DOMINANT MUTATIONS AT *Cer-yy* CHANGE BARLEY SPIKE WAX INTO LEAF BLADE WAX

by

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Seventeen mutations resulting in an apparent loss of the epicuticular wax on barley spikes have been shown by complementation, test cross and F₂ analyses to be dominantly inherited alterations of the *Cer-yy* gene. The chemical composition determined by the four alleles 966, 968, 975 and 984 differs from that determined by the wild type in that none of the β -ketoacyl derived lipids (β -diketones, hydroxy- β -diketones and esters containing alkan-2-ols) are synthesized while the composition of the other wax lipids (alkanes, primary alcohols, aldehydes, esters containing alkan-1-ols and free acids) is markedly altered toward that characteristic of leaf blade waxes. We suggest that the *Cer-yy* gene determines a regulatory component which upon mutation activates the gene(s) in the spike for the leaf blade acyl elongase system and at the same time represses the spike acyl and β -ketoacyl elongase systems.

1. INTRODUCTION

Most induced visible mutations in barley are recessive (13). This is also the case for the *eceriferum* (*cer*) mutants which occur in at least 70 loci (7, 8, 9). In the course of assigning 1355

induced mutants to these gene loci controlling the synthesis and excretion of the epicuticular waxes, 18 dominant mutants were encountered (8). Seventeen of these are alleles of a single gene *Cer-yy*. Their genetic analyses and biochemical

Abbreviations: fas = fatty acid synthetase; GLC = gas liquid chromatography; TLC = thin layer chromatography.

characteristics are presented in this paper. The other dominant mutation is an allele of the gene *cer-n* at which 50 recessive alleles have been previously identified (8). This locus will be the subject of a subsequent paper.

2. MATERIALS AND METHODS

2.1. Isolation of mutants and their genetic characterization

The $M_1(X_1)$ barley plants (*Hordeum vulgare* L.) cv's. Bonus, Mari and Foma were grown at the Swedish Seed Association, Svalöv in plots surrounded by other crops to prevent outcrossing. The *eceriferum* mutants were isolated in $M_2(X_2)$ field material grown either at Svalöv or at the Agricultural Research Department of the Danish Atomic Energy Commission's Research Establishment, Risø. Identification of the mutants and phenotypic classification of progeny in genetic tests were by ocular inspection. Observations on pleiotropic effects associated with the *cer* phenotype were made simultaneously with the latter. That is, the plants were not inspected specifically for time of maturation. Reported results are the sum of observations made every year since the initial isolation of the *cer* mutant. To determine whether the new *cer* mutants were allelic with previously defined *cer* loci the following mutants used in diallelic crosses: *cer-a*¹, -*a*⁹¹³, -*b*¹⁴⁷, -*b*¹²⁶⁷, -*c*¹⁴⁴, -*d*⁵, -*d*⁴⁰, -*e*⁸, -*e*¹⁸, -*f*⁹, -*f*⁷⁶³, -*g*¹⁰, -*h*¹³, -*h*²³³, -*i*²⁵, -*i*⁵¹, -*k*³⁹, -*l*¹⁴, -*m*¹⁵, -*n*²⁰, -*n*⁹⁶³, -*o*²⁸, -*q*⁵⁹⁸, -*r*¹⁹, -*r*⁷⁷³, -*s*³¹, -*t*²², -*t*⁴⁶, -*u*⁷⁷⁶, -*v*⁴⁹, -*v*⁷⁵², -*w*⁴⁸, -*w*²⁸⁰, -*x*⁶⁰, -*y*⁷², -*z**b*³⁸, -*z**b*¹⁰⁵, -*z**c*⁶⁵, -*z**c*²²¹, -*z**i*⁶⁸, -*z**i*⁹⁷⁹, -*z**n*²⁴⁴, -*z**n*³⁶², -*z**o*²²⁹, -*z**o*³⁵², -*z**r*²⁶⁰, -*z**r*⁵²⁸, -*z**s*⁴⁶⁷, -*z**s*⁴⁷⁴, -*z**t*³⁸⁹, -*z**t*⁴⁷⁹, -*z**u*¹²², -*z**x*¹⁰⁰, -*y**c*¹³⁵, -*y**d*¹³⁹, -*y**h*¹¹⁶, -*y**h*⁶⁵⁴, -*y**r*⁴³⁹, -*y**t*⁷⁵⁸ and -*y**t*⁹³⁸ (8). The F_1 's from all these crosses had a *cer* phenotype suggesting that the set of mutants being studied was dominant. This was confirmed by studying the F_2 generations. In scoring F_2 plants when the two *cer* mutations could be distinguished by their effect on the leaf sheath and internode wax, only the phenotype of the spike waxes was noted. Crosses of the dominant mutants with each other and their mother variety were also carried out.

2.2. Observations on and wax collection from Phytotron grown plants

For the chemical studies of spike waxes, seeds of Bonus as well as of the mutants 966, 968, 975 and 984 (Table I) were planted and grown to heading (approximately 56 days later) under optimum conditions for vegetative growth (3, 4) in the Phytotron at the Swedish University of Agricultural Science, Stockholm (15) between 1977 and 1980. Bonus barley for the leaf blade wax analysis was treated identically until just before the flag leaves started to emerge. One group of plants was then placed at 15 °C with 20 hours of light and 4 hours of dark. A parallel group of plants was shifted to 25 °C with the same photoperiod just described. The results are from the former set of plants, but no significant difference between the two temperature treatments was discovered.

