Bull. Inst. Chem. Res., Kyoto Univ., Vol. 61, No. 2, 1983

ORIGINAL REVIEW

Biosynthesis of Leaf Alcohol

Akikazu Hatanaka*

Received April 30, 1983

KEY WORDS: Tea Chloroplats/ Leaf Alcohol/ Biosynthetic Path Way/

INTRODUCTION

Leaf alcohol, *cis*-3-hexenol, and leaf aldehyde, *trans*-2-hexenal¹), are widely distributed in fresh leaves, vegetables, and fruits, and they are responsible for the "Green Odor" characteristics of leaves. They are found in some insect excretions, as functioning attractants and repellents. Investigations on leaf alcohol have been carried out by Takei and Ohno *et al.* since 1938.¹⁻⁸) From 1957, we have been studying leaf alcohol and leaf aldehyde from different approaches: synthetic chemistry, natural products chemistry, and plant biochemistry.⁹⁻¹⁰³)



Scheme 1 Biosynthetic pathway of leaf alcohol.

^{*} 畑中顕和: Department of Agricultural Chemistry, Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida Yamaguchi 753, Japan.

We recently have focused our attention on the biosynthesis of leaf alcohol and aldehyde and have demonstrated the biosynthetic pathway with *Thea sinensis* leaves, as shown in Scheme 1. We know now that the leaf alcohol and aldehyde are produced from *cis*-3-hexenal.^{39,42,49} This C₆-compound, *cis*-3-hexenal, and the C₁₂-compound, 11-formyl-*cis*-9-undecenoic acid,^{61,62} biosynthesized from linolenic acid by enzymatic oxygenative splitting through the addition of oxygen to the double bond between C-12 and C-13. In the oxygenative splitting reaction, two enzymes, *lipoxygenase* (E'_2) and *hydroperoxide lyase* (E''_2) are involved, and 13-L-hydroperoxylinolenic acid is an intermediate. From this *cis*-3-hexenal, *trans*-3-hexenal⁸⁰ and *trans*-2-hexenal are formed by the isomerization and *cis*-3-hexenol is formed by reduction with *alcohol dehydrogenase* $(E_3)^*$. The C₁₂ fragment isomerizes to give 11-formyl-*trans*-10-undecenoic acid,^{61,62} traumatic half aldehyde, which is known as the "wound hormone".

FORMATION OF "GREEN ODOR"

Changes in fatty acid contents.^{39,72)} First, changes in fatty acid contents in lipids were examined. The Table I shows the result of leaves harvested in June and in November. The summer leaves showed a high activity for C₆-aldehyde formation and the winter leaves showed a low activity. Fatty acids were prepared from leaves blended for 3 minutes in a Waring blender or without blending. The homogenate was extracted with chloroform-methanol and separated into phospholipid, neutral fat, and free fatty acid fractions by acetone and ether fractionation. The phospholipids and neutral fats obtained were saponified with potassium hydroxide, then esterified with diazomethane. The obtained fatty acid methyl esters were analyzed and determined by GLC with a 20% PEG-adipate column, 3 mm by 1 m. In this table, the 0 and 3 in parentheses refer to minutes of blending time. With no blending, more than 50% of the fatty acids were found in the neutral fat fraction, and the rest in the

	Blending time (min)					
Fraction	0	3	0	3		
	Linole	eic acid	Linolenic acid			
Free fatty acid	trace (trace)	trace (trace)	trace (trace)	trace (trace)		
Neutral fat	55.6 (18.5)	23.5 (15.8)	218.3 (164.0)	99.3 (145.8)		
Phospholipid	16.8 (13.8)	$13.3 \\ (3.5)$	11.5 (8.4)	10.5 (1.4)		
Total lipid	72.4 (32.3)	36.8 (19.3)	229.8 (172.4)	109.8 (147.2)		

Table I. Changes in fatty acid contents in tea leaves

mg/100 g fresh tea leaves.

On 6th of June, () on 26th of November.

* trans-2-hexenol.⁸¹⁾

A. HATANAKA

phospholipid fraction. The ratio of linolenic acid to linoleic acid was about 3 in summer leaves and 5 in winter leaves. When the summer leaves were blended for 3 minutes, large quantities of linoleic acid and linolenic acid, about a half of the initial content, disappeared from these two lipid fractions, neutral fat and phospholipids. But no free fatty acids were found. In the winter leaves, blending caused a far lower decrease in fatty acid content in lipid fractions compared with the summer leaves. These results indicate that linolenic acid and linoleic acid hydrolyzed are converted to compounds other than free fatty acids.

Hexenal and hexanal formation during the blending.^{39,72)} In Fig. 1, the broken

line indicates the total linolenic acid content in lipids. During the blending of summer tea leaves, linolenic acid content decreased. Therefore, we expected that C_6 -compounds, such as *trans*-2-hexenal and *cis*-3-hexenol derived from linolenic acid would increase during the blending of leaves with decreasing linolenic acid content. In addition to linolenic acid determination, C_6 -compound determinations were performed using essential







Fig. 2. Seasonal changes in the activity of enzyme system producing cis-3-hexenal in fresh leaf homogenate.

oil prepared from blending leaves by steam distillation followed by ether extraction. During the blending, *trans*-2-hexenal, indicated by the open circles and solid line, increased dramatically. *cis*-3-Hexenol, indicated by the dark circles, increased initially at one minute and then reached a plateau. On the other hand, in the first three minutes, 4 μ moles of linolenic acid were lost from lipids and 1 μ mole of *trans*-2-hexenal and *cis*-3-hexenol was newly formed, indicating that one-quarter to one-third of the lost linolenic acid was converted to C₆-compounds, such as *trans*-2-hexenal and *cis*-3-hexenol. In the procedures used here, *cis*-3-hexenal, which is a labile compound in the homogenate, was not detected because of isomerization to *trans*-2-hexenal.

Seasonal changes in the C₆-aldehyde forming activity.^{52,54)} Next we examined the seasonal changes in C₆-aldehydes forming activity from linoleic acid and linolenic acid using tea leaf homogenates. Linolenic acid or linoleic acid was incubated at 35° C for 10 minutes with the leaf homogenate in a sealed flask. At the end of incubation time, the headspace vapor gas was analyzed by GLC as having 20% PEG 20M column in 3 mm by 3 m. Hexanal formation is represented by the dark circles and hexenal formation, by open circles. The dotted lines indicate averages of maximum and minimum temperature for 10 days (Fig. 2). The solid line indicates solar radiation. C₆-Aldehyde forming activity began to increase in late March to April. In July to August, the activity reached a maximum, then gradually decreased and disappeared completely in December. The activity changes were parallel to the temperature changes.

Localization of C₆-aldehyde formation activity.⁴⁷⁾ Since the summer leaves exhibited high activity for C₆-aldehyde formation, we fractionated the tea leaves into subcellular fractions. Tea leaves harvested in August were homogenized with McIlvaine's buffer, pH 6.3, containing 0.4 M sucrose for 3 minutes, and the homogenate was filtered through three layers of gauze. Then the filtrate was subjected to successive centrifugation at 1,000 g, 4,000 g and 19,000 g. The activity for C₆-aldehyde formation in each fraction was determined by the headspace method. Most of the activity for C₆-aldehyde formation was localized in the 1,000 g pellet, which was the chloroplast-rich fraction (Table II). Washing the chloroplast-rich 1,000 g pellet with McIlvaine's buffer, pH 6.3, repeatedly, did not cause a significant decrease in activity. Therefore, the activity for C₆-aldehyde formation was concluded to be localized in the chloroplast lamellae membrane. In the following experiments, the

En et en	C_6 -Aldehydes [μ mol]			
Fraction ~	Hexenals	Hexanal		
1000 g pellet*	117.5 (90)	22.3 (76)		
4000 g pellet	10.7 (8)	3.6 (12)		
19000 g pellet	2.9 (2)	1.1 (4)		
Supernatant	0.4 (0)	2.4 (8)		

Table II. Localization of C_6 -aldehyde formation activity

(183)

A. HATANAKA

pellet obtained by centrifugation of the 1,000 g supernatant at 4,000 g for 10 minutes was used routinely as the chloroplast-rich fraction.

