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Amount and Chemical Characteristics of the Epicuticular Waxes on Leaves at Active Tillering and Heading Stages of Rice Varieties

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벼 品種들의 分蘖盛期 및 出穗期의 葉表面Wax의 量 및 化學的 組成

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ABSTRACT : Differences in the amount and chemical characteristics of the epicuticular waxes on rice leaves were studied for the active tillering and heading stages of rice varieties differing widely in gross leaf-surface property and genetics. The amount of waxes on surfaces of rice leaf-blades was determined by extraction with chloroform and chemical composition of the waxes was characterized by thin layer chromatography, gas liquid chromatography and infrared spectrophotometry.

The amount of waxes varied by variety and significantly with growth stage. The amount at the heading stage was 1. 7 to 3. 6 mg / g fresh weight of leaves, which was two to three times as much as that at the tillering stage of 0. 8 to 1. 8 mg / g fresh weight. The waxes consisted of seven chemical classes, namely diols, fatty acids, fatty alcohols, fatty aldehydes, fatty esters, saturated and unsaturated hydrocarbons.

Diols and unsaturated hydrocarbons were identified as new chemical classes of the rice epicuticular waxes. The polar constituents such as diols, fatty acids and fatty alcohols and the non-polars such as fatty aldehydes, fatty esters, and saturated and unsaturated hydrocarbons were identified at the heading stage, but at the tillering stage only the non-polar compounds were identified.

In the carbon numbers (C) of the chemical classes, diols were composed entirely of C30 and acids were mainly of C30 and C31. In alcohols, primary alcohols were composed of C13 and C32, and the secondary alcohols were of C14, C16 and / or C30 regardless of the rice varieties. The acid portion of fatty esters, mainly composed of C22 and C23, showed low cabon numbers compared with the aldehydes. The alcohol portion of them showed a wide distribution in carbon numbers from C13 to

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C26 depending on the rice varieties. Hydrocarbons had odd carbon numbers, consisting mainly of C29 and C31.

Key words : epicuticular wax, chemical composition, wettability, rice, variety, growth stage.

Epicuticular waxes are deposited on the outer surface of plant leaves and are embeded-ded in cutin matrix[15]. It has been suggested that waxes are to control water balance in plant bodies, protect the plants mechanically from infection of pests and excessive exposure to ultra violet radiation and prevent uptake of xenobiotics applied to the shoots[2,4,16]. The amount of epicuticular waxes on plant surface is more under high light intensity and in low humidity[11, 12, 18] than under low light intesity and in high humidity and is more at high than at low temperature[11]. Wax formation is known to be altered by environmental conditions, to which plant parts are exposed and can be readily analyzed. By a recent gas liquid chromatograph[1, 6] it is now possible to analyze the chemical composition of waxes even in infinitesimal amount. Uchiyama *et al*(1987) reported that epicuticular waxes on rice leaves consisted mainly of alkanes(6. 2% of total wax composition), alkyl esters(27. 8%), alkyl aldehydes(30. 3%) and alcohols (20. 4%)[20]. Emery and Gear(1969) reported that the epicuticular waxes on clover leaves consisted mostly of fatty esters of C16 to C30 and the acid parts were mainly of the alcohols of C26[5]. Other results showed that those on asparagus leaves consisted of alkanes(31. 5%), wax esters(20. 1%), ketones(13. 5%), alcohols(16. 4%) and fatty acids(8. 5%)[17].

Rice is one of the four major food crops of the world. Understanding of the properties of the epicuticular waxes on leaves is very important for breeding for resistance to certain pests as well as proper selection of adjuvants in spray and formulation of pesticides. A few studies have been done on the fine structure and morphology of epicuticular waxes of rice leaves by scientists, but information on the composition and amount of the waxes on rice leaf surfaces is very limited. In particular such information on the difference among rice varieties is absent. The present study reports the amount and chemical composition of epicuticular waxes of rice leaves at active tillering and heading stages of eight varieties differing widely each other in genetical background and gross leaf morphology, i.e. ,Japonica and IndicaxJaponica varieties having glabrous, long hairy, pubescent or water-wettable leaves, so classified by rice breeders.

MATERIALS AND METHODS

Preparation of rice leaves. Eight rice varieties used herein differed in the properties of their leaf surfaces and were obtained from the Crop Breeding Lab. of the Agronomy Department, Seoul National University and Crop Experiment Station of Korea. Table 1 shows the source, leaf surface property, and type of the varieties used. They were cultured in ordinary paddy fields at the HAN-NONG Central Research Institute. Rice leaves for wax extraction were sampled for 50 g fresh of weight of uppermost two leaves at the active tillering (June 20) and the heading stages(August 20).

Extraction and purification of crude epicuticular waxes. A thirty gram sample of leaves was allowed to wilt slightly to assure stomatal closure, and each leaf was dipped for 5 min. in each of two successive 500 ml of redistilled chloroform. The resultant extracts were combined and dried under reduced pressure on a rotary evaporator at a temperature not exceeding 40℃, and then dehydrated over anhydrous sodium sulfate layer and filtered into tared round flasks(50 ml). The flasks were dried to get crude waxes containing chlorophylls. To obtain pure epicuticular waxes free of chlorophylls the resultant crude waxes were eluted with hot chloroform on a glass column chromatograph, (1 cm×30 cm) packed with activated florisil(60−100 mesh). The eluent gathered into tared round flasks(100 ml) was dried to constant weight at 40℃, and the purified wax amount was determind by substraction. Data are expressed in ug/g fresh weight of leaves. Two replications were made for each treatment. A portion of the purified wax was dissolved with hot chloroform(3 to 8 mg wax/ml) and stored in a refrigerator(-40℃) until analysis for its chemical composition.