Subsequent to collection of waxes from the spikes (section 2.3), the lengths from the uppermost node to the tip of the longest awn and to the tip of the highest seed's lemma were measured with a millimeter scale while the stem diameter 1 to 1.5 cm below the same node was determined with the aid of a caliper ruler. Values given are averages of 20 measurements.

2.3. Chemical analyses

Wax was collected from heading spikes (22) or the two uppermost leaf blades as described earlier (14, 16). The resulting chloroform solutions were dried using nitrogen. A Cahn model 4600 electrobalance (Cahn/Ventron Corp., Paramount, California) was used to weigh out 5.000 to 10.000 mg of each wax sample from which dilution series were prepared. The optical density of a 1% solution of each wax sample in 0.5 cm hexane at 273 nm was determined with the aid of a Zeiss PMQ 3 spectrophotometer (Oberkochen, West Germany).

The lipid classes were separated using preparative thin layer chromatography (TLC) on silica gel H (Merck) plates that were developed first in hexane and second in benzene or amylene stabilized chloroform (Merck). Location of the lipids has been described (16). Hydrocarbons, esters, aldehydes and primary alcohols were eluted (16) using chloroform while the free acids

Table 1

Data on *Cer-yy* (– ++ ++)^a barley mutants^a.

Mutant	Mother variety	Year of isolation	Mutagen	Dose	Conditions of treatment ^c	Special remarks ^d
–yy 437	Foma	1967	ethylmethane-sulfonate	0.5 % × 5 h	resting	very early maturity
649	Mari	1967	γ-rays	7,500 rad	chronic recurrent	early maturity
650	Mari	1967	γ-rays	7,500 rad	chronic recurrent	early maturity
849	Bonus	1970	ethylene-oxide	0.14 % × 2 h	resting	early maturity
898	Bonus	1970	iso-propyl-methane-sulfonate	0.6 % × 5 h	resting	early maturity (?)
919	Mari	1972	γ-rays	7,500 rad	chronic recurrent	very early maturity; simultaneous occurrence of mutations for anthocyaninless (<i>ant</i> 41) and resistance to powdery mildew (SR 33), both not linked
920	Bonus	1970	neutrons ^b	300 rad	resting	early maturity, anthocyaninless
923	Bonus	1970	neutrons ^b	300 rad	resting	anthocyaninless
929	Bonus	1970	neutrons ^b	200 rad	resting	early maturity, simultaneous occurrence of mutation for anthocyaninless, not linked
935	Bonus	1970	neutrons ^b	200 rad	resting	early maturity, anthocyaninless
941	Bonus	1970	iso-propyl-methane-sulfonate	0.6 % × 5 h	resting	early maturity, anthocyaninless
966	Bonus	1971	neutrons ^b	200 rad	resting	early maturity, simultaneous occurrence of mutation for anthocyaninless, not linked
968	Bonus	1971	neutrons ^b	200 rad	resting	early maturity
975	Bonus	1973	X-rays	10,000 rad	resting	early maturity (?); simultaneous occurrence of mutations for anthocyaninless and resistance to powdery mildew (SR 50), both not linked
982	Bonus	1971	neutrons ^b	300 rad	resting	early maturity, anthocyaninless
983	Bonus	1971	neutrons ^b	200 rad	resting	early maturity
984	Bonus	1971	neutrons ^b	200 rad	resting	early maturity, anthocyaninless

^a The amount of wax on each organ of the wild type is given as ++, a reduced amount as + and an apparent lack of wax as a – (7). From left to right the phenotypic formula describes spikes, uppermost leaf sheaths plus internodes, leaf blades.

^b Neutrons in reactor center (see 1).

^c Refers to conditions of treated material:

resting = resting seeds with a water content of 11 %.

chronic recurrent = growing plants irradiated chronically from a ¹³⁷Cs source during the whole vegetation period two or three years in succession.

^d Early maturity = heading several days before Bonus: ? = observed to be early in only one year since isolation.

Very early maturity = heading at least one week before Bonus, as does Mari.

SR = Svalöv's resistant mutant stock number to powdery mildew.

were recovered using acetic acid acidified chloroform: methanol (1:1, v/v). The isolated lipid classes and/or their derivatives (see 22) were analyzed via gas liquid chromatography (GLC) using the Hewlett-Packard instrumentation, the 3% SP-2100 and 1% Dexsil-300 columns and the conditions detailed previously (22).

3. RESULTS

3.1. Phenotypic observations

Table I lists the pertinent details concerning the origin and characterization of 17 dominant mutants at locus *Cer-yy* which were isolated from M_2 populations over a seven year period. All 17 mutants are classified as - ++ ++ (see footnote a, Table I). The mutants 649, 650, 919 and 975 were isolated from sister M_2 spikes to spikes which segregated in green house tests for resistance to powdery mildew. Neither 649 nor 650 proved to be resistant upon further investigation. The dominant resistance to powdery mildew induced in the same plant with the *eceriferum* phenotype of 919 and 975 was also present among *cer* plants, but could be segregated away from the *cer* phenotype. The two characters have been shown to be due to mutation in two different loci.