Biosynthetic pathway of C_6 -aldehydes from ¹⁴C-labeled fatty acids.⁴⁹⁾ When uniformly labeled ¹⁴C-linolenic acid was incubated with isolated tea chloroplasts, two radioactive peaks were found at positions of cis-3-hexenal and trans-2-hexenal in headspace vapor gas with radio gas chromatograph, as shown by the spiked line (Fig. 3). Other radioactive peakes, such as hexanal, alcohols, and short chain aldehydes, were not found. When incubation was prolonged, radioactivity in *cis*-3-hexenal decreased, and radioactivity in trans-2-hexenal increased. When ¹⁴C-labeled linoleic acid was used, hexanal was labeled by 14C, but no other volatile compounds were labeled. Thus, the six-carbon aldehydes were produced from linolenic acid or linoleic acid in the presence of isolated tea chloroplasts. When the reaction mixture of $1-^{14}$ C-labeled linolenic acid and isolated tea chloroplasts was incubated, extracted with ether and the extract analyzed by gas liquid chromatograph, five major radioactive peaks were found. One peak was an unreacted substrate peak. Two others were 11-formylcis-9-undecenoic acid and 11-formyl-trans-10-undecenoic acid, as presented here. After cleaving linolenic acid and linoleic acid, these two compounds were expected to be moiety twelve-carbon. Other radioactive peaks were azelaic half aldehyde, 9formyloctanoic acid, and unknown compound. Radioactivity in 11-formyl-cis-9undecenoic acid decreased with prolonged incubation time, while 11-formyl-trans-10undecenoic acid increased. These results indicate that cis-3-hexenal and 11-formylcis-9-undecenoic acid were first formed from linolenic acid and then cis-3-hexenal was isomerized to trans-2-hexenal and 11-formyl-cis-9-undecenoic acid to 11-formyl-trans-10-undecenoic acid.



Fig. 3. Radio gas chromatograms of cis-3-hexenal and trans-2-hexenal from linolenic acid [U-¹⁴C] (left) and of 11-formyl-cis-9- and -trans-10-undecenoic acid from linolenic acid [1-¹⁴C] by isolated tea chloroplast.

Distribution of an enzyme system producing *cis*-3-hexenal and *n*-hexanal from linolenic and linoleic acids in some plants.⁶⁷⁾ The activity of the enzyme system $(E'_2 + E''_2)$ producing C₆-aldehydes from C₁₈-unsaturated fatty acids was investigated using about 40 plants. Green leaves of dicotyledonous plants belonging to the *Sphenopsida*, *Pteropsida*, *Theaceae* and *Leguminosae* showed a high enzyme activity but edible leafy vegetables and fruits and monocotyledonous plants showed a low activity as shown Table III. Seasonal changes in the enzyme activities were observed. The concentrations of *cis*-3-hexenol and *trans*-2-hexenal and the enzyme activities showed a correlation; high concentrations were observed in the summer but they were low in the winter.

Solubilization and properties of the enzyme-cleaving 13-L-hydroperoxylinolenic acid in tea leaves.⁸²⁾ The membrane bound hydroperoxide lyase (E_2'') which catalyses the cleavage of 13-L-hydroperoxides of linolenic and linoleic acids to C₆volatile aldehydes (hexenals and n-hexanal) was found to be localized in the chloroplast lamellae of tea leaves. It was selectively solubilized from the lamellae with 0.5% (w/v) Tween 20. The enzymatic cleavage of the hydroperoxides occurred even under anaerobic conditions. The optimal pH of E_2'' was 7-8. The common structural features shown by substrates of E_2'' were the presence of a 13-L-hydroperoxy group at ω -6 with a conjugated trans, cis-diene at ω -7 and ω -9 in a C₁₈-fatty acid. E_2'' had an apparent Km of 2.5 and 1.9 mM for 13-L-hydroperoxylinolenic and 13-L-hydroperoxylinoleic acids, respectively. No significant differences were found between chloroplast and solubilized (E_2'') .

Participation and properties of lipoxygenase and hydroperoxide lyase in volatile C_6 -aldehyde formation from C_{18} -unsaturated fatty acids in isolated tea chloroplasts.⁸⁴⁾ Isolated tea chloroplasts utilized linoleic acid, linolenic acid and their 13-hydroperoxides as substrates for volatile C_6 -aldehyde formation. Optimal pH values for oxygen uptake (E'_2) , hydroperoxide lyase (E''_2) and the overall reaction $E'_2 + E''_2$ from C_{18} -fatty acids to C_{6} -aldehydes were 6.3, 7.0 and 6.3, respectively. Methyl linoleate, linoleyl alcohol and γ -linolenic acid were poor substrates for the overall reaction, but linoleic and linolenic acids were good substrates (Fig. 4, 5). The 13hydroperoxides of the above fatty acids and alcohol also showed substrate specificity similar to that of fatty acids. Oxygen uptakes (relative Vmax) with methyl linoleate, linoleyl alcohol, linolenic acid, γ -linolenic acid and arachidonic acid were comparable to or higher than that with linoleic acid. In winter leaves, the activity for C₆-aldehyde formation from C_{18} -fatty acids was reduced to almost zero. This was due to the reduction in oxygenation. The findings presented here provide evidence for the involvement of *lipoxygenase* and *hydroperoxide lyase* in C_6 -aldehyde formation in isolated chloroplasts.

Oxygen-isotope effect in enzymatic cleavage raction of 13-L-hydroperoxylinoleic acid to hexanal and 11-formyl-*cis***-9-undecenoic acid**⁸⁶⁾ *Hydroperoxide lyase E*ⁿ₂ solubilized with Tween 20 from tea chloroplasts⁸²⁾ was shown to catalyze cleavage reaction of 13-L-hydroperoxy-*cis*-9, *trans*-11-octadecadienoic acid (13-L-hydroperoxylinoleic acid) to hexanal, and 11-formyl-*cis*-9-undecenoic acid by identification of cleavage products using authentic specimens synthesized through an unequivocal route. An oxygen-isotope effect was first observed in the cleavage