Thin layer chromatography(TLC). The constituents of epicuticular waxes were seperated by thin layer chromatography and their chemical classes were identified by comparison of Rf values with those of the standards shown in Table 2. The wax solutions were spotted (5 ul) on precoated glass plates (Kiesel Gel G60, 250 um, 20×20 cm). Spotted plates were developed in benzene or benzene+n-hexane(1 : 1, v/v) up to 10 cm at 25 ℃. Wax constituents were localized and identified by irradiation with UV lights (254 and 360 nm), by charring (130 ℃) after spraying with H_2SO_4 solution (40%, w/w) mixed with $K_2Cr_2O_7$(5%, w/w), by reacting with iodine vapor or by observing glossiness of each consituent under sunlight. Esters, diols and unsaturated hydrocarbons among the constituents were conformed for chemical structure by an infrared spectrophotometer(a FTIR. model FTS−15/80, Digilab Co. ,USA). For quantitative TLC, the wax solution(1 ml) was streaked as a narrow band (3 mm×9 cm) on a precoated plastic plate prewashed with bezene. The streaked plate was developed with bezene up to 10 cm, and localized by UV irradiation (254 and 360 nm) and exposure to sunlight, and then recovered by modified Stahl's method[19]. The recovered splinters adsorbed with wax constituents were extracted and filtered by syringe filter(25 mm) with the elution of n-hexanes (0.2 ml) for hydrocarbons, tetrahydrofuran (0.5 ml) for primary and secondary alcohols, diols and esters and toluene (0.5 ml) for acids, ketones and aldehydes. Each eluent was directly analyzed by a gas liquid chromatograph.

Gas liquid Chromatography(GLC). Preparation for GLC analysis was done by Barta's method [1] except for column chromatography for ester analysis. Esters were transesterified by TMAH solution (20%, w/v, in methanol) and chromatographed by a glass column (5 mm pasteur tube) under elution of benzene + ethyl ether (95 : 5, v/v) and benzene + ethyl

Table 1. Rice varieties used in the present study.

Variety	Source	Leaf surface property	Type
M101	Cultivar in USA	Glabrous	Japonica
wx817	wx817−1−65−2−1	〃	Indica×Japonica
LK2−7	LK2−7−12−1−1	Long-hairy	Japonica
HP914	HP914−3−2−1−1	〃	Indica×Japonica
Chucheong	Cultivar in Korea	Pubescent	Japonica
Cheongcheong	Cultivar in Korea	〃	Indica×Japonica
HP857	HP857−B−1−2−B−1−1	Water-wettable	Japonica
wx139	wx139−3−64−2−3−1−1	〃	Indica×Japonica

Table 2. Standard compounds and chromatograph adopted for identification of wax constituents.

Compound	Chemical name	Chromatogram
	n−Hexadecane(C16), n−Eicosane(C20)	GLC
Hydrocarbons	n−Tetracosane(C24), n−Octacosane(C28)	TLC
	n−Dotriacontane(C32)	TLC
Esters, Methyl	Methyl eicosanoate(C20)	TLC
	〃 tetracosanoate(C24)	TLC
	〃 triacontanoate(C30)	TLC
Acid	n−Hexadecyl n−pentadecanoate(C31)[a]	TLC
	1−Hexadecanol(C16), 1−Eicosanol(C20)	TLC
Alcohols, Primary	1−Tetracosanol(C24), 1−Hexacosanol(C26)	GLC
	1−Triacontanol(C30)	GLC
Second-ary	2−Hexadecanol(C16)	TLC, GLC
Acids	Stearic acid(C18), Melissic acid(C30)	TLC
	Hexacosanoic acid(C26), Behenic acid(C22)	GLC
Aldehydes	Laurin aldehyde(C21), 1−Heptaaldehyde (C14)	TLC
Ketones	9−Heptadecanone(C16), 10−Nonadecanone (C19)	TLC
Diols	1, 2−Hexadecanediol(C16)	TLC
	1, 2−Octadecanediol(C18)	GLC

a. : Synthesized from n−pentadecanoic acid and 1−hexadecanol through refluxing for 72 hours.

ether (8 : 2, v/v) to collect fatty acid methyl esters and fatty alcohols, respectively. The fatty alcohols eluted were acylated by Barta's method[1] and then injected into GLC. GLC analysis was done with a gas liquid chromatograph (Model 3700, Varian Co. Inc., USA) equipped with a flame ionization detector and a temperature programmer. A stainless steel column(0.3 cm, inside diameter by 4 m long) packed with Chromosorb W/HP (60−80 mesh) coated with SE−30 (2%) was used. Operating conditions were:nitrogen gas flow 40 ml min, injector temp. 320 ℃, detector temp. 340 ℃, and column temp. programmed from 160 to 320 ℃ at 5 ℃ per min. Carbon numbers of wax constituents were identified by comparing the elution times with those of standards shown in Table 2 and the relative quantities were calculated by calibration curve of the standards.

