ethylene determination. The gas sampling was replicated four or five times from 9 o'clock to 15 O'clock on a fine day. At the same time, the fresh weights of the leaf sheaths and the panicles were mea-

sured. The volume of the gas air contained in them was also measured.

Ethylene was determined by gas chromatography, with a flame ionized detector (Hitachi model 163), using a glass column (3 mm × 2 m) with Porapak Q (50/80 mesh). The flow rate of carrier gas (nitrogen) was 100 ml/min and the oven temperature was maintained at 80°C.

### Results

#### Fresh weight of the leaf sheaths and the panicle

The fresh weight of the leaf sheath was increased with the rise in leaf position on the main stem among the leaf sheaths from the 6th leaf (0.13 g) to the 12th leaf (1.02 g) (Fig. 1). The fresh weights of these leaf sheaths were not changed with growth during the experimental period after the expansion. Above the 12th leaf sheath, the fresh weight of the leaf sheath was about 0.8 g to 1.0 g. These leaf sheaths were slightly decreased after the expansion.

The fresh weight of a panicle was about 1.2 g until the heading time (Fig. 1). After heading, the fresh weight of the panicle increased and attained a weight of 2.46 g fourteen days after heading.

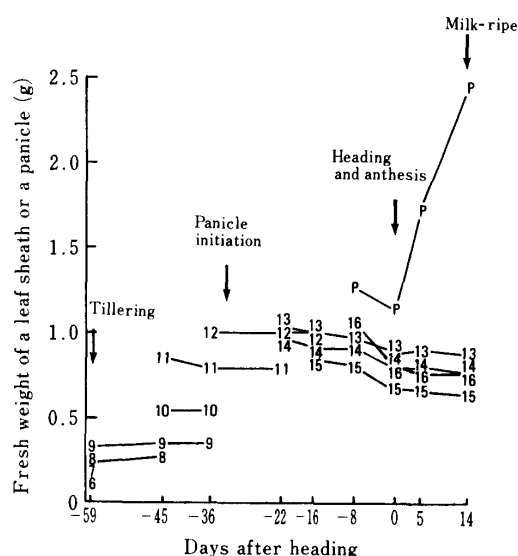


Fig. 1. Fresh weights of the leaf sheaths and the panicle at various growth stages in the rice plant.

Arabic numerals and "P" in this figure indicate leaf position and panicle in the main culm, respectively. The arrows indicate the typical growth stages.

### Volume of the gas air contained in the leaf sheaths and the panicle

Ethylene is released from the cells as a biosynthetic product and stored temporarily in the aerenchyma of leaf sheath or in the space between lemma and palea of glumous flowers. These air spaces may act as the temporal storage organ of ethylene. So the ethylene contained in leaf sheaths and panicles was fluctuated by the volume of the gas air contained in them, that is the volume of the storage organ.

The upper leaf sheaths contained more gas air per a leaf sheath among the leaf sheaths from 6th leaf (0.1 ml) to the 11th leaf (1.1 ml) (Fig. 2) in the same way as the fresh weight of the leaf sheaths (Fig. 1). Therefore, to see the gas air content based on 1 g fresh weight (Fig. 3), the gas air contents of the 6th to 11th leaf sheaths had little difference owing to the leaf position. Above the 11th leaf sheath, the upper leaf sheaths contained less gas air (Fig. 2). And the flag leaf sheath (the 16th leaf) contained about 0.2 ml of gas air per a leaf sheath. Among these leaves, The gas air content per 1 g fresh weight also decreased with the rise in leaf position on the main stem (Fig. 3), as the fresh weight of these leaf sheaths fluctuated a little according to leaf positions (Fig. 1).

The panicle contained about 0.7 ml of gas air per a panicle (Fig. 2) and 0.6 ml of gas air per 1 g fresh weight (Fig. 3) at the heading

and anthesis stage. It is mainly due to the gas air in the glumous flowers. The gas air in the panicle decreased after heading, because the grains developed in the glumous flowers.

### Ethylene levels in the leaf sheaths

The ethylene concentrations in the gas air squeezed from the leaf sheaths ranged from 12 nl/l to 22 nl/l at the 59th day before heading, followed by gradual decrease (Fig. 4), and showed the minimum value (6.0 nl/l to 8.3 nl/l) at the 22th day before heading. To see the change of the ethylene content per leaf sheath (Fig. 5), the ethylene content was higher at the 45th day before heading than the other days, as the 10th and 11th leaf sheaths which were active at that stage had more gas air than the others (Fig. 2). On the other hand, the ethylene content per 1 g fresh weight of leaf sheath was higher at the 59th day before heading and subsequently decreased (Fig. 6), as the leaf sheaths below the 12th leaf had high gas air content per 1 g fresh weight of leaf sheath (Fig. 3). To see the change of the ethylene contents in the same leaf position, the ethylene levels based on the three items were generally decreased from the 45th day until the 22th day before heading.