А. Натапака

Table III. Distribution and activity of enzyme system

1. Hair moss Musci Polytrichaccae Pegonatum inflexum L 2. Horsetail Sphenopsida Osmundaceae Gumund ajeonica L 4. Bracken Poloyoliaccae Osmundaceae Osmunda L Equisetum artense L 5. Ginkgo Ginkgopsida Ginkgooaceae Podocarpaceae Podocarpasemato L L 6. Chinese black pine Coniferopsida Dicotyledoneae Cucurbitaceae Cucurnis mai L 7. Melon Dicotyledoneae Cruciferae Brassica oleracea var. botytis L (green) 9. Cabbage Cruciferae Brassica oleracea var. botytis L (green) L 10. Cauliflower Theaceae Cleyera japonica L L 11. Radish Theaceae Cleyera japonica L L 12. Sakaki Theaceae Cleyera japonica L L 13. Tea Tea Sasanqua L Camellia sasangua L 14. Camellia Sasanqua Ebenaceae Fragaria grandiflora F (red) 15. Sasanqua Leguminosae Prumus persica L		Plant no. and common name	Class	Family	Genus and species	Organ*
1. Hair moss Musci Polytrichacea Pagonatum inflexum L 2. Horsetail Sphenopsida Peropsida Osmunda ceae Comunda joponica L 3. Ozmunda Polypodiaceae Osmunda joponica L Periofium aguilinum L 5. Ginkgo Ginkgopsida Coniferopsida Odocarpaceae Padocaptus macrophylla L 7. Melon Dicotyledoneae Cucurbitaceae Padocaptus macrophylla L (green) 9. Cabbage Cruciferae Brassica oleracea var. L (green) (green) 10. Cauliflower Brassica oleracea var. L (green) Inforet Inforet 13. Tea Thea ceae Cleyera japonica L Camellia japonica L 14. Camellia Thea ceae Diostyros kaki L Camellia sasangua L 15. Sasanqua Ebenaceac Prunus mume L Prassica oleracea var. L 15. Japanese apricot Amygdalaceae Prunus mume L Camellia sasangua L 16. Japanese wistaria Leguminosae Robinia pseudoacacia L Phaseabus vulgaris L </th <th></th> <th></th> <th></th> <th></th> <th></th> <th></th>						
2. Horsetail Sphenopsida Equisetaceae Equisetaceae Equisetam arvense L 3. Osmund Percopsida Osmundaciaponica L Smundaciaponica L 4. Bracken Forken Forkopodiaceae Forkopodiaceae Ginkgo biloba L 6. Chinese black pine Coniferopsida Dicotyledoneae Podocarpaceae Cucumis macrophylla L 7. Melon Dicotyledoneae Cucurbitaceae Cucurbitaceae Cucurbitaceae T L (green) 9. Cabbage Cauliflower Fassica oleracea var. capitata L (green) 10. Cauliflower Brassia oleracea var. botypitis L (green) 11. Radish Theaceae Camellia assangua L L 12. Sakaki Theaceae Camellia assangua L L 13. Tea Lapanese perisimmon Ebenaceae Fragaria grandifora F (red) 13. Sasanqua Leguminosae Prunus mume L L 19. Peach Leguminosae Prusus mume L L 21. False acacia Lovera Aceraceae Aceraceae	1.	Hair moss	Musci	Polytrichaceae	Pogonatum inflexum	L ·
3. Osmund Pteropsida Osmundaceae Polypodiaceae Ginkgoaceae Osmunda japonica L 4. Bracken Ginkgopsida Ginkgoaceae Coniferopsida Osmundaceae Polocarpaceae Dickyledoneae Caucurbitaceae Caucumis malo L 6. Chinese black pine Coniferopsida Dicotyledoneae Cucurbitaceae Caumis saitious F 9. Cabbage Cruciferae Brassica oleracea var. capitata L (green) 10. Cauliflower Brassica oleracea var. capitata L (green) 11. Radish Theaceae Cleyera japonica L 12. Sakaki Theaceae Cleyera japonica L 13. Tea Thea sensuis L Camellia japonica L 14. Camellia Ebenaceae Diospyros kaki L L 15. Sasanqua Ebenaceae Diospyros kaki L L 16. Japanese apricot Amygdalaceae Prunus mume L L 19. Peach L Paaseas catia L Camellia sasanqua L 20. Japanese apricot Amygdalaceae Prunus mume L 21. Peach <td< td=""><td>2.</td><td>Horsetail</td><td>Sphenopsida</td><td>Equisetaceae</td><td>Equisetum arvense</td><td>L</td></td<>	2.	Horsetail	Sphenopsida	Equisetaceae	Equisetum arvense	L
4. Bracken Polypodiaceae Pteridium aquilinum L 5. Ginkgo Ginkgossida Ginkgoaceae Podocarpaceae Ginkgo biloba L 7. Melon Dicotyledoneae Cucurbiaceae Cucurbiaceae Cucurbis mato phylla L 8. Cucumber Dicotyledoneae Cruciferae Brassica oleracea var. L (green) 9. Cabbage Cruciferae Brassica oleracea var. L (green) 10. Cauliflower Brassica oleracea var. floret L (green) 11. Radish Theaceae Cleyra japonica L (green) 12. Sakaki Theaceae Cleyra japonica L L 13. Tea Camellia japonica L Camellia japonica L 14. Camellia Camellia japonica L L 15. Sasanqua Ebenaceae Prous mume L 16. Japanese priot Amygdalaceae Prunus mume L 19. Peach Pruse perioa L L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Amedicago sa	3.	Osmund	Pteropsida	Osmundaceae	Osmunda jabonica	L
5. Ginkgo Ginkgopsida Ginkgoacae Ginkgo biloba L 6. Chinese black pine Dicotyledoneae Podocarpaceae Ginkgo biloba L 7. Melon Dicotyledoneae Cucurbitaceae Ginkgo biloba L 9. Cabbage Cucurbitaceae Cucurbitaceae Cucurbis satituus F 9. Cabbage Cruciferae Brassica oleracea var. capitata L (green) 10. Cauliflower Brassica oleracea var. botrytis L (green) 11. Radish Thea ceae Cleyra japonica L 12. Sakaki Theaceae Cleyra japonica L 13. Tea Thea ceae Disopyros kaki L 14. Camellia Camellia japonica L L 15. Sasanqua Ebenaceae Disopyros kaki L 16. Japanese persimmon Ebenaceae Prunus mume L 19. Peach L Leguminosae Prunus persica L 21. Aile a cacia L Leguminosae L Glycine max L 22. Alfalfa Conter sate acia Solanareeae Solanaru satima <td< td=""><td>4.</td><td>Bracken</td><td>T</td><td>Polypodiaceae</td><td>Pteridium aquilinum</td><td>L</td></td<>	4.	Bracken	T	Polypodiaceae	Pteridium aquilinum	L
6. Chinese black pine Coniferopsida Podocarpaceae Podocarpaceae Podocarpaceae Podocarpus macrophylla L 7. Melon Dicotyledoneae Cucurbitaceae Cucuris saitinus F 8. Cucumber Cruciferae Brassica oleracea var. capitata L (green) 9. Cabbage Cruciferae Brassica oleracea var. capitata L (green) 10. Cauliflower Brassica oleracea var. copitata L (green) 11. Radish Theaceae Cleyera japonica L 12. Sakaki Theaceae Cleyera japonica L 13. Tea Thea sinensis L Camellia japonica L 14. Camellia Camellia japonica L Camellia japonica L 15. Sasanqua Ebenaceae Diopyros kaki L L 16. Japanese persimmon Ebenaceae Prumus persica L L 19. Peach Prunus persica L L L 21. False acacia Moriaceae Moriaceae L Clycine max L 23. White clover Chenopodiaceae Solanaceae Solana	5.	Ginkgo	Ginkgopsida	Ginkgoaceae	Ginkgo biloba	L
7. Melon Dicotyledoneae Cucurbitaceae Cucumis melo L 8. Cucumber Dicotyledoneae Cucurbitaceae Cucumis melo L 9. Cabbage Cruciferae Brassica oleracea var. L (green) 10. Cauliflower Brassica oleracea var. L (green) 11. Radish Theaceae Raphanus sativus L 12. Sakaki Theaceae Camellia japonica L 13. Tea Tea Camellia is asanqua L 14. Camellia Camellia is asanqua L Camellia is asanqua L 15. Sasanqua Ebenaceae Diospyros kaki L L 15. Sasanqua Ebenaceae Diospyros kaki L L 16. Japanese persimmon Ebenaceae Pranus persica L L 17. Strawberry Rosaccae Prunus persica L L 18. Japanese wistaria Leguminosae Wisteria floribunda L 19. Peach Trifolium repens L Z 21. False acacia Moraccae Aceraceae L 23. Sopbean	6.	Chinese black pine	Coniferopsida	Podocarpaceae	Podocarbus macrobhylla	L
8. Cucumber Caumis solitions F 9. Cabbage Cruciferae Brassica oleracea var. capitata L (green) 10. Cauliflower Brassica oleracea var. botrytis L (green) 11. Radish Raphanus satituus L 12. Sakaki Thea ceae Clevera japonica L 13. Tea Thea sinensis L It 14. Camellia Camellia japonica L L 15. Sasanqua Camellia japonica L L 16. Japanese persimmon Ebenaceace Diospyros kaki L 17. Strawberry Rosaccae Fragaria grandifora L 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Loguminosae Wisteria floribunda L 21. False acacia Loguminosae Robinia pseudoacacia L 23. White clover Trifolium repens L L 24. Soybean Aceracea Aceracea L L 25. Kidney bean Aceraceae Solanaceae L L L 26. Mulberry Mora ceae Solanaceae	7.	Melon	Dicotyledoneae	Cucurbitaceae	Cucumis melo	— T.