Association of specific groups of pleiotropic effects with alleles at various *cer* loci has been noted previously and discussed (9). In 10 of the 17 dominant mutants, the simultaneous induction of the absence of anthocyanin (*ant*) with that of the *eceriferum* character has occurred (Table I). In four cases (919, 929, 966 and 975), the *ant* and *cer* phenotypes have been shown to be due to mutations at non-linked loci, by taking advantage of an observed segregation for the presence vs absence of anthocyanin in the *eceriferum* stock. To illustrate, the cross *Cer-yy*⁹⁶⁶ *ant* (anthocyaninless line) × wild type (Bonus) gave an F_2 segregation of 54 *cer* : 14 wild type : 12 *cer ant* : 0 *ant* which is not significantly different from the 9:3:3:1 ratio expected for two independent loci ($\chi^2 = 7.47$). *Cer-yy*⁹⁶⁶ (anthocyanin containing line) × wild type gave 66 *cer* : 18 wild type while *Cer-yy*⁹⁶⁶ *ant* × *Cer-yy*⁹⁶⁶ gave 53 *cer* : 16 *cer ant*; both of which are good fits for 3:1 ratios ($\chi^2 = 0.57$ and 0.12, respectively). Segregation of anthocyanin containing lines in the 920, 923, 935, 941, 982

and 984 *cer* stocks has not been observed, and no attempt has been made thus far to separate the anthocyaninless character from that of the *eceriferum* one. Experiments are in progress to answer this question, as well as to determine how many different *ant* loci are involved.

The other intriguing observation concerning the origin of the 17 mutants is the effect on the time of maturity that occurred simultaneously with 13 and perhaps 15 of the *eceriferum* mutations (Table I). Although we have not tried to determine whether the effect on maturity is a pleiotropic one of the *cer* mutation or is due to simultaneous induction of mutations at one or more additional loci, we believe the latter is more likely. We base this working hypothesis on the observation that in two cases no effect was found, while in two instances the time of maturity was later and in the remaining earlier than that of the mother variety. Given that our hypothesis is correct, it would be interesting to know how many different loci affecting maturity are involved in the 13 (15?) cases.

The four dominant mutants 966, 968, 975 and 984 have been further investigated in the Phytotron. As in the field, plants with these mutations have shiny spikes and wax coats indistinguishable from wild type on the leaf sheaths, internodes and leaf blades. Eight weeks after sowing the mutant plants are markedly shorter than wild type plants. For example, in one experiment the lengths of the spikes including the awns were 18 to 20 cm vs 22 or more cm for those of Bonus. At that time each mutant plant had 5 to 6 tillers with the spikes totally emerged from the flag leaf sheaths vs 2 to 3 tillers for the wild type. Thus, in the Phytotron 975, as well as 966, 968 and 984, would be definitely classified as an early maturer. In this connection the observation (GUSTAFSSON and LUNDQVIST, unpubl.) that 975 does not head at all when grown with a photoperiod of 8 hours light, 16 hours dark is of interest (5). Such behavior is characteristic of eight of the nine *mat* loci and the wild type Bonus, while the ninth *mat-a* (gene giving early maturity in Mari) heads under these conditions (4, 6, GUSTAFSSON and LUNDQVIST, unpubl.). While the uppermost internode of the heading spikes extended 2 to 4 cm above the flag leaf sheath of Bonus, 5 to 12 cm were exposed on the four dominant mutants.

The diameter of the stems of the latter, however, was less. That is, already 1 to 1.5 cm below the uppermost node, the internode diameter of the four studied mutants was 1.1 to 1.3 mm vs a minimum of 1.4 mm for those of the wild type. The 984 mutant stock investigated differed from the other three mutant stocks in lacking visible anthocyanin pigmentation.

3.2. Genetic analyses

All F₁'s arising from crosses between one of the 17 investigated mutants and the wild types Bonus or Mari had the same *eceriferum* pheno-

type (– ++ +++) as did the mutant parent, suggesting dominance of the apparent lack of spike wax over its presence. For 15 of the mutants (Table II), the F₂ progenies segregated 3 *cer* : 1 wild type which is the expected result if the mutant is completely dominant. Deviations from the expected 3:1 ratio as large as those observed for the crosses of the *cer* mutants 650 and 935 to wild type are expected only 0.5 to 1 % of the time due to chance alone. We can offer no explanation as to why these two F₂ segregations differ so greatly from the expected.

