DEVELOPMENTAL AND BIOCHEMICAL EFFECTS OF THE AGROPYROIDES MUTATION IN BARLEY¹

R. T. WIJEWANTHA² AND G. L. STEBBINS

Department of Genetics, University of California, Davis

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ONE of the most important unsolved problems of genetics is how chemical information, which is inherent in the DNA code and is expressed in primary gene action, can be translated into the histological and morphological information which enables a mutant gene to produce a phenotype with altered morphological characteristics. This problem can be solved only by parallel genetic, biochemical, histological, and morphological studies of single gene differences with conspicuous effects on the phenotype. Higher plants provide favorable material for such studies for the following reasons: (1) Many of them are easily grown, both as whole plants and as organ or cell cultures. (2) The overall architecture of flowering plants is sufficiently similar that significant observations made upon one species are likely to be valid, with relatively little modification, for all of them. (3) The various parts of the plant are reproduced serially and repeatedly in a single individual, so that the effects of a single gene difference or a single alteration of the environment can be studied quantitatively on the same genetic material. (4) Development in higher plants is less complex than in higher animals, and in most instances cellular and nuclear divisions at critical stages are much more easily observed.

Barley (*Hordeum vulgare* L.) has additional advantages over many other plants in having seven pairs of large chromosomes, a well developed linkage map on which many genes have been located, and centromeric regions on all chromosomes which are highly heterochromatic, permitting the recognition of the two daughter cells from a particular mitotic division long after that division has been completed. The present contribution is the first of a series on the developmental genetics of genes with morphological effects on the barley spike.

MATERIALS AND METHODS

The mutation used in this study was obtained from Mr. COIT SUNESON of the U.S. Department of Agriculture and the Department of Agronomy, University of California at Davis. It arose in a culture of the variety Early California Mariout, a six-rowed quick flowering, day-neutral variety. The culture, growing in the field, was treated by watering with an aqueous solution of 0.05 ppm "Dalapon," the trade name for an herbicide, consisting essentially of 85 percent sodium salt of 2,2 dichloropropionic acid and 15 percent inert ingredients. Among the progeny of one of

¹ In part an adaptation of a thesis written in partial fulfillment of the requirements for the Ph.D. degree, University of California.

² Present address: Dartonfield, Agalawatta, Ceylon.

the treated plants was a plant which showed pronounced abnormalities of structure in the flowering spike. Selfed progeny from this culture bred true for the abnormalities. The name *agropyroides* has been given because the upper part of the spike resembles that of *Agropyron* (see below).

For the present study of gene expression, the homozygote was grown over four seasons under two environmental conditions, two seasons in the field and two in the greenhouse. Since the expressivity of the gene varies considerably from one spike to another of the same plant, only the first spike on every plant was scored in this investigation. The second and third spikes in every plant grown during the first field season were also scored for expressivity so as to determine whether the mutant expression remains the same or whether it increases or decreases at a later stage in plant growth.

In order to determine the mode of inheritance, the mutant was crossed reciprocally to the typical six-rowed barley variety, California Mariout. The mutant was also crossed reciprocally to *Atlas deficiens*, a two-rowed type. The purpose of the latter cross was to see whether the *deficiens* type, which produces a reduction of the functional spikelets to one per node, would have an epistatic interaction with the *agropyroides* mutation, which produces spikes bearing only one spikelet at some of the upper nodes.

For the studies of spikelet development, seedlings of the mutant and the parental variety were grown in a standard soil mixture of two parts sand and one part sandy loam in 8-inch plastic pots at a density of 15 plants per pot. They were watered with Hoagland's solution of mineral nutrients. Development was hastened by supplementing the normal day length with artificial incandescent illumination to give a 16-hour day length.

Collections were made at 2-day intervals, commencing at the stage when the central awned lemmas were 0.5 cm long. The spikes were fixed in formalin acetic alcohol and stored in 70 percent alcohol for later examination. In older spikes, measurements were made with a millimeter ruler; in younger ones under a binocular dissecting microscope equipped with an ocular micrometer disc.

The method of extraction of free amino acids and their purification was the same as that used by SARKISSIAN, SHAH and STEBBINS (1962). Analysis of the free amino acids of the plant extracts by paper partition chromatography was also similar except that development in n-butanol-acetic acid-water in the long direction was reduced to 38 hours, and 0.2 percent ninhydrin solution in 95 percent ethanol was used in place of a 0.3 percent ninhydrin solution in water saturated n-butanol for color development. These experiments were set up and analyzed as randomized complete block designs. The results are based on 6 replications.