9. Cabbage Cruciferae Brassica oleracea var. capitala L 9. Cabbage Cruciferae Brassica oleracea var. capitala L 10. Cauliflower Brassica oleracea var. botrytis L 11. Radish Raphanus sativus L 12. Sakaki Theaceae Clepera japonica L 13. Tea Thea sinensis L L 14. Camellia Camellia japonica L L 15. Sasanqua Ebenaceae Diospyros kaki L 16. Japanese persimmon Ebenaceae Diospyros kaki L 17. Strawberry Rosaceae Fragaria grandiflora L 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Leguminosae Witteria floribunda L 21. False acacia Leguminosae Robinia pseudoacacia L 22. Alfalfa Medicago sativa L L 23. White clover Phaseolus culgaris L 24. Soybean Chenopodiaceae Spinacia oleracea L 25. Kidney bean Solanaceae Solanareae L <t< td=""><td>-8.</td><td>Cucumber</td><td>201000,100000</td><td>o dour briddoud</td><td>Cucumis saitivus</td><td>F</td></t<>	-8.	Cucumber	201000,100000	o dour briddoud	Cucumis saitivus	F
9. Cabbage Cruciferae Brassica oleracea var. capitata L (green) 10. Cauliflower Brassica oleracea var. botrytis L (pale green) 11. Radish Raphanus sativus L 12. Sakaki Theaceae Cleyera japonica L 13. Tea Theaceae Cleyera japonica L 14. Camellia Theaceae Diospyros kaki L 15. Sasanqua Ebenaceae Diospyros kaki L 16. Japanese persimmon Ebenaceae Pranus mume L 17. Strawberry Rosaceae Fragaria grandiflora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Peach L L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Modicago sativa L L 22. Alfalfa Moraceae Arwas bombycis L 23. White clover Trifolium repens L (green) 24. Soybean Chenopodiaceae Spinacia oleracea L 25. Kidney bean Chenopodiaceae Solanum tuberosum </td <td></td> <td>- ubunnbor</td> <td></td> <td></td> <td></td> <td>Ĩ.</td>		- ubunnbor				Ĩ.
10. Cauliflower L (pale green) 11. Radish Brassica oleracea var. 12. Sakaki Theaceae 13. Tea Theaceae 14. Camellia Cleyera japonica 15. Sasanqua Cleyera japonica 16. Japanese persimmon Ebenaceae 17. Strawberry Rosaceae 18. Japanese persimmon Ebenaceae 19. Peach Amygdalaceae 20. Japanese apricot Amygdalaceae 21. False acacia Leguminosae 22. Alfalfa Medicago sativa 23. White clover Trifolium repens 24. Soybean Chenopodiaceae 25. Kidney bean Chenopodiaceae 26. Mulberry Moraceae 29. Potato Solanaceae 30. Egg plant Solanaceae 31. Tomato Chenopodiaceae 32. Lettuce Compositae 33. Banana Monocotyledoneae 34. Onion Liliaceae 35. Duckweed Lemnaceae 36. Rice Gramineae 37. Wheet Lemnaceae	9	Cabhage		Cruciferae	Brassica oleracea var.	L (green)
10. Cauliflower Brassica oleracea var. botrytis floret 11. Radish Raphanus sativus L 12. Sakaki Theaceae Cleyera japonica L 13. Tea Thea sinensis L 14. Camellia Camellia japonica L 15. Sasanqua Camellia japonica L 16. Japanese persimmon Ebenaceae Distypros kaki L 17. Strawberry Rosaceae Fragaria grandiflora L 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Leguminosae Wisteria floribunda L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False caccia Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Chenopodiaceae Moras bombycis L 26. Mulberry Moraceae Moras bombycis L 27. Japanese maple Chenopodiaceae Solanum melongena L 28. Spinach Chenopodiaceae Solanum tuberosum tuber		CussuBc		oracinorac	capitata	T (Broom)
10. Cauliflower Brassica oleracea var. botytis floret 11. Radish Raphanus sativus L 12. Sakaki Theaceae Clevera japonica L 13. Tea Thea sinensis L 14. Camellia Camellia japonica L 15. Sasanqua Ebenaceae Diospros kaki L 16. Japanese persimmon Ebenaceae Diospros kaki L 17. Strawberry Rosaceae Fragaria grandiflora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Leguminosae Wisteria floribunda L 20. Japanese vistaria Leguminosae Wisteria floribunda L 21. False acacia Moraceae Moticago sativa L 22. Alfalfa Moraceae Morus bombycis L 23. White clover Chenopodiaceae Solanum tuberosum L (green) 24. Soybean Chenopodiaceae Solanum melongena L 25. Kidney bean Solanaceae Solanum melongena L 26. Mulberry Moraceae Solanum melongena L					•	L (pale green)
11. Radish Raphanus satious L 12. Sakaki Theaceae Cleyera japonica L 13. Tea Theaceae Cleyera japonica L 14. Camellia Camellia japonica L 15. Sasanqua Camellia sasanqua L 16. Japanese persimmon Ebenaceae Diospyros kaki L 17. Strawberry Rosaccae Fragaria grandiftora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Peach Prunus persica L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Robinia pseudoacaia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Chenopodiaceae Morus bombycis L 26. Mulberry Moraceae Acer palmatum L (green) 27. Japanese maple Aceraceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena	10.	Cauliflower			Brassica oleracea var. botrytis	floret
12. Sakaki Theaceae Clevera japonica L 13. Tea Thea sinensis L 14. Camellia Camellia japonica L 15. Sasanqua Camellia sasanqua L 16. Japanese persimmon Ebenaceae Diospyros kaki L 17. Strawberry Rosaceae Fragaria grandiflora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Prunus persica L L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Medicago sativa L L 22. Alfalfa Medicago sativa L L 23. White clover Trifolium repens L 24. Soybean Phaseolus vulgaris L 25. Kidney bean Chenopodiaceae Acer palmatum L (green) 26. Mulberry Moraceae Acer palmatum L (green) 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Solanum tuberosum L 29. Potato Solanue	11.	Radish			Raphanus sativus	L
13. Tea Thea sinensis L 14. Camellia Camellia japonica L 15. Sasanqua Camellia sasanqua L 16. Japanese persimmon Ebenaceae Diosyros kaki L 17. Strawberry Rosaccae Fragaria grandiffora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Leguminosae Wisteria floribunda L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Robinia pseudoacacia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Solanum tuberosum L (red) 29. Potato Solanaceae Solanum melongena L 31. Tomato Lopopositae Lactuca sativa L </td <td>12.</td> <td>Sakaki</td> <td></td> <td>Theaceae</td> <td>Cleyera japonica</td> <td>L</td>	12.	Sakaki		Theaceae	Cleyera japonica	L
14. Camellia Camellia japonica L 15. Sasanqua Camellia japonica L 16. Japanese persimmon Ebenaceae Diospyros kaki L 17. Strawberry Rosaceae Fragaria grandiflora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Leguminosae Wisteria floribunda L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Robinia pseudoacacia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Phaseolus vulgaris L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Solanum tuberosum L 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Liliaceae Musaceae Musa paradisiae F 32. Lettuce Compositae	13.	Tea			Thea sinensis	L
15. Sasanqua Camellia sasanqua L 16. Japanese persimmon Ebenaceae Diospyros kaki L 17. Strawberry Rosaceae Fragaria grandiflora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Prunus persica L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Medicago sativa L 22. Alfalfa Medicago sativa L 23. White clover Glycine max L 24. Soybean Glycine max L 25. Kidney bean Moraceae Morus bombycis L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Solanaceae Solanum tuberosum L (red) 28. Spinach Chenopodiaceae Solanum tuberosum L 29. Potato Solanaceae Solanum melongena L 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lactuca sativa L L 32. Lettuce Compositae	14.	Camellia			Camellia japonica	L
