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Endogenous Ethylene Levels in Leaf Sheath and Panicle of Rice Plant*

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Abstract : We examined endogenous ethylene levels in leaf sheath and the panicle of growing rice plants. 1) Endogenous ethylene within the leaf sheaths could be sampled by mechanical squeezing. Ethylene levels reached a maximum in one hour after a lag time of thirty minutes after the harvesting. 2) Levels of endogenous ethylene in leaf sheaths showed diurnal variation with high levels in the day and low in the night. 3) The ethylene levels within leaf sheaths were similar among upper four leaves when measurement was made twenty three days before heading (The flag leaf was not emerged yet). But at the time of anthesis, ethylene level in the leaf sheath of the flag leaf on the main stem showed the highest value among the upper four leaves, decreasing in the order of leaf position from the top. The ethylene level in the panicle after heading was found to be higher than that in the flag leaf.
Key words : Developmental stage, Diurnal variation, Ethylene, Rice plant.

イネの葉鞘および穂における内生エチレンレベルについて：道山弘康***・坂　斉（農林水産省農業生物資源研究所）

要　旨：イネの葉鞘内に通気組織が発達していることおよび若い穂の穎花内に空気が入っていることに着目して，それらを水中で押し潰してガスを捕集することによりエチレンを測定する手法を確立した．この手法を用いて，水田またはポットで生育したイネの葉鞘および穂における内生エチレンの変動を調べ次の結果を得た．

1．イネ葉鞘中のエチレンレベルは，採取30分後まで採取直後と同程度であり，その後急激に上昇して，採取1時間後に最高値を示した．その後は再び低下するものの，採取後3時間の範囲では採取直後のそれより高いレベルを維持した．

2．イネ葉鞘中のエチレンレベルの日変化を見ると，昼間に高く，11：00—15：00頃に最高値を示し，夜間には低下した．

3．出穂23日前のイネでは，上位4葉の葉鞘中のエチレンレベルは同程度であった．しかし，出穂開花期のイネでは，止葉の葉鞘が最高値を示し，葉位が下がるに伴ってエチレンレベルも低下した．しかし，穎花中のエチレンレベルは，葉位間では最高値を示す止葉葉鞘中よりさらに高かった．
キーワード：イネ，エチレン，日変化，発育段階．

Lee et al.[7,8] reported the effect of nitrogenous fertilizer on ethylene evolution and the diurnal variation in ethylene evolution from rice leaves. Kao and Yang[3,4] also tested ethylene production from rice leaves in light and in darkness. In these papers, they measured the ethylene evolved from the excised leaves which were incubated in glass containers. There is no report, however, on endogenous ethylene levels in intact rice plants except in deep water rice[9] and F_1 hybrid between non-floating and floating rice[11].

We report here the relation between environmental conditions and endogenous ethylene levels in growing rice plants, especially in the leaf sheath, in which the aerenchyma is well developed, and in the panicle. More precisely, detailed investigation was made on the effect of wounding at sampling time and the leaf position at a developmental stage on endogenous ethylene levels, and the diurnal variation in its levels by using two rice cultivars grown in a paddy field and a glass house.

Materials and Methods

Experiment 1. Rice plants (cv. Nipponbare) with roots were harvested at the vegetative growth stage from a paddy field at the

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National Institute of Agrobiological Resources (NIAR) in 1985, and incubated in water at about 25°C. The time-dependent gas air was drawn out from the leaf sheaths of three uppermost leaves on the main stem.

Rice plants (cv. Norin 8 and Nipponbare) were also grown in a glass house under natural sun-light conditions in 1985. The gas air from the leaf sheaths of the three uppermost leaves was drawn out immediately after the harvesting of the plants at 11 : 00 a.m. and 11 : 00 p.m. every day in Norin 8 and every three hours during twenty four hours in Nipponbare. The air in the atomosphere was also sampled at the same time.

Experiment 2. Rice plants (Norin 8) were grown in a paddy field at NIAR in 1986. The plants with roots were harvested twenty three days before heading and at the flowering stage. The gas air was drawn out from each of the upper four leaf sheaths and from the panicle, especially from the glumous flowers, on the main stem. The sheath length and volume of the gas air in a leaf sheath were measured at the same time.

Gas sampling from leaf sheaths and from panicles Leaf sheaths and young panicles have more gas air than the other organs because of the aerenchyma and the glumous flowers in rice plants. The gas air was squeezed out of the leaf sheaths and the panicles in water by hand and by the roller (Fig. 1). The gas air from them was trapped using a beaker submerged upside down in the water (Fig. 1). The gas air sample (1 ml) was withdrawn from the beaker by 1 ml tuberculin syringe in water, and was used for the ethylene determination.

