Effects of Potassium Gibberellate on the Production of Alpha- and Beta-Amylase and Protease Activities during the Malting Of Wheat

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Steeping wheat with potassium gibberellate (GA-K) prior to or during germination greatly increased the enzyme activities of the malts. α-Amylase was increased as much as 109%, β-amylase as much as 86%, and protease activity as much as 48%. Response to GA-K was dependent on variety, although this did not appear related to the inherent property of the variety to produce malts of high enzyme activity. The most effective concentration appeared to be approximately 0.005% GA-K in the steep liquor. The treated malts had enzyme activities equivalent to control malts germinated 1 to 3 additional days. Spraying of the steeped grain during germination with solutions of GA-K increased all the measured enzyme activities, but this form of application was slightly less effective than corresponding treatment during the steeping of the grain.

THE GIBBERELLINS have been shown by Hayashi (*3*), Morris (*8*), and Pollock (*12*) to be capable of stimulating the germination of barley seeds. Many others have successfully applied gibberellins to the malting of barley (*1, 6, 9, 13*). These workers reported that malt qualities such as enzyme activities, extract yield, and modification were improved by the use of proper levels of gibberellins during the malting process. Malting times were also materially reduced. Peck *et al.* (*11*) found gibberellic acid (GA) to be innocuous when fed to white rats at the 5% level and they concluded that it is safe for human use. Owades and Chiano (*10*) found it without effect on yeast growth.

The present study was initiated to determine the effect of gibberellins on the production of the α- and β-amylase and protease activities during the malting of wheat.

Experimental

Malting was done on a laboratory scale in which 50-gram portions of grain were customarily used for each lot (*2*). Wheats were steeped to 42% moisture content at 50° F. The steep liquors were changed at 6-hour intervals to prevent damage to the embryos by asphyxiation or from the accumulation of naturally occurring germination inhibitors. The grain was sprouted in a specially designed germination cabinet in which a saturated atmosphere was maintained and temperatures were controlled within ±1° F. Kilning was performed at 104° F. in a forced convection oven for 24 hours.

To determine the most effective concentration range when used during steeping, lots of Pawnee wheat were steeped in GA-K solutions ranging from 0.00001 to 0.10%. The effectiveness of GA-K on the rate of enzyme development during germination was studied by steeping Pawnee wheat in 0.001% solutions to the 42% moisture level and germinating for 12 to 192 hours.

The duration of contact with GA-K during the standard steeping period was investigated. When contact was for less than the full time, it was for the final portion of the steeping period, as shown in Table III. To determine whether single treatments applied during germination were effective, portions of wheat from a single steep were sprayed

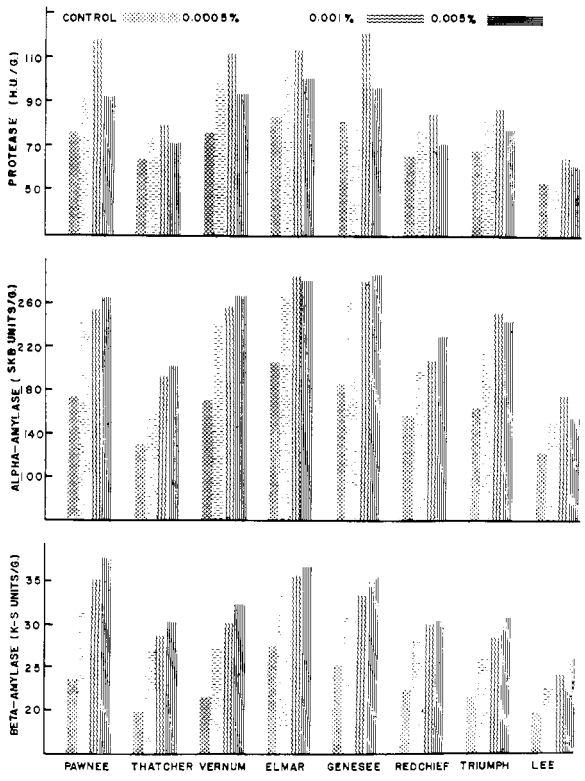

Figure 1. Effect of potassium gibberellate concentration in steep liquor on enzyme activities of various wheat variety malts

with 5 ml. of GA-K solutions of varying concentrations. Treatments were performed at the end of the steeping period and after 1, 2, and 3 days of germination. Eight varieties of wheat were studied to determine the extent to which the responses depended on variety.

α-Amylase was determined by the method of Sandstedt *et al.* (*14*), while β-amylase activity was measured by the Kneen and Sandstedt procedure (*5*). Protease activities were obtained by the Miller and Johnson (*7*) technique. All activities were reported on dry weight basis.

The amount of gibberellin which was absorbed by wheat during steeping was determined by assaying the steep liquor for GA after the steep period by a fluorometric method (*4*).