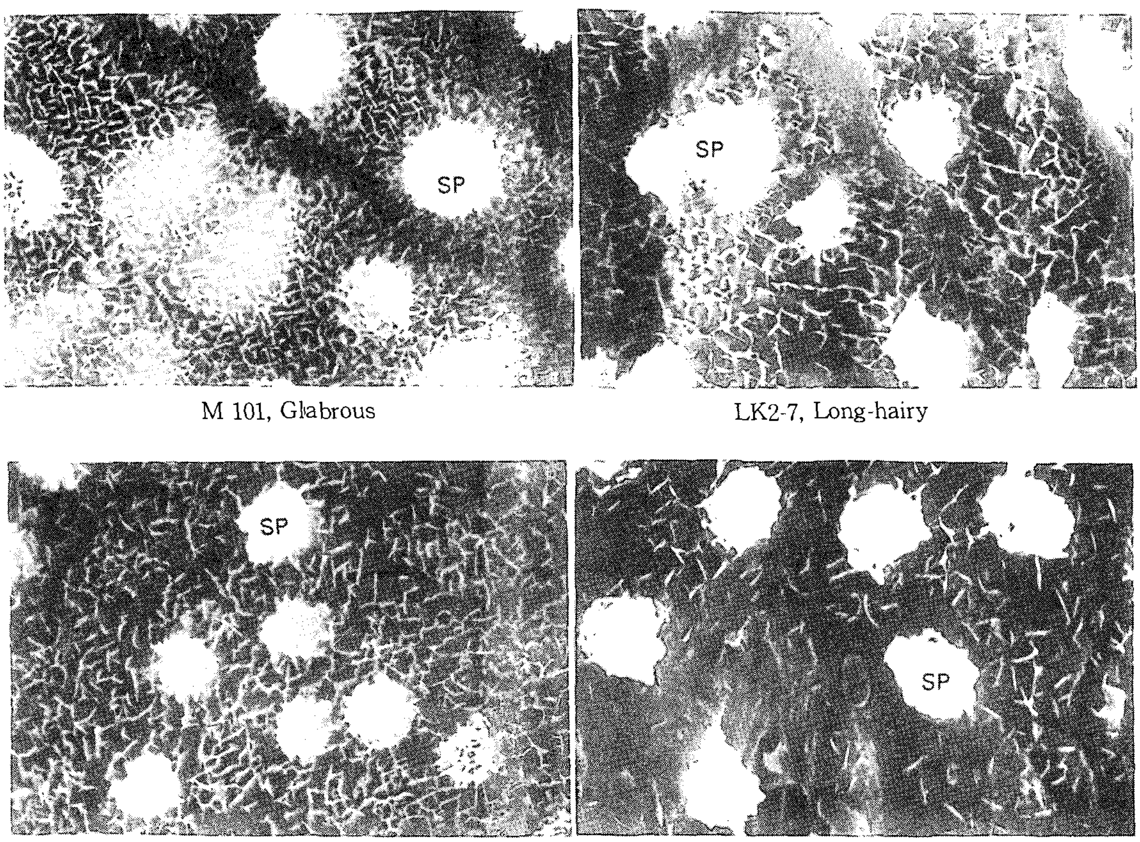
RESULTS AND DISCUSSION

1. Difference in the amount of epicuticular waxes by variety and growth stage.

In the present study the optimum duration of dipping the leaves in the chloroform to extract the epicuticular waxes was investigated prior to the routine extraction of the waxes. Five minutes was found adequate to maximize the wax extraction with minimal dissolution of other components, particularly chlorophylls. But it did not dissolve the whole epicuticular waxes on leaf surface as examined the leaf surace extracted for different period by light and electron microscopy. Dipping the leaves in chloroform for five minutes dissolved the acute portion of the protruded wax platelet and much of basal portion of the wax deposited on the leaf surface.

Chloroform-extracted waxes of fresh rice leaves showed quantitative difference by variety and growth stage of the rice as shown in the Table 3. IndicaxJaponica varieties had more wax on leaves than Japonica varieties in a varietal group having the same leaf surface property as recognized by finger touch of leaf surface, except for the varieties of glabrous leaves. Among the four Japonica varieties the variety having leaves with pubescent surface had the highest amount of wax per unit leaf weight and the wax amount of the varieties was in decreasing order of water-wettable leaf-surface 〉 long hairy leaf-surface 〉 glabrous leaf-surface at the active tillering stage. This varietal difference at the active tillering stage remained unchanged to the heading stage. The order among IndicaxJaponica varieties in the amount of waxes on leaves, however, was somewhat different from that of Japonicas; at the active tillering stage the order was pubescent 〉 long-hairy = water wettable 〉 glabrous and at the heading stage pubescent 〉 long-hairy 〉 water wettable 〉 glabrous.

The amount of epicuticular wax on leaves differed much more by growth stage of the rice plants than between Japonica and Indica x Japonica, by varietal group classified on the



M 101, Glabrous

LK2-7, Long-hairy

Chucheong, Pubescent

HP857, Water-wettable

Fig 1. Scanning electron micrographs of the leaf surface of four Japonica rice varieties having different finger-touch feeling of surface.
(Adaxial side, ×5000 magnification, SP : small papillae)

Table 3. Total amount of the epicuticular waxes on leaf surfaces of rice varieties[a].