The experiments for the production of phenocopies were carried out on plants grown in pots in a well-lighted greenhouse. The 8-inch pots were filled with vermiculite and watered during the course of the experiment with either Hoagland's (HOAGLAND and ARNON 1950) mineral solution, modified to reduce manganese toxicity observed on barley plants grown previously under these conditions, or with modified Hoagland's solution to which one of the following substances had been added: Arginine, at 624 and 312 ppm concentration; cystine, at 120 and 60 ppm; glutamic acid at 1120 and 560 ppm; glutamine, at 4000 and 2000 ppm; cold water and hot water extract of normal seedlings. In a second experiment, the following were used: tyrosine at 200 and 100 ppm; adenine sulphate at 300 and 150 ppm; adenine sulphate at 300 ppm + arginine, at 624 ppm.

The experiments were repeated for arginine, glutamic acid, and tyrosine, using foliar sprays of the same concentrations as those added to the mineral culture solution. In the second experiment for arginine and glutamic acid, plants were grown one per pot, which resulted in much larger plants and a stronger expression of the mutant character.

For the studies of incorporation of C^{14} labelled arginine, seedlings were grown in mineral nutrient solution to the three-leaf stage, and the solution added as a foliar drop on the adaxial as well as the abaxial surface of the leaf. In other experiments, the C^{14} arginine solution was added to the mineral nutrient solution, and so was taken up by the roots. The concentrations and times used are given in the description of the results. For the study of incorporation, spike primordia were fixed in formalin acetic alcohol after treatment with C^{14} labelled arginine, then dehydrated in tertiary butyl alcohol, embedded in paraffin and sectioned at 7.5 μ . After removal

of the paraffin, the most median sections were exposed to strip film for two weeks, the films developed, and the sections stained. The assumption was made that all free arginine was removed from the tissue by the processes of fixation, embedding, and removal of paraffin.

RESULTS

Description and inheritance of the mutant character: In its most characteristic form, the mutant spike has the normal six-rowed condition in its lower half, and the substitution of single, laterally placed glumes for the lateral spikelets at the upper nodes (Figures 1B, 2A). At these nodes the dorsally placed glumes of the central spikelet may be present, variously fused or reduced, or absent. The awn of the central lemma is very often bifid; this condition was found in at least some nodes on eight out of ten spikes scored in detail (Figure 2C). An even more common abnormality is an extra lemma at the central position, usually on the dorsal side of the principal lemma (Figure 2D). Sometimes this extra lemma contains also a palea and rudimentary or partially fertile floret. In addition, the rachilla of either the central or lateral florets may be prolonged behind the lemma, and bear an extra rudimentary floret (Figure 2B). This extra floret may be found on either the central spikelet of the abnormal upper nodes or the florets of the otherwise normal lower nodes.

Consequently, while the number of appendages which is differentiated at the abnormal upper nodes is usually less than normal, this is due entirely to the reduction of the lateral florets to single glumes. At the central position, the number of appendages is usually greater than normal. In five out of ten spikes scored, there were one or two intermediate nodes in which the lateral spikelets were still present and normal, while extra glumes or lemmas were present at the central position, so that the total number of appendages produced at these nodes was greater than normal. The most consistent abnormality produced by the *agropyroides* gene, therefore, is a greater variability in the number and type of appendages differentiated.

Under the cultural conditions employed by us, the mutation consistently showed complete penetrance but incomplete expressivity. On the first three spikes of a plant, the lower nodes had the normal condition of three spikelets, while the upper nodes had various abnormalities, described above. Later emerging spikes, particularly those which were relatively short, often were completely normal in appearance. The average position of the first conspicuously abnormal node was about three fifths of the way up the spike.

The effect of variation in season of growth and cultural conditions on expressivity is shown by the data of Table 1. These data were obtained from the selfed progeny of 20 homozygous plants, each of which was divided into four groups, and grown for two years in the field and the greenhouse. The high degree of variation in expression, which was affected both by the difference between the field and the greenhouse environment, and by the difference between seasons in field grown material, is evident from this table. In general, field conditions permit a stronger expression of the character than those of the greenhouse. This may be associated with the greater size and luxuriance of the field grown plants.