Results and Discussion

The germination rate, indicated by acrospire growth, was appreciably increased by GA-K treatments ranging in concentrations from 0.00005 to 0.005%. Higher concentrations reduced acrospire growth slightly. Rootlet growth was unaffected by concentrations below 0.0005% and was reduced when levels above 0.001% were employed. Malting losses due to the stimulation of acrospire growth and respiration were increased slightly when the GA-K treatments ranged from 0.00005 to 0.005%.

Table I. Effect of GA-K on Enzyme Production

(Fifty grams of Pawnee wheat, steeped to 42% moisture in the presence of 50 ml. of GA-K solution and germinated at 62° F. for 4 days)

GA-K in Steep Liquor, %	α-Amylase Activity,[a] SKB Units[b] G.	Std. Dev.	Response to GA-K, %	β-Amylase Activity[a], KS Units[c] G.	Std. Dev.	Response to GA-K, %	Protease Activity,[a] HU[d] G.	Std. Dev.	Response to GA-K, %
0	158	4.4	..	23.9	1.2	..	70	3.6	..
0.00001	163	3.8	+3	25.0	2.3	+5	73	4.3	+4
0.00005	169	5.5	+6	26.5	1.8	+11	80	4.4	+14
0.0001	179	4.4	+13	28.4	1.1	+19	83	2.7	+19
0.0005	199	6.9	+26	30.3	1.2	+27	87	5.0	+24
0.001	227	5.4	+45	31.1	1.5	+30	92	3.3	+31
0.005	238	8.8	+50	33.7	2.1	+41	86	3.3	+21
0.01	205	5.0	+29	30.4	1.7	+27	72	4.1	+3
0.05	175	4.8	+10	24.1	2.0	+1	64	3.6	−9
0.10	130	4.5	−18	17.8	2.0	−25	57	3.0	−19

[a] Average for 5 replicate malts prepared on different days. [b] SKB Sandstedt-Kneen-Blish units (*14*). [c] KS Kneen-Sandstedt units (*5*). [d] Hemoglobin units (*7*).

Table II. Effect of GA-K on Enzyme Activities after Varying Germination Periods

[Fifty grams of Pawnee wheat, steeped in 50 ml. of water (or 0.001% GA-K solution) to 42% moisture, and germinated for the period indicated at 62° F.]

Germination Period, Hours	α-Amylase Activity: Control, SKB units/g.	α-Amylase Activity: GA-K treated, SKB units/g.	α-Amylase Activity: Response to GA-K, %	β-Amylase Activity: Control, KS units/g.	β-Amylase Activity: GA-K treated, KS units/g.	β-Amylase Activity: Response to GA-K, %	Protease Activity: Control, HU/g.	Protease Activity: GA-K treated, HU/g.	Protease Activity: Response to GA-K, %
12	..	..	..	10.4	13.0	+25	14	20	+41
24	12	20	+66	11.3	15.1	+34	29	43	+47
36	32	54	+70	12.5	16.0	+30	38	55	+44
48	63	98	+56	13.4	19.4	+45	52	72	+38
72	113	168	+49	19.6	24.5	+25	61	82	+35
96	163	235	+44	24.2	30.5	+33	73	96	+31
120	186	251	+35	27.0	34.9	+29	80	102	+27
144	204	271	+33	30.1	37.8	+26	81	101	+25
168	234	304	+30	33.6	39.3	+17	83	104	+25
192	267	338	+25	34.3	41.7	+22	88	108	+23

Table III. Effect of Duration of Treatment with GA-K on Enzyme Production

(Fifty grams of Pawnee wheat, steeped to 42% moisture in the presence of 50 ml. of GA-K solution and germinated for 2 days at 62° F)

GA-K in Steep Liquor, %	Duration of Contact, Hours	Time of Application, Hours	α-Amylase Activity, SKB Units/G.	Response to GA-K, %	β-Amylase Activity, KS Units/G.	Response to GA-K, %	Protease Activity, HU/G.	Response to GA-K, %
0.0005	0	...	73	...	15.8	..	52	..
	1	51–52	101	+38	16.4	+4	63	+21
	3	49–52	109	+49	16.4	+4	63	+21
	6	46–52	112	+53	17.1	+8	68	+31
	12	40–52	119	+63	18.8	+19	73	+40
	18	34–52	126	+72	20.4	+29	74	+40
	24	28–52	129	+76	21.1	+33	77	+48
	36	16–52	129	+76	22.0	+39	73	+40
	52	0–52	134	+83	23.8	+50	74	+42
0.005	0	...	73	...	15.8	..	52	..
	1	51–52	126	+72	18.8	+19	57	+10
	3	49–52	133	+82	20.8	+32	59	+13
	6	46–52	138	+89	22.2	+41	63	+21
	12	40–52	146	+100	25.6	+62	67	+29
	18	34–52	154	+109	25.6	+62	68	+31
	24	28–52	148	+103	27.5	+74	73	+40
	36	16–52	151	+107	29.5	+86	70	+35
	52	0–52	152	+108	28.8	+82	72	+38

The effects of varying the amounts of GA-K in the steep liquor on enzyme production are summarized in Table I. The 0.005% concentration was optimal for the production of α- and β-amylase, while protease activity was greatest when the 0.001% level was used. Munekata and Kato (*9*) also reported that the higher concentrations of GA-K had a greater effect on the amylases than on the protease activity of barley malt.