At the reproductive stage, the ethylene

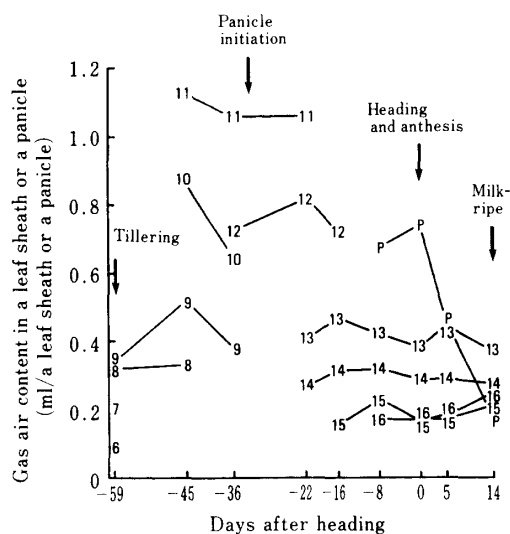


Fig. 2. Gas air contents in the leaf sheaths and in the panicle at various growth stages in the rice plant. See the note in Fig. 1.

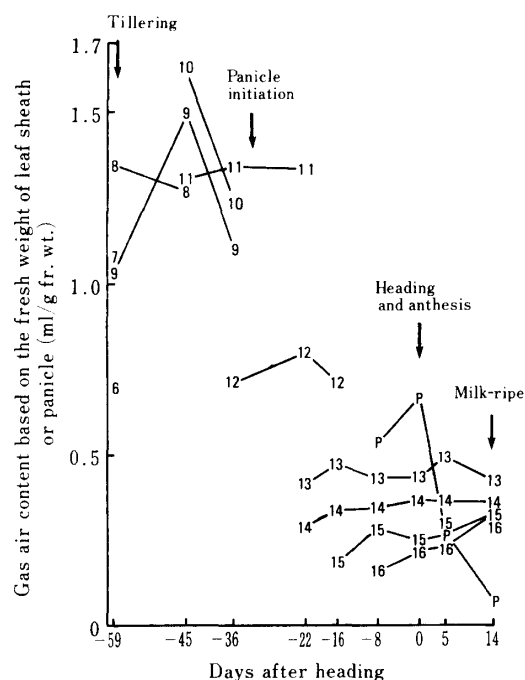


Fig. 3. Gas air contents based on the fresh weight of leaf sheath or panicle at various growth stages in the rice plant. See the note in Fig. 1.

concentration increased up to the heading and anthesis stage, and rapidly decreased after that (Fig. 4). As a result, the ethylene concentration showed a peak (16–18 nl/l) at the heading and anthesis stage. The ethylene content per a leaf sheath (Fig. 5) and per 1 g fresh weight of leaf sheath (Fig. 6) also showed a peak at that stage.

To see the difference of the ethylene level according to leaf position, the 4th leaf sheath counted down from the uppermost expanded leaf showed higher ethylene concentration than the upper three leaf sheaths at the vegetative stage (on and before the 36th day before heading in Fig. 4). But at the reproductive stage (on and after the 22nd day before heading), the difference in the ethylene concentrations owing to the leaf position at the same growth stage was not clear. The ethylene content based on the other items was affected by the gas air contents according to leaf position.

#### Ethylene level in the panicle

The ethylene concentration in the gas air squeezed from the panicle was 29 nl/l at the boot stage (8 days before heading, Fig. 4). Then the concentration increases and was 97 nl/l at the heading and anthesis stage. After

heading, the concentration continued to increase during the experimental period. The concentration was greatly higher than the concentration in the gas air from the leaf sheaths. The ethylene concentration in the panicle at the 14th day after heading (220 nl/l) was about ten times as much as the concentrations in the leaf sheaths at the heading and anthesis stage.

The ethylene content per a panicle (Fig. 5) and the ethylene content per 1 g fresh weight of the panicle (Fig. 6) showed a peak at about the heading and anthesis stage. At the heading and anthesis stage, the content in the panicle was about ten times as much as in the leaf sheaths, too.