FIGURE 1.—(A) Spikes of tester strain, Early California Mariout, showing typical 6-rowed condition. At left, intact spike; center, spike with upper awns removed, to show arrangement of spikelets; right, single node, showing three spikelets. (B) *Agropyroides*, typical spikes and a single node, prepared as in Figure 1A. All reproduced $\times 0.3$.

FIGURE 2.—Single upper nodes from *agropyroides* (*ag ag v v*) spikes. (A) Single central lemma and lateral glumes. (B) Lateral spikelet, showing extra floret. (C) Single central lemma with bifid awn, two lemma-like glumes, and two lateral glumes. (D) Single central lemma with lemma-like glume and lateral glume. All reproduced $\times 1.7$.

TABLE 1

*Effect of environment on expression of agropyroides character.
Shown as percentage abnormal nodes per first spike**

Progeny of Plant No.	Field 1960	Greenhouse 1960	Field 1961	Greenhouse 1961
1	43.7	13.3	47.1	05.9
2	17.6	41.7	64.3	06.7
3	56.2	05.8	50.0	23.5
4	36.8	41.7	36.8	16.7
5	16.7	05.8	56.0	41.2
6	47.1	06.7	43.7	41.7
7	23.5	23.5	46.1	50.0
8	15.4	16.7	50.0	27.3
9	47.1	30.8	30.8	17.6
10	26.7	06.7	35.7	07.7
11	46.1	16.7	16.7	35.0
12	33.3	35.7	43.7	06.7
13	31.6	33.3	47.1	23.5
14	51.8	23.5	35.0	22.2
15	05.9	37.5	25.0	13.3
16	38.5	13.3	50.0	26.7
17	64.3	41.7	27.7	33.3
18	33.3	16.7	50.0	15.0
19	22.2	41.2	22.2	16.7
20	26.3	50.0	51.8	36.8
$\bar{X}$	34.2	25.1	41.5	23.4

Means (expressed as percentage abnormality per spike):

Year 1960—29.6 percent

1961—32.4 percent

Location* 1960—37.8 percent

1961—24.2 percent

* Location significant at 1 percent level.

Aceto-carminc squashes of pollen mother cells from the early California Mariout (tester) parent, *agropyroides*, and their F_1 heterozygotes showed essentially normal meiosis at all stages. All genotypes showed high pollen fertility and normal seed setting. Consequently, *agropyroides* should be regarded as a point mutation, according to the usual operational definition of this term in higher plants.

The *agropyroides* mutant was crossed reciprocally with two barley varieties from MR. SUNESON's collection; the six-rowed Late California Mariout and the two-rowed Atlas *deficiens* which has been produced by incorporating the *deficiens* (V^t) gene into the background of the originally six-rowed Atlas variety. Both California Mariout and Atlas are long-day spring varieties adapted to the Mediterranean type of climate, and so they resemble each other in many respects.

The F_2 of the cross *agropyroides* $\times$ Late California Mariout was segregating for earliness *vs.* lateness as well as for the *agropyroides* character. In respect to the latter, the progeny contained 394 normal: 126 *agropyroides*, which is a close fit to the expected 3:1 ratio ($\chi^2 = 0.164$). In respect to lateness *vs.* earliness the figures are similar, 392 late: 128 early.

TABLE 2

*Interaction between regular vs. irregular (Ag/ag) and early vs. late (Ec/ec)
in the reciprocal cross California Mariout × Agropyroides*

Phenotype	Observed	Expected on 9:3:3:1 basis	Expected with linkage (8.5% crossing over)
Regular 6-row—late	372	292.5	368.90
Regular 6-row—early	22	97.5	21.11
Agropyroides—late	20	97.5	21.11
Agropyroides—early	106	32.5	108.88
		$\chi^2 = 312.94$ P very low	$\chi^2 = .198$ P = .990—.975

The dihybrid ratio showed a strong association between earliness and *agropyroides* (Table 2). Using Immer's tables for calculating linkage intensities (IMMER 1930), and the product method, the data were found to fit the hypothesis of linkage between earliness and *agropyroides*, with 8.5 percent crossing over. The early gene (*ec*) associated with the tester material in which the *ag* mutant arose had been shown by RAMAGE and SUNESON (1958) to be in the "g" or 6th chromosome, the *ag* locus can be assigned to the same linkage group.