Ethylene determination Ethylene was determined by gas chromatography, with a flame ionization detector (Hitachi model 163), using a glass column (3 mm×2 m), packed 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 and Discussion

Endogenous ethylene in intact plant Ethylene level in the leaf sheath aerenchyma (about 35 nl/l), which was not changed for thirty minutes after harvesting (Fig. 2), increased to the highest level (about 110 nl/l) after one hour. After that, time-dependent decrease of the

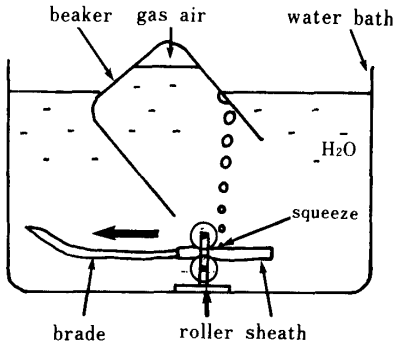

Fig. 1. Sampling method of the gas air from leaf sheaths and from panicles of rice plants.
Note : The gas air was squeezed out of leaf sheaths or panicles in water by hand and by roller. The gas air was trapped using a beaker submerged upside down in the water. The trapped gas air was withdrawn from the beaker by 1-ml tuberculin syringe in water and used for the ethylene determination.

ethylene level was observed, although the levels higher than those in the beginning of the experiment were maintained. On the other hand, Kao and Yang[4] reported that in detached rice leaves, the rates of ethylene production increased immediately after excision and reached a maximum in 12 hours, and subsequently declined. Boller and Kende[2] reported that the rate of wound ethylene formation increased after a lag time of thirty minutes in tomato fruits. Our result, which was consistent with this report, shows that we can detect the endogenous ethylene levels in leaf sheath aerenchyma of intact adult rice plants by the method described here, if the gas air sample is drawn out from the leaf sheaths within thirty minutes after harvesting.

Our method (mechanical squeeze method) for obtaining leaf sheaths ethylene was compared with that, which uses the vacuum apparatus system[1] manufactured by Nissan Chemical Industries Co., Ltd. (unpublished data). Results showed a good coincidence between these two methods. Then, our present method was suitable for the gas sampling from the leaf sheaths and the young panicle of rice plants, although this method was not useful for other tissues and organs of rice plant in which the aerenchyma system was not so developed.

Diurnal variation of ethylene levels Endogenous ethylene levels in the leaf sheath

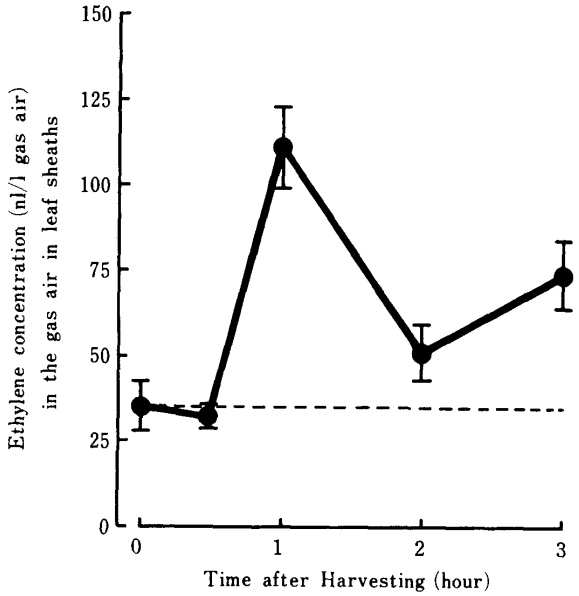

Fig. 2. Changes in ethylene concentration in the gas air in leaf sheaths of rice plants (cv. Nipponbare) of vegetative growth stage after harvesting.
Note: Vertical bars indicate standard error of five determinations.

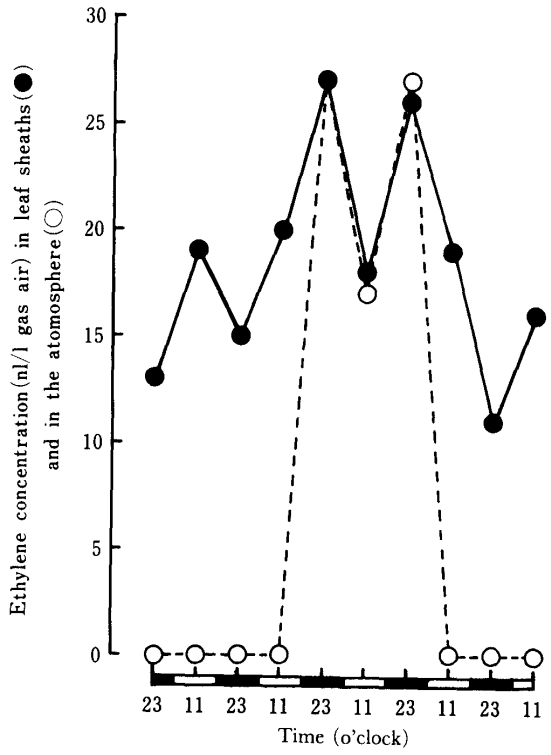

Fig. 3. Day and night variations in ethylene concentration in the gas air in leaf sheaths of rice plants (cv. Norin 8).

aerenchyma were high in the day and low in the night. This diurnal variation, however, was disordered by atomospheric conditions because the atomospheric air occasionally contains ethylene as an air pollutant (Fig. 3). Lee et al.[8] reported the ethylene evolution from the excised leaves of the rice plants markedly increased in the night. Kao and Yang[3,4] also reported that ethylene production was found to reduce under the light and this light inhibition of the conversion of ACC to ethylene was mediated through carbon dioxide. The discrepancy found in the diurnal change of ethylene level may be caused, in part, by the difference of plant organs used, although more detailed investigations are needed.