#### Discussion

In this paper, we showed the dynamic changes of ethylene levels in the leaf sheaths of various leaf positions and in panicle during growth in rice plants, and we discuss the changes in more detail than the previous paper<sup>6)</sup>. The results do not indicate directly the relationship between the ethylene level and the expression of growth phenomena. But the results are important in order to make clear the role of endogenous ethylene and the

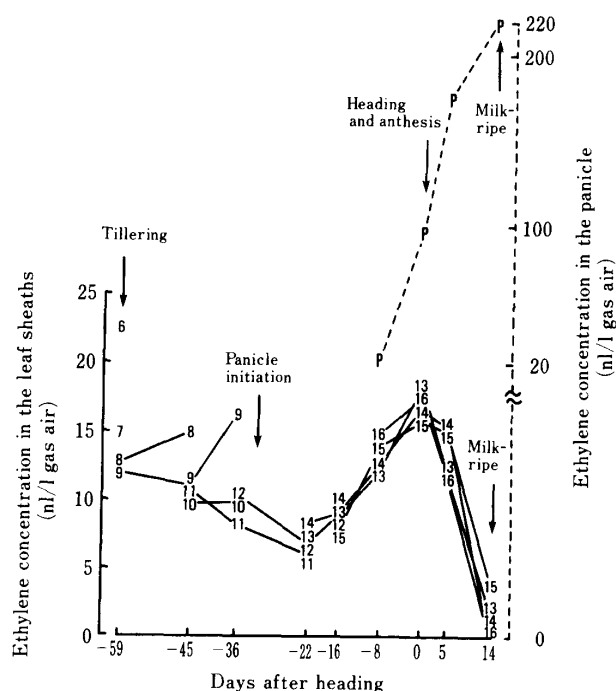


Fig. 4. Ethylene concentrations in the leaf sheaths and in the panicle at various growth stages in the rice plant. See the note in Fig. 1.

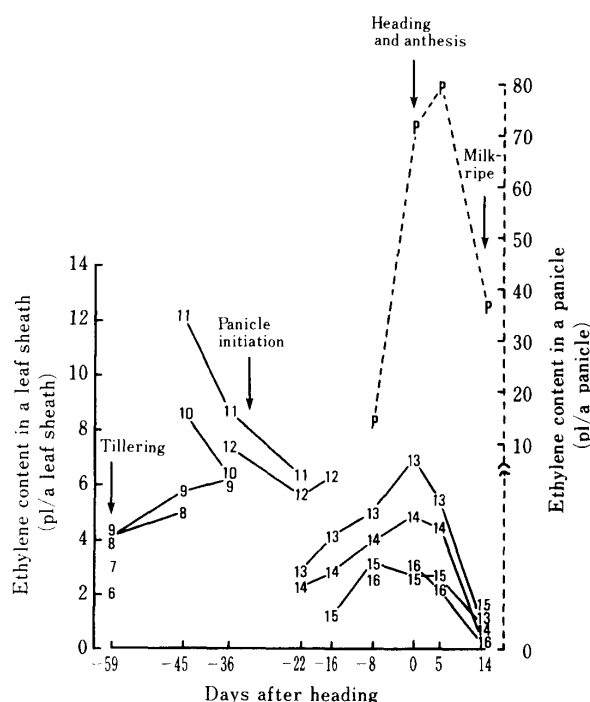


Fig. 5. Ethylene contents based on one leaf sheath or one panicle at various growth stages in the rice plant. See the note in Fig. 1.

interaction of ethylene and the other plant hormones on the expression of various growth phenomena.

To see the changes in the endogenous levels of the other plant hormones in shoots of rice, the levels of indoleacetic acid<sup>5)</sup> and abscisic acid<sup>8)</sup> in shoots were scarcely changed throughout the life cycle. The levels of trans-ribosylzeatin and trans-zeatin (cytokinins) in shoots were high at the active-tillering stage<sup>9)</sup>. The level of gibberellin A<sub>19</sub>, one of the major gibberellins in rice plants, was high at the active-tillering stage and then decreased until the panicle initiation stage<sup>4,8)</sup>. The pattern of gibberellin A<sub>1</sub> level fluctuation in shoots (another major gibberellin in rice plants) showed a peak at the internode elongation stage just before the anthesis stage<sup>4)</sup>.

The levels of the other plant hormones were expressed by the content per 1 g fresh weight of plant materials. But the ethylene contents were influenced by the gas air contents in the plant as the volume of the temporal storage organ, because ethylene is a gaseous body. So the ethylene level can not be discussed only through the content per 1 g fresh weight of the plant materials as well as the other plant hormones. In this study, the ethylene levels in

the leaf sheaths were expressed by using three bases i. e. one milli-liter of gas air from the leaf sheath, one leaf sheath, and one gram fresh weight of leaf sheath. But in all cases of the expression methods, the ethylene levels in leaf sheaths were high during the tillering stage and decreased until early in the panicle initiating stage as well as the levels of cytokinins<sup>9)</sup> and gibberellins<sup>4,8)</sup> in shoots. At the stage when the ethylene level decreased, the production of tillers was ceased, and the differentiation of various organs of the panicle and the rapid elongation of internodes were commenced. Our results showed the possibility that the ethylene might have some roles on the expression of the growth phenomena during the transition stage from vegetative to reproductive stage, although the ethylene levels were measured only in the leaf sheaths in this experiment.