Surface property	Variety type	Name	Wax amount (ug / g of uppermost 2 leaves) Tillering stage	Heading stage
Glabrous	Japonica	M101	950 ± 12.0	2,000 ± 32.5
	Indica×Japonica	wx817	810 ± 8.0	1,700 ± 27.0
Long-hairy	Japonica	LK2−7−12	1,000 ± 8.0	2,421 ± 21.5
	Indica×Japonica	HP914	1,370 ± 7.5	3,120 ± 19.0
Pubescent	Japonica	Chucheong	1,290 ± 14.5	2.581 ± 23.0
	Indica×Japonica	Cheongcheong	1,780 ± 11.0	3,606 ± 15.0
Water-wettable	Japonica	HP857	1,260 ± 11.5	2,530 ± 5.5
	Indica×Japonica	wx139	1,370 ± 13.0	2,845 ± 7.5

a. Epicuticular waxes were extracted by hot chloroform immersion for 5 min. two times and purified by column chromatography.

basis of finger touch leaf-surface property or by individual varieties. The amount of waxes increased much with advance in growth stage of the rice plants in all of eight varieties tested. Total amount at the heading stage ranged from 1.7 mg to 3.6 mg / g fresh weight, which was twice to three times as much as that at the tillering stage (0.8mg / g fresh weight). Netting *et al*[13] and Norris *et al*[14] have reported that epicuticular wax amount on aged plants was more than that on young plants.

Our scanning electron microscopic study on these rice varieties has shown noticeable difference among the varieties in coverage of leaf surface by the waxes as shown in the Fig. 1 and as reported in our another report[3] ; the pubescent leaves had less even wax coverage than the glabrous leaves ; the water wettables leaves had very sparse wax coverage on surface ; Japonica type had more coverage of surface by wax in the long-hairy, the pubescent and the water-wettable rice varieties than the IndicaxJaponica type, but the Indicax Japonica variety had more wax than the Japonica variety in the glabrous. However, the amount of waxes extracted with chloroform of the varieties is seemingly inversely related to their degrees of wax coverage of leaf surface. We have not measured the thickness of the wax deposit on leaf surface so that it is not certain how the above inverse relation holds for the amount of waxes extractable and the wax coverage among varieties.

2. Chemical composition of the epicuticular waxes of rice leaves by variety and growth stage.

Various kind of standard chemicals as shown in the Table 2 were employed to identify the chemical components of the epicuticular waxes in the process of chemical analyses. In thin layer chromatographic analysis of the chemical classes of the components of waxes the Rf value of the standards varied with developing solvent system(Table 4). Benzene resolved better the polar and non-polar compounds into major chemical classes than bezene + n-hexane (1 : 1, v/v). In detection I_2 vapor and $K_2Cr_2O_7$ solution (5%) were destructive for wax consituents, but UV light (254 nm and 360 nm) and sunlight were not. I_2 vapor could not detect n-hydrocarbons, but $K_2Cr_2O_7$ solution could detect all chemical classes of the standards. Long UV light (360 nm) could detect diols, primary and secondary alcohols, and aldehydes in the polar class, but short UV light (254 nm)

Table 4. Rf values of the standards for wax composition analysis on TLC[a].

Wax standards	Rf values in solvent system[a]		Detection(Solvent I)				
			I_2	$K_2Cr_2O_7$[b]	UV light		Sun light
	I	II			254nm	360nm	
Diols	0.00	0.00	0.00	0.00	–	0.00	–
Acids	0.02	0.00	0.02	0.02	–	0.02	–
P. & Sec. alcohols	0.05	0.04	0.05	0.06	–	0.05	–
Aldehydes	0.32	0.23	0.32	0.32	0.31	0.31	–
Esters(Methyl)	0.45	0.32	0.45	0.45	–	–	0.46
Ester(C31)	0.50	0.36	0.50	0.50	–	–	0.51
Ketone	0.48	0.34	0.48	0.48	0.49	–	–
n–Hydrocarbon	0.72	0.63	–	0.72	0.73	–	0.73

a : Solvent system : I –benzene, II –benzene+n–hexane(1 : 1, v/v)

b : $K_2Cr_2O_7$ 5%(w/w) $K_2Cr_2O_7$ in 40%(w/w) H_2SO_4 solution.

could identify ketones and n-hydrocarbons in the non-polar class. Sunlight could easily detect the esters and hydrocarbons appearing glossy on a TLC plate. Use of UV irradiation and sunlight was proper to detect chemical classes without destruction of the waxes.

The epicuticular waxes on rice leaves were clearly resolved by TLC into seven chemical classes, being identified as diols, acids, alcohols, aldehydes, esters, unsaturated and saturated hydrocarbons and an unknown (Table 5 and Fig. 2). Each of the major components seperated by TLC of the epicuticular waxes of the variety LK2-7 was further analyzed by Fourier transform infrared spectrophotometry to get IR spectra as shown in Fig. 3. Putative diols absorbed at the band of 3000 to 3600 cm^{-1}, which denotes -OH bond, but the acylated constituent did not. This result indicates it a diol. Esters absorbed at the band of 1735 to 1750 cm^{-1}, which denotes the -COO- bond. Hydrocarbons were confirmed to be mixture of the normals and the unsaturateds, in which the IR spectrum showed absorption bands in the range of 1620 to 1680 cm^{-1}, included with carbon double bond. Among chemical classes diols and the unsaturated hydrocarbons were identified as new classes of the epicuticular waxes on rice leaves, not observed by Uchiyama *et al*[20]

Table 5. Rf values of rice epicuticular waxes [a].

Fraction No.	Epicuticular waxes	Rf value
1	Diols	0.00
2	Acids	0.02 – 0.06
3	Alcohols	0.06 – 0.12
4	Unknown	0.15 – 0.19
5	Aldehydes	0.35 – 0.40
6	Esters	0.50 – 0.58
7	Hydrocarbons (C=C)	0.63 – 0.68
8	〃 (normal)	0.68 – 0.75

a : Solvent : benzene. Detection : I_2, $K_2Cr_2O_7$, UV light and sun light.