All possible complementation tests among the 17 mutants (except 849 × 929 and 650 × 984 which have not yet been made) were negative, that is the plants had completely waxless appearing spikes. F₂ data is available for 125 out of the 136 possible combinations among these mutants. Should two dominant mutants of the same phenotype represent different loci, F₂ segregations of 15 mutant : 1 wild type would be expected. That this is not the case here is shown in Figure 1 which gives the number of plants scored in each F₂ progeny, all of which had *cer* spikes. In three of the missing combinations, one of the mutant parent lines was observed to segregate wild type individuals and the original cross has to be repeated. Data is not yet available for the other eight missing combinations. Despite the fact that the data are not quite complete and some of the F₂ progeny sizes are rather small, we feel confident that the results presented demonstrate that the 17 investigated mutants are dominant alleles at a single locus.

Genetic crosses have also been carried out to ascertain whether these dominant mutants belong to one of the other *cer* loci affecting wax on the spikes. Since the representative alleles at all these other loci are recessive to the wild type, the expected F₂ segregations arising from their being crossed with the dominant *eceriferum* mutants is 13 *cer* : 3 wild type. Table III presents the F₂ segregations found after crossing the dominant mutants 898, 941 and 982 with 25 different *cer* loci. Data for these three dominant mutants is presented since with one exception it is complete. Of the 350 possible combinations among the other 14 dominant *cer* mutants and the 25 specified *cer* loci, 309 of them have given F₂ data similar to that presented in Table III. In every instance wild type plants were found in the F₂.

Table II

F₂ segregations from *Cer-yy* × Bonus or Mari crosses.

<i>Cer-yy</i>	Number of plants observed		χ^2 for 3:1
	<i>Cer</i>	Wild type	
437	2316	726	2.08
649	710	256	1.16
650	1933	728	7.89
849	152	60	1.23
898	99	41	1.37
919	296	81	2.49
920	154	40	1.98
923	207	60	0.91
929	263	87	<0.01
935	212	46	7.07
941	96	30	0.09
966	257	79	0.40
968	271	93	0.06
975	214	61	1.16
982	292	108	0.85
983	294	90	0.50
984	145	51	0.11

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Table III**F₂ segregations from crosses of three *Cer-yy* alleles with recessive alleles at 25 *cer* loci^a.**

Phenotype of F ₂ :	898		<i>Cer-yy</i> 941		982	
	<i>cer</i>	WT	<i>cer</i>	WT	<i>cer</i>	WT
× I. <i>cer-d</i>	45	6	181	45	96	20
<i>e</i>	59	11	388	119	95	28
<i>h</i>	66	12	195	72	82	24
<i>i</i>	55	7	90	25	70	16
<i>o</i>	23	9	53	24	52	12
<i>t</i>	87	17	340	79	220	41
<i>v</i>	123	21	291	106	168	32
<i>w</i>	27	10	254	68	120	24
<i>zb</i>	78	28	60	11	90	19
<i>zc</i>	158	29	99	23	90	17
<i>zn</i>	117	20	166	49	70	12
<i>zo</i>	62	16	236	87	107	26
<i>yc</i>	39	9	41	12	55	14
<i>yd</i>	45	7	48	15	51	7
<i>yh</i>	32	5	120	48	78	12
<i>yt</i>	113	27	73	11	118	18
II. <i>cer-k</i>	49	27	54	10	25	4
<i>l</i>	30	6	56	13	49	19
<i>zr</i>	40	9	211	50	138	28
<i>zs</i>	132	28	152	29	123	20
<i>zt</i>	46	9	231	68	129	25
III. <i>cer-a</i>	46	5	57	19	39	14
<i>b</i>	91	19	53	17		
<i>x</i>	118	20	64	16	63	12
<i>c, q, u</i> ^b	71	17	144	44	195	50

^a The 25 loci are divided into three groups on the basis of their phenotypic formula. I = - ++ ++; II = + ++ ++; III = - - ++.

^b Data combined since they represent a single locus. Phenotypic formula for *cer-u* = + + ++.

and 64:10. Similar F₂ segregations were obtained for 96 of the 160 possible combinations among the other 16 dominant mutants and the ten specified loci. These include a minimum of three dominant mutants crossed with each of the 10 loci under discussion. For the recently identified (8) *cer-yr* locus (+ + ++), the only F₂ data thus far obtained is from crossing with the dominant mutant 929. A ratio of 53 *cer*:13 wild type occurred. We thus conclude that the dominant mutants being investigated are not allelic with these 11 *cer* loci. Results are not yet available for two other recently defined (8) loci, namely *cer-yk* and *-yn*, which also have the phenotypic formula + + ++.

Despite the absence of genetic tests with five of the *cer* loci affecting barley spike waxes, the results presented demonstrate that the 17 dominant mutants under investigation belong to a *cer* locus which is not allelic with 36 of the previously defined spike loci. This new locus has been designated *Cer-yy* (8).

3.3. Composition of waxes on spikes of four *Cer-yy* mutants compared with that on spikes and leaf blades of Bonus

The low optical densities (0.07, 0.06, 0.05 and 0.05) of waxes from *Cer-yy* spikes (966, 968, 975 and 984, respectively) compared to that

Figure 2. Composition of the epicuticular waxes on spikes of Bonus (+) and *Cer-yy*⁹⁶⁶ as revealed by TLC.

TLC patterns of the *Cer-yy* mutants 968, 975 and 984 can not be distinguished from that shown for 966. The lipid classes were visualized by spraying with 55 % H₂SO₄ containing 0.6 % (w/v) K₂Cr₂O₇, followed by charring.