From the reciprocally made cross *agropyroides* × Atlas *deficiens* allele (V^t) is incompletely dominant; its heterozygote with the recessive allele (v^t) for the six-rowed condition could be recognized as semi-*deficiens*. Consequently, six phenotypes were identified as follows:

- (1) *Deficiens* regular ($Ag-V^tV^t$). Similar to the Atlas *deficiens* parent.
- (2) Semi-*deficiens* regular ($Ag-V^tv^t$). Similar to (1) except for stronger development of the lateral florets.
- (3) Six-rowed ($Ag-v^tv^t$). Similar to the early California Mariout and Atlas six-rowed varieties.
- (4) *Deficiens* irregular ($ag ag V^tV^t$). Lower part of the spike with fertile central and rudimentary lateral florets, but upper part with extra glumes, extra lemmas, or even a second fertile spikelet, irregularly distributed (Figure 3A).

TABLE 3

F_2 segregation for regular vs. irregular (Ag/ag) and 2-rowed vs. six-rowed (V^t/v)
in the reciprocal cross Atlas *deficiens* × Agropyroides

	Phenotypic classes						χ^2	Probability of 6:3:3:2:1:1 segregation
	Deficiens	Semi- <i>deficiens</i>	6-rowed normal	Deficiens irregular	Semi- <i>deficiens</i> irregular	Agropy- <i>roides</i>		
Atlas <i>deficiens</i> × Agropyroides (5 families)	78	171	58	25	51	27	1.355	P = .95 — .90
Agropyroides × Atlas <i>deficiens</i> (4 families)	47	116	59	15	41	14	3.932	P = .75 = .50

FIGURE 3.—Whole spikes and single upper nodes of (A) *Atlas deficiens* ($Ag Ag V^t V^t$) and (B) *agropyroides deficiens*, $\times 0.3$. (C), (D), and (E); single upper nodes from (C) *Atlas deficiens*, (D) and (E), *agropyroides semi-deficiens* ($ag ag V^t v$), showing extra dorsal florets at the central position. All reproduced $\times 1.7$.

(5) Semi-deficiens irregular (*ag ag V^tv^t*). Similar to (4), but with better developed lateral spikelets.

(6) Typical *agropyroides* (*ag ag v^tv^t*). Similar to the spikes already described and illustrated.

As shown in Table 3, the 702 F₂ plants contained in the nine families segregated according to the ratio expected with independent assortment between *ag* and *v^t*. Since the *v^t* locus is on chromosome 1 (SMITH 1951), this result was to be expected.

The unexpected result was the interaction between *ag* and *V^t*. On its original *v^t* (six-rowed) background, *ag* usually produces a reduction in the number of spikelet parts produced at the upper nodes, the lateral florets being replaced by laterally situated glumes, and the dorsally placed glumes present in normal six-rowed barley being often absent. In the F₂ *ag ag V^tV^t* segregates, however, the lateral florets may be replaced by glumes in some of the upper nodes, but in many of them both lateral florets are as fully formed as in typical *deficiens* plants. At such nodes, moreover, additional glumes or lemmas may be differentiated on the dorsal side of the fertile lemma, and in some instances small but fertile spikelets are produced at this position (Figure 3D, E). In general, therefore, the action of *ag* on the *V^t* background is to increase the number of structures differentiated at the upper nodes of the spike.

Developmental studies: The study of the expression of the *ag* gene during development was limited to a determination of developmental growth rates as expressed in the external dimensions of the glumes and awned lemmas of the central and lateral spikelets. A survey of earlier developmental stages showed that the spike develops in these varieties in the same manner as that described by BONNETT (1935). Spikelets are first differentiated near the base of the spike, and in its upper third differentiation is somewhat later. During the earliest stages of spikelet differentiation, no conspicuous histological differences could be seen between *Ag Ag* and *ag ag* plants.

FIGURE 4.—(A) Graph showing growth curves of central florets of tester (*Ag Ag v v*) and *agropyroides* (*ag ag v v*) genotypes. (B) Graph showing growth curves of lateral florets of tester and *agropyroides* genotypes.

Preliminary observations indicated that the spikelets at the fourth node above the base of the spike, which are among the earliest to develop, also have the most constant rates of growth. Consequently all measurements were made on the spikelets borne on this node. The first measurements were made when the length of lemma + awn was 0.9 cm in the central florets and 0.3 cm in the lateral florets. As can be seen from Table 4 and Figure 4A, subsequent measurements of central spikelets made at intervals of two days up to 14 days after the initial measurements reveal a typical sigmoid growth curve for both genotypes, with no significant difference between them at any stage.