16. Japanese persimmon Ebenaceae Diospyros kaki L 17. Strawberry Rosaceae Fragaria grandiflora F (red) 18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Leguminosae Wisteria floribunda L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Medicago sativa L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Aceraceae Morus bombycis L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Solanum tuberosum L (red) 28. Spinach Solanaceae Solanum melongena L 29. Potato Solanaceae Solanum melongena L 30. Egg plant Lycopersicon F (Pink) 31. Tomato L Loupoes ativa L 32. Lettuce Comp	15.	Sasangua			Camellia sasangua	L
17. StrawberryRosaccaeFragaria grandifloraF (red)18. Japanese apricotAmygdalaceaePrunus mumeL19. PeachPeachPrunus persicaL20. Japanese wistariaLeguminosaeWisteria floribundaL21. False acaciaRobinia pseudoacaiaL22. AlfalfaMedicago sativaL23. White cloverTrifolium repensL24. SoybeanGlycine maxL25. Kidney beanPhaseolus vulgarisL26. MulberryMoraceaeMorus bombycisL27. Japanese mapleAceraceaeAcer palmatumL (green)28. SpinachChenopodiaceaeSolanum tuberosumtuber30. Egg plantSolanaceaeSolanum tuberosumtuber31. TomatoCompositaeLactuca sativaL32. LettuceCompositaeLactuca sativaL33. BananaMonocotyledoneaeMusaceaeMusa paradisiaeF34. OnionLiliaceaeAllium cepaL35. DuckweedLemnaceaeLemnaceaeLemnaceaeL36. RiceGramineaeOryza sativaL37. WheatLTriticum aestirumL	16.	Japanese persimmon		Ebenaceae	Diospyros kaki	L
18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Prunus persica L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Robinia pseudoacacia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato L Compositae Lactuca sativa L 32. Lettuce Monocotyledoneae Musaceae Musa paradisiae F 33. Banana Monocotyledoneae Musaceae Allium cepa L 35. Duckweed Lemnaceae Allium cepa L 36	17.	Strawberry		Rosaceae	Fragaria grandiflora	F (red)
18. Japanese apricot Amygdalaceae Prunus mume L 19. Peach Prunus persica L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Robinia pseudoacacia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Solanum tuberosum Luber 29. Potato Solanaceae Solanum tuberosum Luber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato L Lycopersicon esculentum F 32. Lettuce Compositae Musa paradisiae F 33. Banana Monocotyledoneae Musa ceae Musa paradisiae F 34. Onion Lemnaceae Lemnaceae Allium cepa L 35. Duckweed						L`́
19. Peach Prunus persica L 20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Robinia pseudoacacia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acera palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanuceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato L Compositae Lactuca sativa L 32. Lettuce Compositae Musa paradisiae F 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L L 35. Duckweed Lemnaceae Lemnaceae Coryza sativa L <	18.	Iapanese apricot		Amygdalaceae	Prunus mume	L
20. Japanese wistaria Leguminosae Wisteria floribunda L 21. False acacia Robinia pseudoacacia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato L L L 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L L 35. Duckweed Lemnaceae Gramineae Oryza sativa L 37. Wheat Triticum aestirum L Triticum aestirum L <td>19.</td> <td>Peach</td> <td></td> <td>70</td> <td>Prunus persica</td> <td>L</td>	19.	Peach		70	Prunus persica	L
21. False acacia Robinia pseudoacacia L 22. Alfalfa Medicago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L L 35. Duckweed Lemnaceae Lemnaceae Lonythiza L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestirum L Triticum aestirum L	20.	Iapanese wistaria		Leguminosae	Wisteria floribunda	L
22. Alfalfa Mediago sativa L 23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) L 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 1 35. Duckweed Lemnaceae Lemnaceae L 1 37. Wheat Triticum aestivam L 1 1	21.	False acacia			Robinia bseudoacacia	·L .
23. White clover Trifolium repens L 24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L L 35. Duckweed Lemnaceae Lemnaceae L 3 37. Wheat Triticum aestivam L Triticum aestivam L	22.	Alfalfa			Medicago sativa	L
24. Soybean Glycine max L 25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 1 35. Duckweed Lemnaceae Lemna polyrhiza L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestirum L	23.	White clover			Trifolium repens	L ·
25. Kidney bean Phaseolus vulgaris L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Morus bombycis L 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemnaceae Coryza sativa L 37. Wheat Triticum aestimum L Triticum aestimum L	24	Sovhean			Glycine max	L
26. Mulberry Moraceae Morus bombycis L 26. Mulberry Moraceae Morus bombycis L 27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon esculentum F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemnaceae L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	25	Kidney bean			Phaseolus vulgaris	L
27. Japanese maple Aceraceae Acer palmatum L (green) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L L 35. Duckweed Lemnaceae Lemnaceae L L 37. Wheat Triticum aestimum L L	26.	Mulberry		Moraceae	Morus bombycis	L
21. Jopanese Impression Filtering Partmann L (red) 28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L L 35. Duckweed Lemnaceae Lemnaceae L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	20.	Iananese manle		Aceraceae	Acer balmatum	L (green)
28. Spinach Chenopodiaceae Spinacia oleracea L 29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato Lycopersicon F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemnaceae L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	4/.	Japanese maple	÷	1 iceraceae	22001 paulitations	L (red)
29. Potato Solanaceae Solanum tuberosum tuber 30. Egg plant Solanaceae Solanum melongena L 31. Tomato L 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemna polyrhiza L 36. Rice Gramineae Oryza sativa L 37. Wheat L	28	Spinach		Chenopodiaceae	Shinacia oleracea	L .
30. Egg plant Solanum relongena L 31. Tomato Lycopersicon esculentum F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemnaceae L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	- <u>7</u> 0. 90	Potato		Solanaceae	Solanum tuberosum	- tuber
31. Tomato Lycopersicon esculentum F (Pink) 32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemnaceae L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	23.	Faa plant		Juliaccac	Solanum melongena	L
32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemnaceae L 36. Rice Gramineae Oryza sativa L 37. Wheat L Triticum aestinum L	30. 31.	Tomato			Lycopersicon esculentum	F (Pink)
32. Lettuce Compositae Lactuca sativa L 33. Banana Monocotyledoneae Musaceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemna polyrhiza L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestinum L						L
33. Banana Monocotyledoneae Musa ceae Musa paradisiae F 34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemna polyrhiza L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	39	Lettuce		Compositae	Lactuca sativa	L
34. Onion Liliaceae Allium cepa L 35. Duckweed Lemnaceae Lemna polyrhiza L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	32.	Banana	Monocotyledoneae	Musaceae	Musa paradisiae	F
35. Ouckweed Lemnaceae Lemna polyrhiza L 36. Rice Gramineae Oryza sativa L 37. Wheat Triticum agetinum L	99. 94	Onion		Liliaceae	Allium ceba	L
36. Rice Gramineae Oryza sativa L 37. Wheat Triticum aestimum L	25	Duckweed	ter ter en en en	Lemnaceae	Lemna holyrhiza	Ē.
37 Wheat Triticum aestinum I.	36	Rice		Gramineae	Orvza sativa	ī.
	27	Wheat			Triticum aestinum	L