The ethylene levels in leaf sheaths showed a peak at the heading and anthesis stage, being consistent with the result of the previous paper<sup>6)</sup>. The level of GA<sub>1</sub>, which was considered to be active GA in rice plants<sup>3,4)</sup>, also showed the peak just before the anthesis stage<sup>4,8)</sup>. The combined application of ethylene and GAs exerted a cooperative effect on internodal elongation in floating rice<sup>7)</sup>. Our results showed that the endogenous ethylene, which increased conspicuously at the heading and anthesis stage, might play an important role cooperatively with the endogenous GAs on internodal elongation and anthesis.

During the vegetative growth stages, the ethylene concentration in the gas air from the old leaf sheath was higher than the concentration in the gas air from the younger leaf sheaths. Endogenous ethylene plays an important role on the leaf senescence and abscission<sup>1,2)</sup>. The results of this study indicate that the endogenous ethylene might have some roles on leaf senescence in rice plants. But at the reproductive growth stages, the ethylene level of the older leaf sheath was not higher than the younger leaf sheaths, although the ethylene concentrations were different owing to leaf positions at the heading and anthesis stage in the previous paper<sup>6)</sup>.

In the panicle, the level of ABA<sup>8)</sup> was high at the milk-ripe stage. On the other hand, the levels of GAs showed a peak at the anthesis stage<sup>8)</sup>. The endogenous levels of IAA and

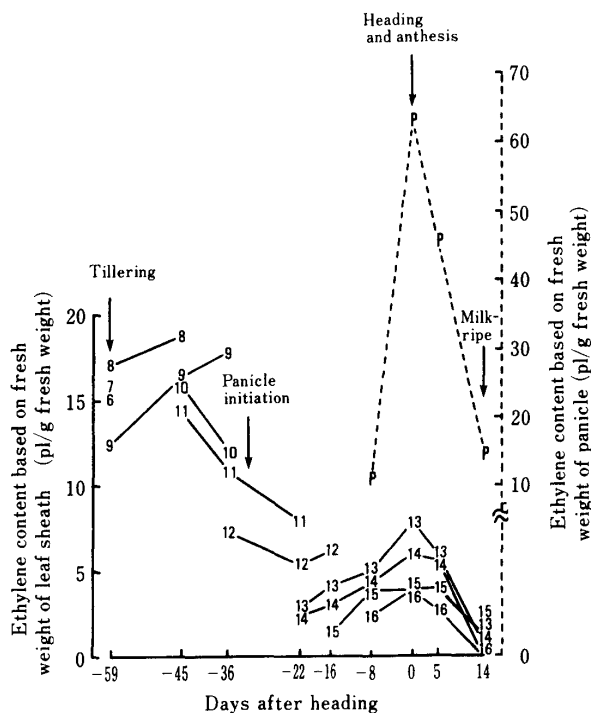


Fig. 6. Ethylene contents in the leaf sheaths and in the panicle based on fresh weight at various growth stages in the rice plant. See the note in Fig. 1.

conjugated IAA were high at the anthesis stage and milk ripe stage<sup>5)</sup>. The levels of cytokinins were high at the early growth stage after heading, namely at the heading, anthesis or milk ripe stage<sup>10)</sup>

In this study, ethylene concentration in the gas air in the panicle also increased after the boot stage to the milk ripe stage. But the grains developed in the glumous flowers. The gas air content per panicle decreased after anthesis. As a result, the ethylene content per panicle and per 1 g fresh weight of panicle showed a peak about the heading and anthesis stage as well as the other plant hormones. These results indicated that ethylene was produced rapidly in glumous flowers after anthesis, although the air space in the glumous flowers, which acted as the temporal storage organ of ethylene, became narrower during grain filling. In this case, the ethylene content per 1 g fresh weight of panicle can not be handled as well as the other plant hormones.

During these fluctuations, the endogenous ethylene level in the panicle was remarkably higher than the ethylene levels in the leaf sheaths, as well as the endogenous levels of the other hormones<sup>4,5,8,9,10)</sup>. These results indicate that the plant hormones including ethylene play exceedingly important role on the development and the maturation of grains in rice plants. In this experiment, we measured the ethylene levels in leaf sheaths and panicles, but the levels in leaf blades, stems and roots were not measured. So it is expected that the ethylene levels in every part of the plant should be measured and the ethylene levels and its roles of the plant parts are compared with one another.

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