(0.72; average of measurements from six different wax samples) of wild type spikes, suggested little if any β -diketones and/or hydroxy- β -diketones would be present in these mutant waxes (see 17). Figure 2 illustrates the difference in composition of the waxes found on wild type and *Cer-yy* spikes. All four investigated alleles at this locus determine waxes lacking β - and hydroxy- β -diketones and having relatively increased proportions of primary alcohols, making the latter one of the three prominent lipid classes. The primary alcohols, however, do not dominate the wax as they do that from leaf blades and the lower leaf sheaths and internodes (see 14). The distribution of *Cer-yy* spike lipid classes is most similar to that from wild type awns (14).

The composition of the hydrocarbons (Table IV) present on Bonus spikes differs significantly from that on the leaf blades in that the C₃₁

homologue predominates (72%) on the former whereas the C₃₃ homologue does so (53%) on the latter. While the C₃₁ homologue is also the major hydrocarbon in wax from the four *Cer-yy* mutants, the C₃₃ homologue is more pronounced than in the wild type, amounting to 16% in the case of 966. In the more than 30 thus far investigated *cer* spike mutants, the C₃₃ homologue has always accounted for less than 4% of the hydrocarbons. Unlike the wax from leaves but similarly to that from spikes of wild type, the four mutants have relatively minor amounts of the 25 and 27 homologues.

An examination of the data in Table V reveals that the compositions of the free primary alcohols in the spike waxes of the four investigated mutants are indistinguishable from that characteristic for wild type leaf wax in which the C₂₆ homologue predominates. This homologue accounts for only half the primary alcohols on Bonus spikes where the C₂₈, 30 and 32 chain lengths are also important components. Interestingly, in *cer-c*³⁶ and *-u*⁶⁹ spike waxes, a major portion of the C₃₂ homologue is contributed by the awns (21, 22). This suggests that mutation of the *Cer-yy* locus influences synthesis of primary alcohols on the awns as well as on those cuticle surfaces of the spikes which in the wild type bear β -diketones. An increased prominence of the C₂₆ chain length is also evident in the aldehyde distributions (Table VI) of the three *Cer-yy* mutants 968, 975 and 984. This homologue is an important member of the aldehydes from wild type leaf but not spike wax. However, unlike leaf wax in which the 28 carbon chain is the other major homologue, the 32 carbon chain is conspicuous in the aldehydes of the wild type as well as in those of the three *Cer-yy* mutants mentioned above. The fourth mutant, 966, has the greatest amount (61%) of the C₃₂ homologue, and in addition 19% of the aldehydes are present as 34 carbon chains.

The distributions of chain lengths among the free acids from wild type leaves and spikes are quite similar, although somewhat less of the shorter 18 and 20 carbon chains and somewhat more of the longer 32 and 34 ones are found in waxes from the former vs the latter (Table VII). A greater difference is seen when comparing the free acid distributions from the four *Cer-yy* mutant waxes with those from the wild type

Table IV**Composition of hydrocarbons present in the wax from spikes of four *Cer-yy* mutants plus spikes and leaves of wild type Bonus (weight %).**

Number of carbons ^a	Spikes					Leaves
	Bonus	966	968	975	984	Bonus
21	0.3	0.2	0.3	0.2	0.3	
22	0.2	0.1	tr	tr	tr	
23	1.5	2.7	2.9	1.1	2.3	
24	0.2	0.3	0.2	tr	0.1	
X		0.3	0.3			tr
25	2.0	2.9	3.3	2.1	4.1	8.5
26	0.1	0.1	0.1	0.2	0.2	1.6
X	0.1	0.4	0.4	0.1	tr	tr
27	2.0	2.8	3.2	1.8	3.3	6.8
28	0.1	0.2	0.2	0.2	0.2	0.3
X	0.1	0.4	0.6	0.2	0.1	tr
29	14.7	7.5	12.9	10.2	13.5	8.6
30	1.3 ^b	1.1 ^b	1.2 ^b	0.9 ^b	1.4 ^b	0.2
31 ^c	72.1	64.2	66.0	73.0	68.0	19.0
32	1.0	1.1	0.9	0.9	0.6	tr
33	4.1	15.8	7.5	9.1	5.8	52.8
34						tr
35						2.1

^a X represents a member of a second homologous series whose retention times are slightly less than the following normal hydrocarbon.^b Percent is overestimated as the C₃₀ homologue has not been integrated separately from an unknown of approximately the same amount as C₃₀.^c Includes the longest member of the X series which was too small to be resolved from the C₃₁ hydrocarbon under the GLC conditions used.

tr = trace (<0.1 %).