* L: leaf. F: fruit.

† Plant materials were harvested in late May-July 1976, except for alfalfa in September.

‡ Total hexenals: cis-3- and trans-2-hexenal.

	A	ctivity of e	nzyme sy	stem pro	ducing C	3-aldehydes	†		Isome	rization
F	resh tissue (µg/g	homogena fr. wt)	ite	4000 g precipitate fraction $(\mu g/g \text{ ppt.})$				- ra Fresh	Fresh	
cis-3- hexenal	trans-2- hexenal	total hexenals‡	n- hexenal	cis-3- hexenal	trans-2- hexenal	total hexenals‡	n- hexenal	Degree of activity	tissue homo- genate	ue 4000 g no- pellet ate
-0	0	0	19	90	16	106	230	L	0	2
1004	57	1061	1507	1776	481	2557	1877	н	5	21
214	107	321	966	3816	54	3870	2298	н	33	1
85	321	406	702	290	458	748	1580	H	79	61
0	24	24	6	132	63	195	48	L	100	32
577	165	742	109	1710	284	1994	388	H	22	14
2921	316	3237	430	_				H	10	
26	14	40	42	162	27	189	62	L	35	14
0	0	0	88	24	8	32	23	L	0	25
532	123	655	52	150	71	221	117	Μ	19	32
555	33	588	36	600	347	947	184	М	6	37
49	, 7	56	12	48	19	67	48	L	13	28
1012	93	1105	357	216	101	317	781	Η	. 8	32
854	14	868	292	2544	189	2733	1403	H	2	7
332	279	661	318	3000	134	3134	1785	H	42	4
339	5	392	150	498	0	498	604	Μ	2	0
105	7	112	61	792	102	894	209	L	6	11
621	79	700	141	0	1712	1712	3427	H	11	100
11	21	32	55	108	115	223	101	L	66	52
692	61	753	256	1512	95	1607	1137	н	8	6
623	85	708	342	3000	521	3521	1115	\mathbf{H}	12	15
386	14	400	52				·	Μ	.4	
68	9	77	154	288	27	315	56	\mathbf{L}	1	9
1037	263	1300	439	5376	499	5875	1124	H	20	8
1029	127	1156	509				`	н	11	<u> </u>
566	104	670	110	36	324	360	250	\mathbf{M}	16	90.
299	70	369	1375	552	402	954	995	H	19	42
1025	410	1435	61	360	324	684	209	Μ	29	47
820	58	878	124	576	133	709	144	М	7	19
769	9	778	368	2820	101	2921	1510	н	2	3
555	2	557	287	3372	303	3675	3014	H	0	8
179	86	265	73	96	0	96	94	\mathbf{L}	33	0
0	0	0	27	96	16	112	41	L	• 0	14
224	38	262	30	``			_	М	15	
26	0	26	6	240	38	278	71	L	0	14
171	203	374	81	348	0	348	174	L	54	0
0	0	0	6	60	8	68	17	L	0	12
0	175	175	84	360	177	537	115	L	100	33
38	0	38	30	12	8	20	38	\mathbf{L}	0	40
54	0	54	36	510	55	565	200	\mathbf{L}	0	10
26	0	26	. 1	300	8	308	17	\mathbf{L}	0	-3
288	57	345	15	0	55	55	27	Т.	17	100

§ H, L show high, moderate and low enzyme activity, respectively.

|| Isomerization rate (%) = $\frac{trans-2-hexenal}{total hexenals} \times 100.$

(187)





Fig. 4. C₆-Aldehyde formation from LA, LNA and their 13-hydroperoxides by isolated tea chloroplasts. Ten milliliters of the chloroplast suspension was incubated at 35°C for 10 min with a single substrate (A or B) or mixed substrates (C) at the concentrations indicated in a sealed 50-ml flask. Numbers given as the substrate concentrations in (C) are the sums of LA and LNA (1: 1). At the end of incubation, 6 ml of headspace vapor was analyzed by GLC as described in the text. A, hexanal formation from LA (●) or LAHPO (▲). B, hexenal formation from LNA (○) or LNAHPO (△). C, hexanal (●) and hexenal (○) formation from the mixute of LA and LNA as substrate. (□) shows the total C₆-aldehydes (hexanal and hexenals) produced from the mixed substrates.

LNA: Linolenic acid, LA: Linoleic acid, LNAHPO: 13-L-Hydroperoxy-(cis, trans, cis)-9, 11, 15-octadecatrienoic acid, LAHPO: 13-L-Hydroperoxy-(cis, trans)-9, 11-octadecadienoic acid.

Fig. 5. Changes in the amounts of substrate and the products formed during C_6 -aldehyde formation. A chloroplast suspension (10 ml) first was incubated for 1 min at 35°C, then transferred to a sealed 50-ml flask which contained LA or LNA (final concentration, 0.6 mM). This mixture was incubated at 35°C. At the times indicated, 6 ml of headspace vapor from the sealed flask was analyzed by GLC. Immediately after headspace analysis, 1 ml of 2 N HCl was added to the reaction mixture, then the contents of the flask were treated with 5 ml of hexane. The hexane extract was concentrated to 2 ml, and 25 μ l was used in HPLC analysis to determine the C_{18} -fatty acid hydroperoxides. The rest of the concentrate was esterified with diazomethane, then analyzed by GLC to determine the unreacted C_{18} -fatty acids. A, hexanal formed from LA (\odot), LAHPO from LA (\bigcirc), unreacted LA (\triangle) and the total amount detected as LA, LAHPO from LNA (\bigcirc) and the total amount detected as LNA, LNAHPO from LAN (\bigcirc), unreacted LAN (\triangle) and the total amount detected as LNA, LNAHPO from LNA (\bigcirc).

reaction of ¹⁸O-labeled 13-L-hydroperoxylinoleic acid by solubilized E''_2 (Scheme 2, Fig. 6, Table IV). The ¹⁸O-atom of hydroperoxide was not detected in carbonyl group of hexanal formed from ¹⁸O-labeled 13-L-hydroperoxylinoleic acid.





e 2 Procedure for the preparation of [18O]-labeled hydroperoxide and analysis of hexanal during $E_2^{"}$ reaction.