On the other hand, conspicuous differences were found in the growth rate of the lateral spikelets. Beginning two days after the first measurement and continuing until 12 days after this time, the lemma of the lateral florets in *ag* was only 0.5 to 0.67 as long as the corresponding lemmas of *Ag*. At later stages, the proportional difference between the two genotypes was less, and at maturity it was very little evident. As Figure 4B shows, the greatest difference between genotypes was at the period of most rapid growth. This indicates that the principal effect of the *ag* allele on the lower nodes of the spike is a retardation of the growth rate of the lateral spikelets, which is more or less proportional to their overall growth rate in the normal genotype.

Free amino acid content of AG and ag plants: The content of free amino acids was obtained by the chromatography method (see MATERIALS AND METHODS) at three different stages of development: six-day seedlings, plants with the spikes of the main tiller at the early stage of differentiation designated the double-ridge stage (BONNETT 1935), and plants with spikes at the stage of early awn differentiation, after all of the floral parts had been formed and were developing. The morphological difference between the *Ag* and *ag* genotypes becomes first evident part way between the last two of these stages.

The results of the three series of experiments are summarized in Table 5. Although the means for the six replicates were somewhat different for most of the amino acids at one or more of the three stages, these differences were significant with respect to only glutamic acid, glutamine, ornithine, and arginine. Glutamic acid and ornithine were deficient only in six-day seedlings, glutamine only at the double-ridge stage, and arginine at all stages. At the later stages, the content of ornithine in both genotypes was so low that it could not be measured. In respect

TABLE 4
*Relative growth of central lemma length to lateral spikelet lemma
length in two genotypes of barley differing in a single gene*

<i>Agropyroides</i>		Early California Mariout	
Length of central lemma (cm)	Length of lateral lemma (cm)	Length of central lemma (cm)	Length of lateral spikelet lemma (cm)
3.9	1.2	4.1	2.1
4.6	1.4	4.6	2.6
7.1	2.3	7.4	3.8
7.8	2.6	7.9	4.1
12.2	4.2	12.4	6.3
12.9	4.8	13.1	6.8

to arginine, both genotypes had a much higher content in the seedlings than at later stages. In *Ag* the arginine content rose slightly between the double ridge and late differentiation stages, while in *ag* it remained at a low level. Since glutamic acid and ornithine are both well-known precursors of arginine, the upset of metabolism produced by the *ag* allele appears to be principally in an early step of arginine synthesis.

Production of phenocopies by exogenous amino acids: Since the *agropyroides* phenotype is associated with a deficiency of glutamic acid, glutamine, and arginine at various stages of development, one might expect that these amino acids, supplied exogenously to growing plants, would correct the deficiency and produce normal plants. Consequently, experiments were set up to test this possibility. The methods used are described in an earlier section. Four experiments were carried out. In the first, *agropyroides* plants growing in Hoagland's solution were supplied in different replications with arginine, cystine, glutamic acid, glutamine, hot water and cold water extracts of normal seedlings. For the latter, each pot received an extract obtained from 15 six-day old seedlings. The amounts of the other substances were given in Table 6.

Cystine at both 60 ppm and 120 ppm produced toxic effects, as did also glutamine at 4000 ppm. The data from these replicates are, therefore, omitted from the table. As shown in the table, arginine and glutamic acid at both concentrations used, as well as hot water extract from normal plants, produced a significant reduction in the expression of the *agropyroides* character, all to about the same degree. The effect of glutamine was not significant, and that of cold water extract

TABLE 5

Quantities of free amino acids of agropyroides (ag ag) and g-tester parent (Ag Ag) in 6-day barley seedlings. (Quantities are means of six observations in µg/gm fresh weight)