Table V**Composition of free primary alcohols present in the wax from spikes of four *Cer-yy* mutants plus spikes and leaves of wild type Bonus (weight % as acetates).**

Number of carbons	Spikes					Leaves
	Bonus	966	968	975	984	Bonus
20		0.9	1.5	0.4	0.6	tr
22	1.5	0.3	0.5	0.4	0.3	1.0
23	tr	tr	tr	tr	tr	tr
24	6.5	2.9	2.7	2.4	2.7	4.1
25	0.5	0.8	0.7	0.5	0.6	0.3
26	50.8	85.8	85.9	83.7	86.6	89.7
27	0.6	0.2	0.4	0.2	0.2	0.2
28	16.4	4.3	5.9	5.9	6.7	3.8
29	1.1	tr	0.1	tr	tr	tr
30	12.0	0.5	1.2	1.4	1.0	0.5
31	1.2	0.1	0.1	0.1	tr	tr
32	9.1	3.2	1.1	4.3	1.3	0.2
33		tr		tr		
34	0.2	1.2		0.6		0.2

tr = trace (<0.1 %).

Table VI

Composition of aldehydes present in the wax from spikes of four *Cer-yy* mutants plus spikes and leaves of wild type Bonus (weight % as alcohol acetates).

Number of carbons	Spikes					Leaves
	Bonus	966	968	975	984	Bonus
20	tr	tr	0.2	0.5	2.0	tr
22	0.1	0.1	0.7	1.1	3.5	0.3
23		tr	tr	tr	tr	tr
24 ^a	0.9	1.3	5.6	3.5	4.8	1.1
25		0.1	0.7	0.6	0.7	0.5
26 ^a	4.1	10.2	22.0	29.9	32.8	39.7
27	0.2	0.3	1.7	0.3	0.5	1.1
28 ^a	8.4	3.9	9.1	7.5	10.8	45.9
29	0.5	tr	0.5	0.4	0.3	0.7
30 ^a	27.0	4.1	14.9	9.5	13.7	6.3
31	1.9	0.4	0.9	1.1	1.0	tr
32 ^a	54.8	60.8 ^b	41.5	40.4	29.3	2.9
33	tr		tr	tr		tr
34	2.1	18.8	2.3	5.1	0.7	1.5

^a Includes minor amount of a member of a second homologous series whose retention times are slightly greater than those of the normal alcohol acetates.

^b Includes minor amount of C₃₃ which was not integrated separately.

tr = trace (<0.1 %).

Table VII

Composition of free acids present in the wax from spikes of four *Cer-yy* mutants plus spikes and leaves of wild type Bonus (weight % as methyl esters).

Number of carbons ^a	Spikes					Leaves
	Bonus	966	968	975	984	Bonus
16	4.8	3.8	1.8	3.2	3.7	2.0
17	tr	tr	tr	tr	tr	0.2
18	9.7 ^b	2.8	1.9	2.5	2.3	2.7
19	tr	tr	tr	0.1	0.4	0.1
20	9.9	5.1	5.0	9.1	5.7	4.1
21	0.3	0.4	0.4	0.5	0.4	0.3
22	14.0	32.9	29.6	29.5	24.5	13.9
23	0.5	0.9	0.9	0.7	0.8	0.2
24	12.9	16.6	19.5	14.9	16.5	7.3
25	0.3	0.4	0.6	0.3	0.4	1.5
26	9.2	16.8	27.5	18.9	20.9	15.6
27	0.2	tr	0.1	tr	0.7	2.2
28	16.2	4.8	8.0	7.4	10.0	18.8
29	0.7	0.1	tr	0.1	0.2	1.9
30	12.0	2.5	2.7	4.0	6.7	15.3
31	0.5	0.1	tr	tr	tr	tr
32	6.1	8.2	1.8	7.4	6.0	9.0
33	tr	tr		tr	tr	tr
34	0.3	2.7	tr	1.3	0.8	4.9
36	0.1					
Unknown ^c	2.1	1.8	0.1	tr	tr	tr

^a The trace to very small amounts of C₁₄ often observed are not included since their volatility leads to preferential losses during isolation and preparation of the methyl esters.

^b Probably overestimated because of contamination of wax sample by internal lipids.

^c Primarily two components which elute shortly before normal C₁₈ and C₂₀ acid methyl esters.

tr = trace (<0.1 %).

Table VIII

Composition of the ester acids present in the wax from spikes of four *Cer-yy* mutants plus spikes and leaves of wild type Bonus (weight % as methyl esters).

Number of carbons	Spikes					Leaves
	Bonus	966	968	975	984	Bonus
16	11.0	5.9	6.0	6.1	11.4	3.2
17		tr	tr	tr	tr	tr
18	12.3	7.3	7.7	9.8	11.5	11.8
19	0.1	tr	0.2	tr	0.2	tr
20	57.1	36.2	41.7	54.2	42.0	34.4
21	tr	0.6	0.8	0.6	0.5	0.2
22	16.5	31.1	27.4	22.7	23.0	35.4
23	tr	0.2	0.2	tr	tr	tr
24	2.9	12.9	11.6	5.5	7.6	5.7
25	tr	0.2	0.4	tr	tr	tr
26	0.1	5.5	4.0	1.0	3.5	9.0
27		tr	tr			tr
28		tr	tr		0.2	0.2
30						tr
Unknowns ^a			tr	tr		tr

^a Consists of two components which have slightly shorter retention times than normal C₁₈ and C₂₀ acid methyl esters.

tr = trace (<0.1 %).