А. Натанака

	Hexanal [µmol]				
Enzyme	[¹⁶ O]* ⁵	[¹⁸ O]*6			
Tea leaves*1	2.42 (100)*7	1.27 (52)			
Tea chloroplasts*2	3.68 (100)	1.62 (44)			
Solubilized $E_2^{\prime *3}$	2.70 (100)	1.23 (46)			
Watermelon seedlings*4	0.83 (100)	0.45 (54)			

Table IV Comparison of oxygen-isotope effect in E_2'' reaction by plant tissues.

*1; 0.5 g fresh weight.

*2; 0.1 g [corresponded to 0.5 g leaves (fresh weight)].

*3; 1 ml (corresponded to 0.1 g chloroplasts).

*4; 10 ml (corresponded to 3 g fresh seedling).

*5; hexanal formation from [¹⁶O]-13-L-hydroperoxide.

*6; hexanal formation from [18O]-13-L-hydroperoxide.

*7; numbers in parentheses represent relative values (%).

REFERENCES

- (1) S. Takei and Y. Sakato, Bull. Inst. Physic. Chem. Res., Tokyo, 12, 13 (1933).
- (2) S. Takei, Y. Sakato, M. Ohno and Y. Kuroiwa, Bull. Agric. Chem. Soc. (Japan), 14 (6), 709 (1938).
- (3) S. Takei, M. Ohno, Y. Kuroiwa, T. Takahata and T. Sima, Bull. Agric. Chem. Soc. (Japan), 14 (6), 717 (1938).
- (4) S. Takei and M. Ohno, Bull. Agric. Chem. Soc. (Japan), 15 (2), 193 (1939).
- (5) S. Takei, M. Ohno and K. Sinosaki, Bull. Agric. Chem. Soc. (Japan), 16 (8), 772 (1940).
- (6) S. Takei and M. Ohno, Bull. Agric. Chem. Soc. (Japan), 18 (1), 119 (1942).
- (7) S. Takei, T. Imaki and Y. Tada, Ber. Chem., 68 (1), 954 (1935).
- (8) S. Takei, M. Ohno and K. Sinosaki, Ber. Chem., 73 (9), 950 (1940).

Original references

- (9) A. Hatanaka, M. Hamada and M. Ohno, Bull. Agr. Chem. Soc. (Japan), 24 (2), 115 (1960).
- (10) A. Hatanaka, M. Hamada and M. Ohno, Botyu-kagaku, 24, 151 (1959).
- (11) A. Hatanaka and M. Ohno, Bull. Agr. Chem. Soc. (Japan), 24 (5), 532 (1960).
- (12) A. Hatanaka and M. Ohno, Z. Naturforsch., 15 (b), 415 (1960).
- (13) A. Hatanaka and M. Ohno, Bull. Agr. Chem. Soc. (Japan), 24 (6), 614 (1960).
- (14) A. Hatanaka and M. Ohno, Agric. Biol. Chem., 25 (1), 7 (1961).
- (15) M. Ohno and A. Hatanaka, Botyu-kagaku, 25, 168 (1960).
- (16) A. Hatanaka, M. Ohno and Y. Inouye, Angew. Chem., 74 (8), 291 (1962).
- (17) M. Ohno, A. Hatanaka and Y. Inouye, Agric. Biol. Chem., 26 (7), 460 (1962).
- (18) M. Ohno and A. Hatanaka, Bull. Inst. Chem. Res., Kyoto Univ., 40 (5-6), 322 (1962).
- (19) E. Honkanen, T. Moisio, M. Ohno and A. Hatanaka, Acta Chem. Scand., 17 (7), 2051 (1963).
- (20) M. Ohno and A. Hatanaka, Agric. Biol. Chem., 28 (12), 908 (1964).
- (21) A. Hatanaka and M. Ohno, Agric. Biol. Chem., 28 (12), 910 (1964).
- (22) M. Ohno and A. Hatanaka, Bull. Inst. Chem. Res., Kyoto Univ., 42 (4), 227 (1964).
- (23) M. Ohno and A. Hatanaka, Bull. Inst. Chem. Res., Kyoto Univ., 42 (4), 232 (1964).
- (24) A. Hatanaka, T. Kajiwara and M. Ohno, Agric. Biol. Chem., 29 (7), 662 (1965).
- (25) M. Ohno, Y. Inouye, A. Hatanaka and T. Kajiwara, Bull. Inst. Chem. Res., Kyoto Univ., 43 (3), 231 (1965).
- (26) A. Hatanaka, T. Kajiwara and M. Ohno, Agric. Biol. Chem., 31 (8), 964 (1967).

- (27) A. Hatanaka, T. Kajiwara and M. Ohno, Agric. Biol. Chem., 31 (8), 969 (1967).
- (28) M. Ohno, A. Hatanaka, T. Kajiwara and H. Miyawaki, Bull. Inst. Chem. Res., Kyoto Univ., 45 (3), 184 (1967).
- (29) T. Kajiwara, A. Hatanaka, Y. Inouye and M. Ohno, Agric. Biol. Chem., 33 (3), 409 (1969).
- (30) A. Hatanaka, O. Adachi and M. Ameyama, Agric. Biol. Chem., 34 (10), 1574 (1970).
- (31) A. Hatanaka and M. Ohno, Agric. Biol. Chem., 35 (7), 1044 (1971).
- (32) A. Hatanaka, O. Adachi, T. Chiyonobu and M. Ameyama, Agric. Biol. Chem., 35 (7), 1142 (1971).
- (33) A. Hatanaka, O. Adachi, T. Chiyonobu and M. Ameyama, Agric. Biol. Chem., 35 (8), 1304 (1971).
- (34) M. Hamada, Y. Nagata and A. Hatanaka, Agric. Biol. Chem., 36 (2), 324 (1972).
- (35) A. Hatanaka and T. Harada, Agric. Biol. Chem., 36 (11), 2033 (1972).
- (36) A. Hatanaka, Bull. Inst. Chem. Res., Kyoto Univ., 50 (3), 135 (1972).
- (37) A. Hatanaka and T. Ohgi, Agric. Biol. Chem., 36 (7), 1263 (1972).
- (38) A. Hatanaka, Bull. Inst. Chem. Res., Kyoto Univ., 50 (3), 192 (1972).
- (39) A. Hatanaka and T. Harada, Phytochemistry, 12 (10), 2341 (1973).
- (40) A. Hatanaka, T. Kajiwara and S. Tomohiro, Agric. Biol. Chem., 38 (10), 1819 (1974).
- (41) A. Hatanaka, T. Kajiwara, S. Tomohiro and H. Yamashita, Agric. Biol. Chem., 38 (10), 1835 (1974).
- (42) T. Kajiwara, T. Harada and A. Hatanaka, Agric. Biol. Chem., 39 (1), 243 (1975).
- (43) T. Kajiwara, Y. Odake and A. Hatanaka, Agric. Biol. Chem., 39 (8), 1617 (1975).
- (44) J. Sekiya, W. Kawasaki, T. Kajiwara and A. Hatanaka, Agric. Biol. Chem., 39 (8), 1677 (1975).
- (45) A. Hatanaka, T. Kajiwara and T. Harada, Phytochemistry, 14 (12), 2589 (1975).
- (46) H. Aoshima, T. Kajiwara, A. Hatanaka, H. Nakatani and K. Hiromi, Agric. Biol. Chem., 39 (11), 2255 (1975).
- (47) J. Sekiya, S. Numa, T. Kajiwara and A. Hatanaka, Agric. Biol. Chem., 40 (1), 185 (1976).
- (48) A. Hatanaka, J. Sekiya and T. Kajiwara, Phytochemistry, 15 (4), 487 (1976).
- (49) A. Hatanaka, T. Kajiwara and J. Sekiya, Phytochemistry, 15 (7), 1125 (1976).
- (50) A. Hatanaka, T. Kajiwara, J. Sekiya and H. Hirata, Agric. Biol. Chem., 40 (11), 2177 (1976).
- (51) H. Aoshima, T. Kajiwara, A. Hatanaka, H. Nakatani and K. Hiromi, Biochim. Biophys. Acta, 486, 121 (1977).
- (52) A. Hatanaka, T. Kajiwara and J. Sekiya, Phytochemistry, 15 (12), 1889 (1976).
- (53) A. Hatanaka, J. Sekiya and T. Kajiwara, Plant & Cell Physiol., 18 (1), 107 (1977).
- (54) J. Sekiya, T. Kajiwara and A. Hatanaka, Plant & Cell Physiol., 18 (1), 283 (1977).
- (55) J. Sekiya, T. Kajiwara, T. Miura and A. Hatanaka, Agric. Biol. Chem., 41 (4), 713 (1977).
- (56) J. Sekiya, H. Aoshima, T. Kajiwara, T. Togo and A. Hatanaka, Agric. Biol. Chem., 41 (5), 827 (1977).
- (57) J. Sekiya, T. Kajiwara and A. Hatanaka, Phytochemistry, 16 (7), 1043 (1977).
- (58) H. Aoshima, J. Sekiya, T. Kajiwara and A. Hatanaka, Agric. Biol. Chem., 41 (9), 1787 (1977).
- (59) J. Sekiya and A. Hatanaka, Plant Science Letters, 10, 165 (1977).
- (60) T. Kajiwara, J. Sekiya, Y. Odake and A. Hatanaka, Agric. Biol. Chem., 41 (8), 1481 (1977).
- (61) T. Kajiwara, J. Sekiya, Y. Kido and A. Hatanaka, Agric. Biol. Chem., 41 (9), 1793 (1977).
- (62) A. Hatanaka, T. Kajiwara, J. Sekiya and Y. Kido, Phytochemistry, 16 (11), 1828 (1977).
- (63) H. Aoshima, T. Kajiwara, A. Hatanaka, H. Nakatani and K. Hiromi, Int. J. Peptide Protein Res., 10, 219 (1977).
- (64) A. Hatanaka, T. Kajiwara, J. Sekiya and T. Koda, Phytochemistry, 17 (3), 548 (1978).
- (65) H. Aoshima, T. Kajiwara, A. Hatanaka and H. Hatano, J. Biochem., 82 (6), 1559 (1977).
- (66) T. Kajiwara, J. Sekiya, K. Fujimura and A. Hatanaka, Agric. Biol. Chem., 41 (11), 2249 (1977).
- (67) A. Hatanaka, J. Sekiya and T. Kajiwara, Phytochemistry, 17 (4), 869 (1978).
- (68) J. Sekiya, T. Kajiwara and A. Hatanaka, Plant & Cell Physiol., 19 (4), 553 (1978).
- (69) J. Sekiya, T. Kajiwara and A. Hatanaka, Agric. Biol. Chem., 42 (3), 677 (1978).