	6-day seedlings		Early differentiation (double-ridge)		Late differentiation	
	<i>ag ag</i>	<i>Ag Ag</i>	<i>ag ag</i>	<i>Ag Ag</i>	<i>ag ag</i>	<i>Ag Ag</i>
Cystine	77.1	87.5	26.5	32.4	17.5	22.2
Aspartic acid	86.7	92.8	12.1	14.7	101.3	117.4
Glutamic acid	117.0**	150.3**	113.4	128.0	108.2	116.8
Serine	70.7	71.3	65.8	58.9	33.4	33.0
Glycine	18.9	16.8	9.7	10.9	11.0	11.1
Lysine	51.2	44.5	3.9	3.1	4.5	4.2
Arginine	34.9**	42.4**	8.7**	12.3**	6.5**	17.6**
Threonine	100.3	103.8	80.1	93.8	57.5	63.6
α-Alanine	93.5	76.9	67.6	53.0	42.4	39.2
γ-Aminobutyric	99.5	84.8	21.2	21.2	39.8	47.1
Valine	139.3	146.5	25.8	28.6	41.8	40.8
Asparagine	166.2	175.2	69.6	71.7	42.2	43.2
Glutamine	830.0	844.0	98.4**	158.4**	64.0	70.8
Methionine	274.5	270.0	...	...	...	...
Proline	346.6	336.0	...	...	...	...
Ornithine	16.0**	35.7**	...	...	...	...

** Significant at the 1 percent level

TABLE 6

Effect of exogenous substances on the expression of ag: Summary of results from the first experiment

		Arc-sine treatment means	Treatment means retransformed to percent abnormality per spike
Arginine	624 ppm	21.70*	13.6
Arginine	312 ppm	19.33*	10.9
Glutamic acid	1120 ppm	20.82*	12.6
Glutamic acid	560 ppm	17.73*	9.3
Hot water extract		17.84*	9.4
Cold water extract		26.50†	19.9
Glutamine	2000 ppm	28.99	22.0
Control		46.70	52.9
(Differences between treatment means required for significance = 19.89)			

barely so. In a second experiment, conducted under the same conditions, hot water and cold water extracts of mutant plants were added in separate replicates to growing seedlings of the normal tester variety. These treatments were completely without effect.

In the third experiment, growing plants of the *ag* mutant were treated with tyrosine, adenine sulphate, and adenine sulphate + arginine. As shown in Table 7, tyrosine at 100 ppm applied either as a spray or a supplement to the mineral solution, produced a significant reduction in expression of the *agropyroides* phenotype, as did also adenine sulphate at both 150 ppm and 300 ppm. On the other hand, the effects of tyrosine at 200 ppm and adenine sulphate at 300 ppm + arginine at 624 ppm were either insignificant or barely so.

In a fourth experiment, plants grown singly in 8-inch pots were treated with foliar sprays of arginine at 400 and 200 ppm and glutamic acid at 600 and 300

TABLE 7

Effect of exogenous substances on the expression of ag: Summary of third experiment

		Treatment means of arc-sine data	Means retransformed to percent abnormality per spike
Tyrosine sol	200 ppm	24.80*†	17.7
Tyrosine sol	100 ppm	16.49*	8.0
Tyrosine spray	200 ppm	26.28	20.4
Tyrosine spray	100 ppm	16.77*	8.3
Adenine sulphate	300 ppm	17.49*	9.0
Adenine sulphate	150 ppm	15.32*	7.0
Adenine sulphate	300 ppm	27.33	21.1
plus arginine	624 ppm		
Control		46.70	52.9
(Differences between treatment means required for significance = 23.81)			

* Significantly different from control.

*† Barely significant.

TABLE 8

Results of fourth experiment on addition of exogenous substances to ag plants growing in mineral solution

Treatment	Mean number of abnormal nodes per spike
Arginine, 200 ppm	45.7
Arginine, 400 ppm	45.2
Glutamic acid, 300 ppm	49.8
Glutamic acid, 600 ppm	44.2
Controls, mineral solution only	83.6

ppm. The treatments were repeated at four-day intervals, commencing at the tenth day from germination and continuing until the differentiation of the flowering spike was complete. The first spike on every plant was scored at maturity for percentage of abnormality. As shown in Table 8, the untreated controls showed a much higher percentage of abnormal nodes (83.6 percent) than in earlier experiments (52.9 percent). As before, however, the added amino acids produced a significant reduction in character expression, although the percentage of abnormal nodes (44.2 to 49.8) was considerably higher than in the first experiment. We can conclude from these experiments, therefore, that arginine and one of its precursors, glutamic acid, as well as tyrosine and adenine sulphate, can produce a significant change in expression of the *agropyroides* character toward normality. The significance of the positive results with adenine sulphate and tyrosine is not yet understood.