As shown in Fig. 2, there was no significant qualitative difference in chemical classes of the waxes among rice varieties, but difference was found between two growth stages. The waxes extracted at the tillering stage included only the non-polars as aldehydes, esters and hydrocarbons. On the other hand, those at the heading stage included not only non-polar but also the polars such as diols, acids and

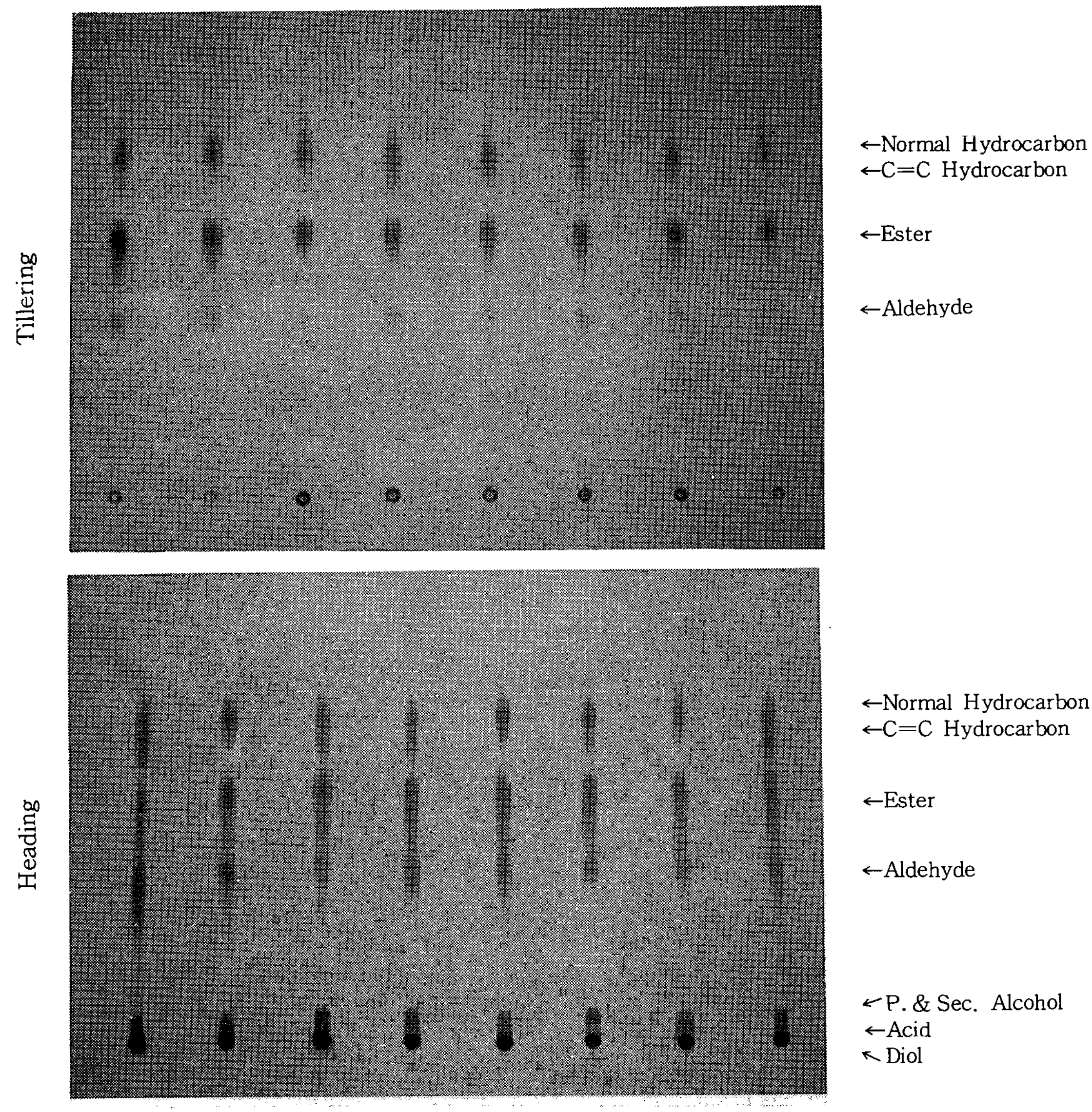


Fig. 2. Thin laver chromatograms of epicuticular waxes of rice varieties.
Solvent : Benzene, Detection : Benzene, Detection : 5% $K_2Cr_2O_7$ in 40% H_2SO_4 solution.
Ⅰ : M101, Ⅱ : wx817, Ⅲ : LK2-7, Ⅳ : HP914, Ⅴ : Chucheong, Ⅵ : Cheongcheong
Ⅶ : HP857, Ⅷ : wx139

alcohols. This results are consistent with our observation that rice leaves at the heading stage could be much easily wetted than those at the tillering stage by spray droplets of pesticides. This result is affirmed in Fig. 4 which shows relative composition (%) of chemical classes of the leaf epicuticular waxes at two growth stages of eight varieties. The waxes at the tillering stage consisted mainly of aldehydes and esters, except for the variety wx139, in which n-hydrocarbons were the main constituents. Those at the heading stage, on