A. HATANAKA

- (70) H. Aoshima, T. Kajiwara, A. Hatanaka and H. Nakatani, Agric. Biol. Chem., 43 (1), 167 (1979).
- (71) T. Kajiwara, J. Sekiya and A. Hatanaka, Agric. Biol. Chem., 42 (6), 1293 (1978).
- (72) A. Hatanaka, T. Kajiwara and J. Sekiya, Annual Rept. Lab. Protein Chem. Yamaguchi Medical School, 10, 45 (1978).
- (73) A. Hatanaka, T. Kajiwara, J. Sekiya and K. Fujimura, Agric. Biol. Chem., 43 (1), 175 (1979).
- (74) A. Hatanaka, J. Sekiya, T. Kajiwara and T. Miura, Agric. Biol. Chem., 43 (4), 735 (1979).
- (75) J. Sekiya, T. Kajiwara and A. Hatanaka, Agric. Biol. Chem., 43 (5), 969 (1979).
- (76) T. Kajiwara, T. Koda and A. Hatanaka, Agric. Biol. Chem., 43 (8), 1781 (1979).
- (77) A. Hatanaka, T. Kajiwara and T. Koda, Agric. Biol. Chem., 43 (10), 2115 (1979).
- (78) T. Kajiwara, N. Nagata, A. Hatanaka and Y. Naoshima, Agric. Biol. Chem., 44 (2), 437 (1980).
- (79) A. Hatanaka and T. Kajiwara, Nippon Kagaku Kaishi, 8 (5), 684 (1981).
- (80) A. Hatanaka and T. Kajiwara, Z. Naturforsch., 36 (b), 755 (1981).
- (81) H. Aoshima, T. Kajiwara and A. Hatanaka, Agric. Biol. Chem., 45 (10), 2245 (1981).
- (82) A. Hatanaka, T. Kajiwara, J. Sekiya, M. Imoto and S. Inouye, Phytochemistry, 21 (1), 13 (1982).
- (83) A. Hatanaka, T. Kajiwara, J. Sekiya, M. Imoto and S. Inouye, *Plant & Cell Physiol.*, 23 (1), 91 (1982).
- (84) J. Sekiya, T. Kajiwara, M. Imoto, S. Inouye and A. Hatanaka, J. Agric. Food Chem., 30 (1), 183 (1982).
- (85) J. Sekiya, H. Kamiuchi and A. Hatanaka, Plant & Cell Physiol., 23 (4), 631 (1982).
- (86) A. Hatanaka, T. Kajiwara, J. Sekiya and T. Fukumoto, Z. Naturforsch., 37c (9), 752 (1982).
- (87) A. Hatanaka, J. Sekiya, T. Kajiwara and K. Munechika, Agric. Biol. Chem., 46 (11), 2705 (1982).
- (88) T. Kajiwara, J. Sekiya, M. Asano and A. Hatanaka, Agric. Biol. Chem., 46 (12), 3087 (1982).
- (89) A. Hatanaka, T. Kajiwara and J. Sekiya, J. Agric. Food Chem., 31 (1), 176 (1983).
- (90) J. Sckiya, T. Kajiwara, K. Munechika and A. Hatanaka, Phytochemistry, 22, in press (1983).
- (91) J. Sekiya, K. Munechika and A. Hatanaka, Agric. Biol. Chem., in press (1983).

Original review

- (92) A. Hatanaka, Botyu-kagaku, 28, 110 (1963).
- (93) A. Hatanaka, Bull. Facul. Agric., Yamaguchi Univ., 19, 1107 (1968).
- (94) A. Hatanaka, The Koryo, 101, 29 (1972).
- (95) A. Hatanaka, *Chemistry*, **30** (5), 347 (1975).
- (96) A. Hatanaka, The Koryo, 111, 37 (1975).
- (97) A. Hatanaka and J. Sekiya, "Methods in enzymes and proteins of plants", edited by Y. Morita, M. Shin, K. Asada and S. Ida, Kyoritsu Pub. Tokyo, 225 (1976).
- (98) A. Hatanaka, KASEAA, 14 (12), 788 (1976).
- (99) A. Hatanaka, KASEAA, 15 (1), 39 (1977).
- (100) A. Hatanaka, The Koryo, 117, 23 (1977).
- (101) A. Hatanaka, The Koryo, 125, 11 (1979).
- (102) A. Hatanaka, Seikagaku, 52 (12), 1268 (1980).
- (103) A. Hatanaka, Journal of Synthetic Organic Chemistry, (Japan), 39, 142 (1981).