A final series of experiments, using C^{14} arginine, was designed to show whether this substance is absorbed and incorporated into the tissues of normal and mutant plants. In the first of these, mutant seedlings at the three-leaf stage were treated with 10 μ l containing 0.183 microcuries of C^{14} arginine as a foliar drop to the adaxial surface of the leaf. After 24 hours the seedlings were dried, the leaves separated, and mounted on sensitive film. Photographs of dried seedlings showed that the arginine was transported to the meristematic regions of leaf bases and shoot apex. In two subsequent experiments, 400 μ l containing 7.32 microcuries of C^{14} were added to one-half strength HOAGLAND's solution and the seedlings transferred to this solution for periods of one, two, three, and four days. The arginine accumulated in the meristematic regions more slowly than it did with leaf applications, but at four days as much arginine was detected in the primordium as after the 24-hour treatment on the adaxial leaf surface. When *Ag* and *ag* plants were treated simultaneously for four days with 7.32 microcuries added to the mineral solution, the amount of label detected in dried shoot apices of *ag* was markedly greater than that in *Ag*.

In order to find out whether the two genotypes differ in the rate at which exogenous arginine is incorporated into protein, plants of both genotypes were treated by both of the treatments described above, and after treatment the spike primordia were fixed, dehydrated in tertiary butyl alcohol, embedded in paraffin, and sectioned at 7.5 μ , exposed to sensitive film, and stained as described in the methods section. As can be seen from Figures 5A and 5B the *ag* plants incorpo-

FIGURE 5.—Basal portions of the primordial spikes from (A) *agropyroides* and (B) tester plants, fixed 24 hours after application of C^{14} arginine, sectioned, stained, and photographed with radiosensitive film.

rated a large amount of C^{14} into their tissues, and the control *Ag* plants almost none (with the aid of Dr. S. YAMAGUCHI, a small amount of label was detected in the apices of *Ag* which is not evident in the photographs). Since the fixation and washing in tertiary butyl alcohol could be expected to remove all of the soluble arginine not incorporated into protein, we can conclude from this experiment that during a four-day period under our experimental conditions, the *ag* mutant incorporates a large amount of exogenous arginine, or other substances derived from it, into insoluble components of the living material, presumably protein, but that such incorporation is virtually absent in the normal *Ag* genotype during the same period of time.

DISCUSSION

The significant deficiency in arginine at all three stages of development examined, both before and after the stage when the histological and morphological

effects of the *ag* mutation are initiated, indicates that the altered biochemical information produced by this gene affects arginine metabolism at all stages in the life cycle of the plant. The visible *ag* effect, therefore, is not due to a time limited initial action of the gene, but apparently to the fact that the unbalance of arginine metabolism affects critical stages of cellular differentiation. This situation is comparable to that found by one of us and his coworkers in the hooded mutation of barley (SARKISSIAN, SHAH and STEBBINS 1962; YAGIL and STEBBINS 1963). As yet, the precise cellular abnormalities which initiate the peculiar differentiation processes of *ag* have not been identified. Careful comparison of spike apices of *Ag* and *ag* plants, fixed at these critical stages and stained by several methods, including the methyl green pyronin method to reveal the distribution of ribonucleic acids, failed to reveal any obvious differences between them before the actual differentiation of primordia.

Whatever these differences are, they apparently affect two different types of developmental processes. The primary morphogenetic effect of *ag* is, as described above, to change the number of organ primordia which are differentiated from the upper nodes of the spike. On the background of the *v* (six-row) allele, this number is usually decreased; in the presence of *V^t* ("two row") it is usually increased. A secondary effect was revealed by the comparative studies of growth rate in lateral spikelets of California Mariout and *agropyroides*; on its original background, *ag* also reduces the growth rate of the lateral spikelets after their formation. Since no comparable comparative studies were made of *Ag Ag V^tV^t* and *ag ag V^tV^t*, the effect of *ag* on growth rate when placed on the background of *V^t* is unknown.

With these facts in mind, we can construct a model which may lead to an understanding of the interaction between *ag* and *v*. The alleles at the *v* locus have no effect on the initial differentiation of parts from the spike primordium. The number of glumes and lemmas present even in the *V^tV^t* or *deficiens* genotype is exactly the same as in *v v*, or six-rowed. Rather, the effect of *V^t* is to inhibit the growth of lateral spikelets after they have been differentiated.