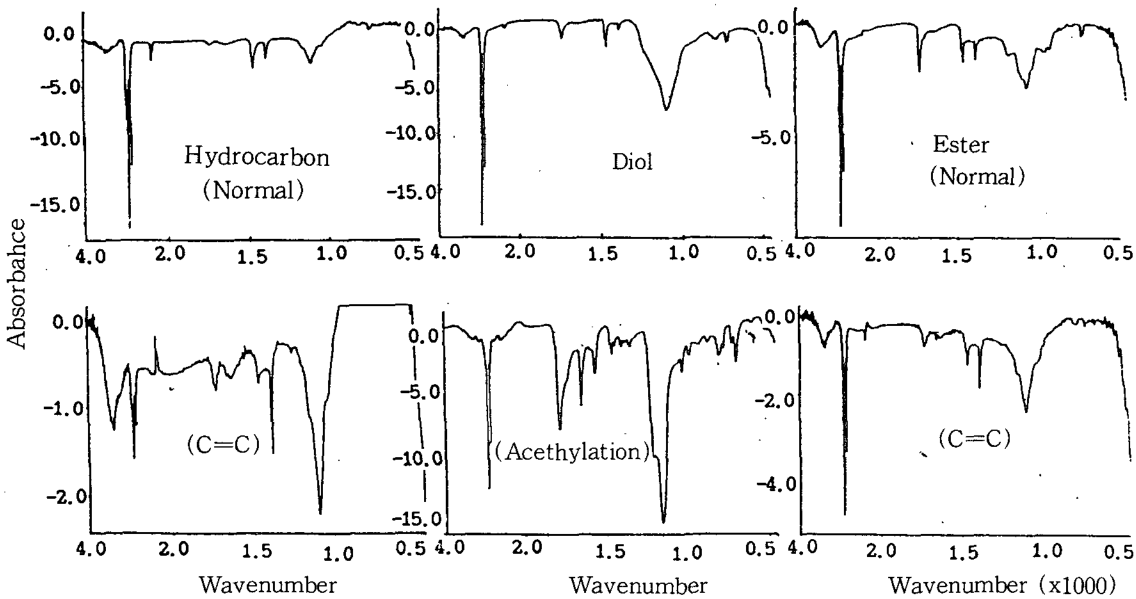


Figure 3. IR spectra of hydrocarbon, diol, and ester of the epicuticular wax components of rice variety, LK2-7.

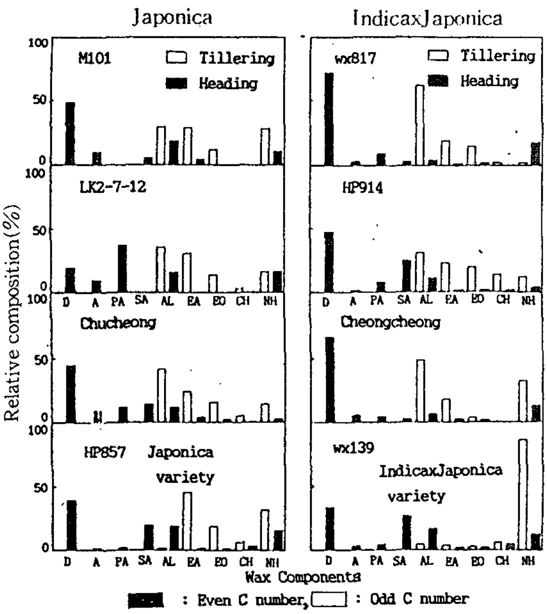


Figure 4. Relative composition(%) of rice leaf epicuticular waxes at two growth stages of 8 varieties.

Note〉 D : diols, A : acids, PA : primary alcohols, SA : secondary alcohols, AL : aldehydes, EA : acid esters, EO : alcohol esters, CH : C=C hydrocarbons, NH : normal hydrocarbons.

the other hand, consisted mainly of diols, except for the variety LK2-7, in which primary alcohols were the main constituents. Amount of diol and ketone in the glaborus, long-hairy and pubescent varieties were in general greater in the IndicaxJaponica varieties than in the Japonicas. In the water-wettable varieties diols and ketones were less in the IndicaxJaponicas than in the Japonicas. On the contrary, the relative amount of hydrocarbons was greater in the IndicaxJaponicas than in the Japonicas.

Carbon numbers of the polar classes were analyzed and identified by GLC. Diols were entirely composed of C30 and acids were of C30 and C31. In alcohols, primary alcohols were composed of C13 and C32 and the secondaries were of C14, C16, and/or C30 regardless of rice variety (Fig. 5). Carbon numbers of the primary alcohols in this study were different from those reported by Uchiyama *et al*[20] where those consisted mainly of C30. Carbon numbers in the non-polar classes (Fig. 6,7,8 and 9) were similar in aldehydes, ranging from

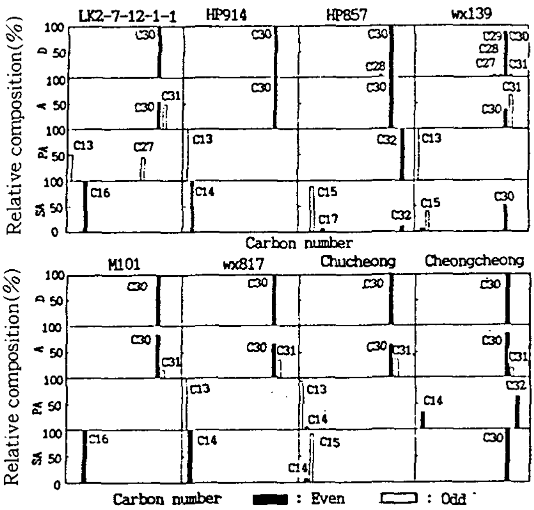


Figure 5. Relative Composition(%) of polar components of rice leaf epicuticular waxes in carbon number as analyzed for 8 varieties at heading stage.

Note〉 D : diols, A : acids, PA : primary alcohols, SA : secondary alcohols.