Table IX

Composition of the ester alcohols present in the wax from spikes of four *Cer-yy* mutants plus spikes and leaves of wild type Bonus (weight % as acetates).

Number of carbons	Spikes Leaves					Leaves
	Bonus	966	968	975	984	Bonus
Alkan-2-ols ^a	12.4					
Alkan-1-ols						
16		0.8	1.2	tr	tr	
17		tr	0.1			
18	0.3	2.4	4.7	1.8	4.6	2.0
19	tr	0.1	0.1	tr	tr	tr
20	1.8	6.6	6.2	4.8	7.1	0.7
21 ^b	tr	tr	tr	tr	tr	tr
22	19.0	13.4	16.4	13.3	18.2	4.7
23	0.4	0.4	1.6	0.1	0.4	tr
24	20.7	13.0	15.3	11.4	15.7	4.5
25	0.5	1.6	1.9	0.6	0.8	0.1
26	32.3	58.4	48.4	62.3	49.9	82.3
27	0.2	0.2	0.2	0.2	0.3	0.1
28	5.8	2.6	3.2	4.3	2.8	5.1
29	0.2	tr	tr	tr	tr	tr
30	2.1	0.2	0.5	0.5	0.1	0.4
31	0.3	tr		tr	tr	
32	4.1	0.3	tr	0.7	0.2	

^a Includes C₁₃, C₁₅ and C₁₇. Underestimated as the volatility of the C₁₃ homologue leads to preferential losses during preparation of the alcohol acetates (20). May also include some 16 carbon alkan-1-ol (see Table X, footnote a).

^b Presence of C₂₁ is assumed since both C₁₉ and C₂₃ are found. The amount is not measurable as contamination by a phthalate having the same retention time occurs during sample preparation (22).

tr = trace (<0.1 %).

Table X

Composition of esters present in the wax from spikes of four *Cer-yy* mutants plus spikes and leaves of wild type Bonus (weight %).

Number of carbons	Spikes					Leaves
	Bonus	966	968	975	984	Bonus
Alkan-2-ol esters						
29	tr					
31	0.7					
33	11.2					
35	16.9					
37 ^a	3.2					
Unknowns ^b	tr					
Alkan-1-ol esters						
36			0.1	tr	0.3	
37			tr	tr	tr	
38	2.1	1.1	3.5	2.3	3.8	tr
39	m	tr	tr	tr	0.1	
40	5.7	6.4	6.9	4.7	7.4	0.2
41	m	0.3	0.5	0.4	0.5	tr
42	12.6	19.9	17.1	14.2	18.7	4.6
43	m	0.3	0.3	0.3	0.5	tr
44	14.9	18.3	18.1	17.5	17.8	12.3
45	m	0.5	0.5	0.7	0.7	tr
46	17.4	26.5	25.5	37.1	26.3	31.7
47	m	0.8	0.6	1.0	1.1	tr
48	7.4	16.2	15.4	15.7	14.2	32.8
49	m	0.3	m	m	0.5	tr
50	4.9	7.2	8.5	4.8	6.6	6.6
51	m	m	m	tr	m	tr
52	3.0	2.0	2.9	1.2	1.6	8.1
53						tr
54			0.1			2.8
56						0.8

^a Probably contains a small amount of 36 carbon alkan-1-ol esters (see 20). On the GLC columns used, 37 carbon alkan-2-ol and 36 carbon alkan-1-ol esters appear as a single peak.

^b Semi-log plots of apparent carbon number vs retention time from isothermal GLC on 3% SP-2100 columns suggest that they are C₃₀, C₃₂, C₃₄ and C₃₆ alkan-2-ol containing esters.

tr = trace (<0.1%).

m = minor odd chain length alkan-1-ol containing ester integrated together with preceeding even chain homologue from which they were not completely separated by the 1% Dexsil-300 columns used. Visually they are of the same size or smaller than the shorter odd chain length homologues which were better separated by the columns.

waxes. In the mutant fatty acids the C₂₂ homologue is quite important. Furthermore, whereas the sum of the 22, 24 and 26 homologues in the four mutants accounts for 62 to 77% of this wax class, it amounts to only 37% of the wild type's free acids. The C₂₈ and

longer homologues are less important members of the mutant free acids than they are of those of the wild type.

The data in Table VIII indicate that the compositions of the ester acids from the mutants 966, 968 and 984 are not like that of the wild

type spike wax, but tend to be more similar to that of the wild type leaf wax. Specifically, the importance of the C_{20} homologue is decreased to the benefit of the C_{22} , C_{24} and C_{26} homologues. When the compositions of the ester alcohols are examined (Table IX), a decided shift towards that typical for leaf wax is apparent for all four mutants. As characteristic for leaf wax esters, no alkan-2-ols are found. While the C_{26} homologue accounts for at least half of the ester alcohol moieties, it is not so dominating as in the wild type leaf wax esters (83%), but certainly more important than in the wax esters from the wild type spikes (32%). The total ester compositions are compared in Table X. The distributions confirm the conclusion drawn from the ester alcohol data, namely, that the mutant and wild type leaf blade esters contain only alkan-1-ols. If the chain length distribution of the alkan-1-ol containing esters from Bonus spikes is calculated ($C_{38} = 3.1$, $C_{40} = 8.4$, $C_{42} = 18.5$, $C_{44} = 21.9$, $C_{46} = 25.6$, $C_{48} = 10.9$, $C_{50} = 7.2$ and $C_{52} = 4.4$) and then compared with the ester distributions from the mutants, no striking differences are apparent. Although a small increase in the 46 carbon chain ester of the four mutants has occurred, this chain length is nowhere near so prominent as in the wild type leaf blade ester spectrum. In this connection it is interesting that mutant 975 which showed the greatest modification of its ester alcohol moieties (Table IX) had ester acids (Table VIII) almost identical to those of the wild type.