We examined in detail the pattern of diurnal variation of the ethylene levels on the day when the atomospheric ethylene was not detected. Since 9 : 00 a.m. the ethylene level gradually increased, and reached the highest level at 3 : 00 p.m. The level was maintained at low level throughout the night (Fig. 4). The increasing pattern of ethylene level in the leaf sheaths of rice plants in the daytime was similar to that in *Vicia faba*[2]. The diurnal variation of endogenous ethylene levels may be correlated with the water stress or stomatal aperture function in the leaves.

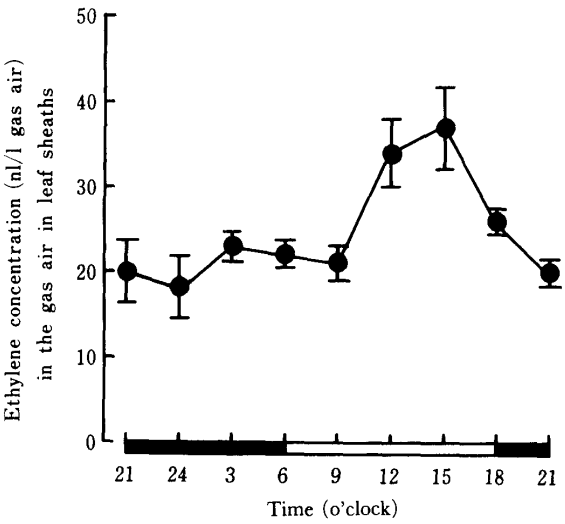

Fig. 4. Diurnal variation in ethylene concentration in the gas air in leaf sheaths of rice plants (cv. Nipponbare) on the day when the atomospheric ethylene was not detected.
Note: Vertical bars indicate standard error of five determinations.

Changes in endogenous ethylene levels with the growth stage and leaf position on the main stem
Levels of ethylene within leaf sheaths were not

Table 1. Ethylene contents in the gas air in leaf sheaths of the rice plants (cv. Norin 8) twenty three days before heading.

	Leaf position on the main stem			
	1 (upper)	2	3	4 (lower)
Length of leaf sheath (cm)*	24.5±0.1	23.3±0.2	22.1±0.3	17.2±0.3
Ethylene concentration in the gas air in leaf sheaths (nl/l gas air)**	12±1.3	10±1.5	9±2.5	12±1.7
Ethylene content per a leaf sheath ($\times 10^{-3}$ nl/sheath)**	9.5±1.0	13.3±1.4	11.8±3.5	9.6±1.2

Notes: *Each figure is an average of fifteen leaf sheaths with standard error.
**Each figure is an average of five determinations with standard error.
Three leaf sheaths were used for one determination.

Table 2. Ethylene contents in the gas air in leaf sheaths and the panicle of rice plants (cv. Norin 8) at anthesis.

		Leaf position on the main stem			
	Panicle****	1 (flag leaf) (upper)	2	3	4 (lower)
Length of leaf sheath (cm)*	—	34.2±0.2	23.2±0.1	23.1±0.2	23.8±0.1
Ethylene concentration in the gas air in leaf sheaths** or a panicle (nl/l gas air)	88±9.2	32±4.0	22±2.2	15±4.9	6±2.8
Ethylene content per a leaf sheath** or a panicle*** (10^{-3} nl/sheath or panicle)	83.9±9.5	17.0±2.8	7.9±1.2	8.1±2.2	4.3±2.1

Notes: *Each figure is an average of twenty five leaf sheaths with standard error.
**Each figure is average of five determin ations with standard error.
***Each figure is an average of four determinations with standard error.
Three panicles were used for one determination.
****One panicle had 96.8 glumous flowers on an average.

so different among the upper four leaves when measurement was made twenty three days before heading (Table 1). On the day, the flag leaf was not emerged yet. But at the flowering stage, the flag leaf showed the highest ethylene level, decreasing in the order of leaf position from the top of the stem (Table 2). Furthermore, the ethylene level in the gas air in the flag leaf sheath at the flowering stage (Table 2) was higher than the levels in the gas air in the sheaths of the upper four leaves twenty three days before heading (Table 1). The ethylene level in the gas air in the panicle was found still higher than that in the leaf sheath of the flag leaf (Table 2). This is interesting because the levels of other plant hormones, i.e. gibberellins (GAs), auxins, cytokinins and abscisic acid, in the panicle of a rice plant were remarkably increased around the heading time[6,12]. Suge[10] reported the combined application of ethylene and GAs exerted a cooperative effect on internodal elongation in floating rice. Our result shows that the endogenous ethylene, which increased conspicuously at heading time, might play an important role cooperatively with the other

plant hormones on internodal elongation and maturation.

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