C29 to C34 and those in acid portion of the esters were of C22 and C23, lower carbon numbers compared with the aldehyde's. Those in the alcohol portion of the esters ranged widely from C13 to C26 depending on rice variety. Hydrocarbons had odd carbon numbers, consisting mainly of C29 and C31, which is consistent with the results of Flore *et al*[7] and Foy *et al*[10]. Unsaturated hydrocarbons with carbon double bond had simple distribution in carbon number as n-hydrocarbons.

Through the present study it has been clear that the rice varieties differ each other in the amount and chemical composition of chloroform extractable epicuticular waxes on leaves and the amount and chemical composition vary more with growth stage than by variety. The amount of waxes extractable was not proportional to the coverage of leaf surface by waxes, indicating that thickness of the wax deposits is also an important factor determining the amount of waxes on leaf surface. Although the amount of waxes on leaf surface was two to three times greater at heading stage of the rice plants than at tillering stage, the rice leaves had an increased wettability at the later stage due to increased polarity of the waxes. Water wettability of the so called water-wettable genotypes seems to owe to sparse wax deposition on leaves while the glabrous leaf variety seems to owe to even wax cover on leaf surface for glabrousness. The amount and composition of epicuticular waxes as well as the leaf surface coverage by wax and fine structure of the waxes might be under genetic control and should have some bearings on the varietal difference in water relations, resistance to diseases and insects and penetration of exogeneously applied chemicals.

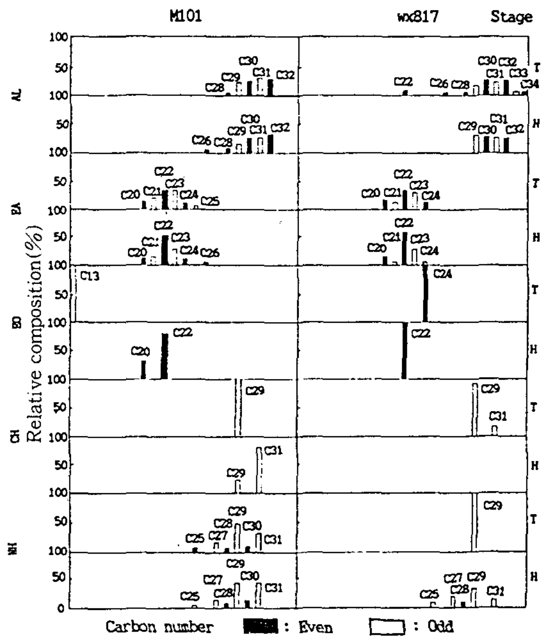


Figure 6. Realtive composition(%) of nonpolar components of rice leaf epicuticular waxes in carbon number as analyzed for glaborus varieties at two growth stages.

Note〉 T : tillering, H : Heading, NH : normal hydrocarbons, EO : alcohol esters, CH : C=C hydrocarbons, EA : acid esters, AL : aldehydes.

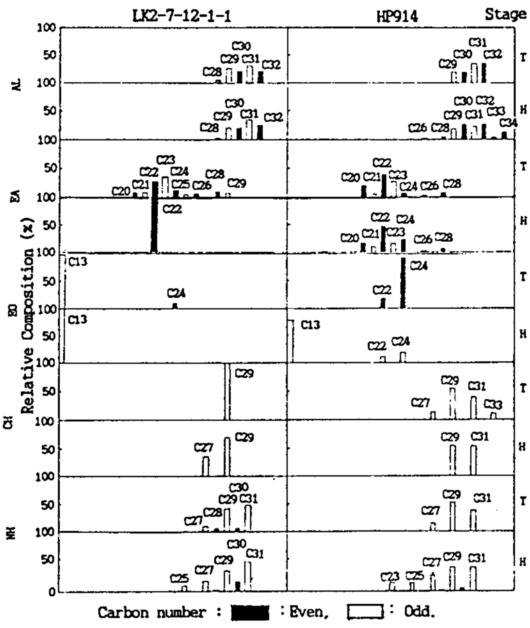


Figure 7 Relative composition(%) of nonpolar components of rice leaf epicuticular waxes in carbon number as analyzed for long-hairy varieties at two growth stages.

Note〉 T : tillering, H : heading AL : aldehydes, EA : acid esters, NH : normal hydrocarbons, EO : alcohol esters, EA : acid esters, CH : C=C hydrocarbons.

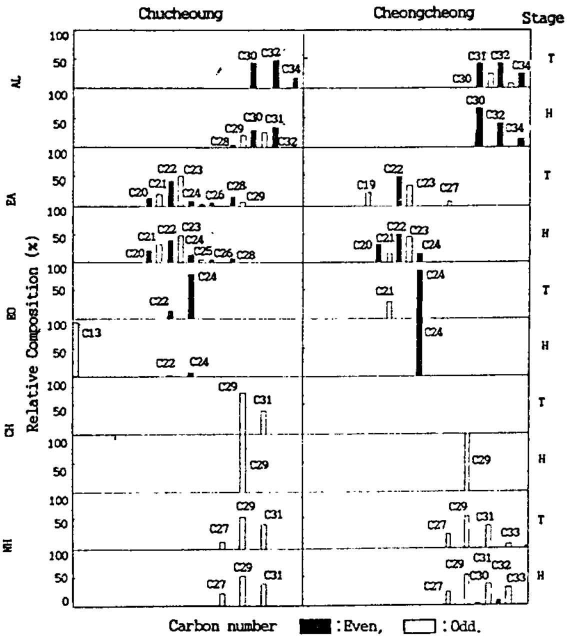


Figure 8 Relative composition(%) of nonpolar components of rice leaf epicuticular waxes in carbon number as analyzed for pubescent varieties at two growth stages.