ZOHARY (1959) has pointed out that in spite of the statements to the contrary by other workers, the only truly wild form of the *Hordeum vulgare* complex is the ("two-rowed") *H. spontaneum*, which is homozygous for a dominant *V* allele. Furthermore, all other species of the genus *Hordeum* have sterile, rudimentary lateral florets, and so are uniformly homozygous for *V*. As ZOHARY has pointed out, the lateral florets aid in seed dispersal and in the penetration of the seed into the ground. Consequently, there is a selective advantage to their regular, even differentiation and growth, and we would expect these processes to be canalized, in the sense of WADDINGTON (1957).

The mutation from *V* to *v* has been produced artificially by radiation (NÖTZEL 1952) and by chemical means (EHRENBERG, GUSTAFSSON and LUNDQUIST 1961). The fact that it is recessive suggests that it represents the loss of an enzymatic function. Consequently, we may postulate that *v* differs from *V* or *V^t* in its inability to produce a substance which retards or inhibits the growth of lateral spikelets. Since six-rowed barley would be successful only if all of the parts of the

lateral spikelets were well formed, the v allele would be selected and incorporated into a cultivated variety only if it occurred in a genetic background already canalized or buffered against any effect on differentiation which might be produced by differences in the amount of the growth retarding substance produced by V , so that V^t , V and v genotypes produce the same number of normally differentiated structures. On the basis of this hypothesis, we can postulate that the dominant allele at the Ag locus plays a large role in this buffering process, so that $ag\ ag$ mutants are poorly buffered against differences in the amount and type of organ differentiation produced by differences in the quantity of V substance present. If this substance is present in excess, as in the case in the V^t genotypes, a greater amount of differentiation takes place, particularly in the later produced, upper nodes in which larger quantities of this substance may have accumulated. If the V substance is deficient, less differentiation than normal takes place in the upper nodes, which are produced after it has been largely used up by the lower nodes. If this hypothesis is correct, we might postulate that on the background of V (normal two-rowed or *H. spontaneum*), the ag mutation would differ only slightly from Ag in the number of parts differentiated at the upper nodes. Crosses to obtain these genotypes and test this point are now being made.

The connection between the morphological effect of the ag gene and its disturbance of arginine metabolism is still unclear, but is open to attack. Since arginine is an important component of nuclear histones, an attractive hypothesis is that arginine deficiency, particularly at the early stages of spike development, becomes so acute that it reduces the rate at which these basic proteins are synthesized. Since histones apparently regulate the rate at which certain genes produce RNA (HUANG and BONNER 1962; ALLFREY, LITTAU and MIRSKY 1963), histone deficiency may be directly responsible for failure of the differentiation processes to proceed normally. Appropriate experiments to test this hypothesis will be undertaken in the near future.

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SUMMARY

The recessive mutation *agropyroides* appeared in a culture of the barley variety early California Mariout which had been exposed to the action of the weed killer "Dalapon" (a sodium salt of 2,2 dichloropropionic acid). Its effects are to alter the number of appendages differentiated at the upper nodes of the spike. On the original six-rowed ($v\ v$) background, the number of appendages is usually decreased, the lateral spikelets being replaced by single, laterally placed glumes. On the *deficiens* (V^tV^t) background, the number of appendages is usually in-

creased through the addition of extra glumes or an extra lemma. The gene *ag* is on the 6th or "g" chromosome, 8.5 crossover units from the gene *ec* for earliness. Plants of *ag* analyzed at three different developmental stages for free amino acid content showed a significant deficiency for arginine compared to corresponding *Ag* plants at all stages. In six-day old seedlings, free ornithine and glutamic acid were also significantly deficient, while glutamine was deficient at the double ridge stage. When *ag* plants were grown in mineral nutrient solution to which was added either arginine, glutamic acid, tyrosine, or adenine sulphate, the *ag* expression was much reduced, and spikes were nearly normal in appearance. Addition of C¹⁴ arginine to seedlings as well as to plants in the early flowering stage resulted in greater uptake of the labelled tracer by *ag* as compared to *Ag* plants. In the *ag* plants, this compound was extensively incorporated into insoluble substance, presumably protein, while in *Ag* plants almost no incorporation took place. The suggestion is offered that the connection between the primary effect of the *ag* gene on arginine metabolism and its final effect on differentiation is via an abnormal or deficient synthesis of histone proteins.

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