4. DISCUSSION

The complex of enzymes known as fatty acid synthetase (fas) carries out de novo synthesis of fatty acids. That is, to an activated primer a number of short carbon chains donated by an activated elongating agent are successively added until a given chain length, most frequently C_{16} , is attained. For each addition and preparation of the growing chain for the next, as many as seven different enzymatic reactions can be involved. The enzyme system(s) carrying out further elongation of fatty acyl chains to those lengths ($\geq C_{20}$) characteristic of the epicuticular wax lipids is known as an elongase. Since the addition of each C_2 -unit from the donor malonyl-CoA presumably requires a similar series of reactions

to those occurring in de novo synthesis, an elongase must be an enzyme complex very similar to fas. Presently available evidence suggests that de novo fas synthesis in plant epidermal cells gives rise to the substrates for the wax elongase which is located in the plasma-lemma and/or cell walls of the same cells (see 11). Differential modification of the biosynthesis of the wax carbon chains by inhibitors, genetic mutation and photoperiodic treatments are best interpreted by the existence of several elongases (see 18, 2, 19). Before arriving on the cuticle surface, the resulting pools of fatty acyl chains can either serve as substrates for associated pathways such as decarboxylation or reduction, or simply be released from the elongase(s) as free acids (see 18).

The most clearly differentiated elongases are those in barley giving rise to the alkanes and β -diketones (see 18, 10). The former system, known as an acyl elongase (12), presumably uses C_{16} and/or C_{18} fatty acyl chains as substrates to yield the spectrum of carbon chains composing the alkanes. The latter system, known as a β -ketoacyl elongase (12), utilizes only C_{16} or shorter fatty acyl chains to construct the carbon skeleton of the β -diketones (96% hentriacontan-14,16-dione; 10). In the following discussion no attempt will be made to differentiate between the various sequential and/or parallel elongation systems that might be involved in the synthesis of the non β -ketoacyl elongase derived lipids (18). Exactly how these two elongase systems differ is not known. Two possibilities can be envisaged: either the β -ketoacyl elongase system is involved in modifying the fas product(s) to β -ketoacyl chains which are then elongated by the acyl elongase and/or the β -ketoacyl elongase requires β -ketoacyl chains as the initial substrate for elongation. While an acyl elongase must function in all barley epidermal cells, e.g., those of the leaf blades, the β -ketoacyl elongase is restricted to given epidermal cells such as those of the spike lemmas, paleas and glumes (14) where it and the acyl elongase both function in the same cell.

Synthesis of the β -diketone lipids is either eliminated or reduced by mutations at all investigated *cer* spike loci. A reduction in amount is not accompanied by an alteration in structure or chain length composition (VON

WETTSTEIN-KNOWLES, unpubl.). Reduction is expected as the mutants are selected on the basis of a change of color from blue-grey to yellow-green that is correlated with the amount of β -diketones present in the wax (see 18). Some of the spike *cer* loci have no effect on the other wax lipids, e.g., the *cer-cqu* region (22). Such genes must influence reactions unique to the β -ketoacyl elongase system. Mutations at many *cer* spike loci, however, also alter the composition of one or more of the non β -ketoacyl elongase derived wax lipid classes. This behavior suggests that the acyl and β -ketoacyl elongase systems have structural or regulatory components in common (18). The mutations in the *Cer-yy* gene fall into the latter category as the data in this paper demonstrate. The *Cer-yy* locus can be distinguished from all the other *cer* loci in the same category because the chain length distributions of the non β -ketoacyl elongase derived lipids are modified markedly towards those characteristic for leaf blade waxes. A second unique attribute of the *Cer-yy* gene is that all 17 identified mutant alleles are dominant, indicating that the gene product is a regulatory component which can affect the expression of genes on both homologous chromosomes. Only one other dominant mutation has been identified out of the 1338 mutations assigned to the various *cer* loci (8).

The pertinent question to be asked at this point, is how to interpret the *Cer-yy* gene in light of our present understanding of the synthesis of the epicuticular wax lipids. We should like to suggest that the *Cer-yy* gene determines a regulatory component that upon mutation activates in the spike the genes for the leaf blade acyl elongase system and at the same time represses both the spike acyl and β -ketoacyl elongase systems which in the mutants are functional in the epidermal cells of the uppermost leaf sheaths. The testing of this hypothesis awaits the isolation and characterization of the *fas* and various elongase systems from barley epidermal cells, a project that has been initiated.

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