Note〉 T : tillering, H : heading, AL : aldehydes, EA : acid esters, NH : normal hydrocarbons, EO : alcohol esters, EA : acid esters, CH : C=C hydrocarbons.

摘　　要

本 硏究는 葉表面 特性 및 遺傳的 特性이 현저히 다른 벼 品種들의 葉表面 wax의 量과 化學的 組成을 밝히고자 遂行 되었다. 벼品種은 葉의 形態的 特性面에서 glabrous한 것, long-hairy한 것, pubescent한 것, water-wettable한 것을 選定했고 이들 各各에 대한 日本型 品種과 統一型(Indica ×Japonica) 品種을 1개씩 供試하였으며, 供試品種들의 分蘖期 및 出穗期에 葉表面 wax를 chloroform으로 抽出하고 그 量 및 化學的 組成을 TLC, GLC 및 FTIR로 分析했으며, 그 主要結果는 다음과 같다.

1. 벼의 葉表面 wax는 chloroform을 溶媒로 抽出할 때 대체로 葉表面 wax 全體에 대해 均等하게 作用하며 wax 小片의 一部 및 尖銳한 部分을 많이 溶解시키나 常溫에서 chloroform이 葉全面에 均一하게 接觸하고 wax 以外 物質을 抽出하지 않기 위해서 벼 잎을 5分以內 浸漬함이 적당했지만 5分間 抽出로도 葉面積 wax 全量을 抽出하지는 못하였다.

2. 供試 品種들의 葉表面 wax는 大部分 小片狀(platelet)으로 葉表面에 蓄積되어 있고 品種에 따라 wax蓄積의 稠密性이 달랐는데 Glabrous 品種이 제일 緻密했고 Long-hairy 品種, Pubescent 品種들이 그 다음으로 緻密했으며, Water-wettable 品種들은 wax 小片들이 적고 엉성하여 큐티클層 露出이 많았다. 또한 잎의 前面과 裏面間 wax 蓄積은 品種에 관계없이 裏面보다 前面이 더 緻密하였다.

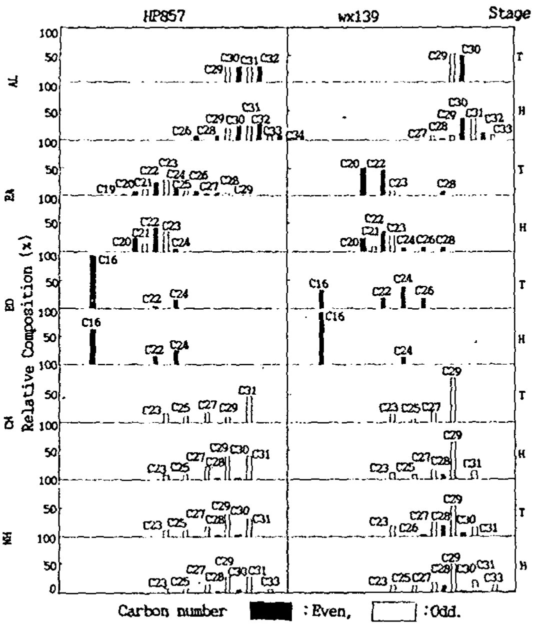


Figure 9 Relative composition(%) of nonpolar components of rice leaf epicuticular waxes in carbon number as analyzed for water-wettable varieties at two growth stages.

Note〉 T : tillering, H : heading, AL : aldehydes, EA : acid esters, NH : normal hydrocarbons, EO : alcohol esters, EA : acid esters, CH : C=C hydrocarbons.

3. Chloroform으로 5分씩 2回 浸出한 葉表面 wax의 量은 벼品種間 差異보다 生育時期間 差異가 컸는데, 分蘖期는 0.8~1.8 mg/g f.w. 水準, 出穗期에는 1.7~3.6 mg/g f.w. 水準으로 出穗期에 分蘖期보다 2~3倍 정도 더 많았다.

4. 벼 葉表面 wax의 化學組成은 TLC상에서 크게 7成分으로 分離 同定되었는데 ketone類는 確認할 수 없었고 diol類, fatty alcohol類, fatty acid類, fatty aldehyde類, fatty ester類, 飽和와 不飽和 hydrocarbon類 等이 確認되었다.

5. 葉表面 wax의 TLC上의 化學組成은 벼品種間보다 生育時期別로 달랐는데 分蘖期는 極性 成分인 diol, fatty acid, fatty alcohol類들을 確認할 수 없었지만 出穗期에는 이들과 非極性 成分인 fatty aldehyde, fatty ester, 飽和 및 不飽和 hydrocarbon類를 確認할 수 있었다.

6. 벼 葉表面 wax를 이루고 있는 組成을 化學成分別로 炭素數面에서 보면 diol類는 C30 單一物質로, fatty acid類는 C30과 C31로 構成되어 있었고, fatty alcohol類中 primary인 경우 C13과 C32가 主宗이었고 secondary는 C14, C16, C30으로 構成되어 있었으며, aldehyde類는 C29 - C34까지 비교적 비슷한 比率로 分布되어 있었다. Fatty ester중 acid 部位는 aldehyde 部位보다 低炭素數 分布를 보였고 主로 C22, C23이었고, alcohol類는 C13, C22, C24, C26 等으로 品種에 따라 다른 構成을 보였다. Hydrocarbon類는 品種과 生育時期에 관계없이 炭素數가 홀수인 物質이었으며 主로 C29와 C31이었다.

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