A similar trend was noted when additional varieties of wheat were studied (Figure 1). The 0.005% concentration appeared to be the most effective for stimulating α- and β-amylase activities. Maximum protease production was achieved when GA-K was used at 0.001% (Figure 1). The α-amylase production in Pawnee, Thatcher, Vernum, and Genesee wheats was increased by approximately 50% when steep liquor concentration of GA-K was 0.005%. RedChief, Triumph, and Elmar were affected to a somewhat lesser degree while the response of Lee wheat was the least of all the varieties tested. In most instances, when amylase production was appreciably increased, that of protease was also augmented. Munekata and Kato (*9*) reported that barley varieties differed in their response to GA-K treatment. Sandegren and Beling (*13*) found that the concentration of GA-K which elicited the greatest response was the same for all the varieties tested. The foregoing data have indicated that the same trends were true for wheat.

Data concerning the influence of GA-K on malt enzyme activities after varying periods of germination are presented in Table II. The stimulation of production of all three enzymes appeared to be greatest during the first three days of growth but continued for the full period of growth studied. The enzyme activities of the GA-K–treated malts, in most instances, were equivalent to control malts that had been germinated for 1 to 3 additional days. These data thus indicate that significant reduction in malting times should be possible by use of GA-K.

The treatments, considered thus far, have consisted of continuous steeping of grain in GA-K solutions. The data given in Table III concern the effect of lesser periods of contact with GA-K. The maximum stimulatory effects with 0.00005% GA-K on all enzyme productions were after 18 to 24 hours of contact. Significant stimulation of enzyme activities was induced, however, even by shorter periods of contact (1 to 6 hours). Treatment with 0.005% GA-K was more effective than with the 0.0005% level.

The amounts of GA absorbed by 50-gram lots of wheat during the final portions of the steep period are given in Table IV. The quantities increased as the concentration or the period of contact was increased, but were relatively minor when the total amount available in the steep was considered.

The loss of significant amounts of GA-K in the steep liquor indicated that another mode of application was desirable. The results of a preliminary study of the effect of applying gibberellin to the wheat during germination are given in Table V. Appreciable increases in enzyme activity were induced when germinating wheat was sprayed with GA-K solutions. α-Amylase production was influenced to the greatest degree when applied during the early portion of the germination period. β-Amylase and protease production appeared to be influenced to much the same extent by applications of GA-K at all of the stages of germination studied. The increase in enzyme activity induced by spray treatments was

Table IV. Effect of Time and Concentration on Absorption of GA-K from Steep Liquor

Contact Time, Hours	Original GA-K Concn., μg./50 Mg.	GA-K Absorbed,[a] μg./50 G.	Std. Dev.	GA-K Absorbed, %
1	250	61	13.6	24.4
	500	76	12.4	15.2
	1000	131	19.4	13.1
	2500	230	23.6	9.1
6	250	80	12.4	32.0
	500	110	13.4	22.0
	1000	166	21.6	16.6
	2500	345	27.7	13.8

[a] Average of 15 determinations.

Table V. Effect of GA-K Applied during Wheat Germination on Enzyme Production

(Fifty grams of Pawnee wheat, steeped to 42% moisture in 50 ml. of water, and germinated at 62° F. for 4 days)

Mg. of GA-K per 50 Grams of Wht.	Time of Treatment with GA-K	α-Amylase Activity, SKB units/g.	α-Amylase Total response, %	α-Amylase Response due to GA-K, %	β-Amylase Activity, KS units/g.	β-Amylase Total response, %	β-Amylase Response due to GA-K, %	Protease Activity, HU/g.	Protease Total response, %	Protease Response due to GA-K, %
Control										
0	Immediately after steeping	163	..	..	22.5	..	..	71	..	..
2.5		166	+2	..	23.3	+4	..	73	+3	..
1.5		196	+20	+18	26.7	+19	+15	79	+11	+8
1.0		204	+25	+22	27.3	+21	+17	83	+31	+28
0.5		209	+28	+26	25.8	+15	+11	87	+23	+20
		190	+16	+14	24.9	+11	+7	83	+17	+14
0	After germinating 1 day	171	+5	..	24.1	+7	..	73	+3	..
2.5		186	+14	+9	26.9	+20	+13	83	+17	+14
1.5		199	+22	+7	27.9	+24	+17	90	+27	+23
1.0		203	+25	+20	26.5	+18	+11	90	+27	+23
0.5		194	+19	+14	25.5	+13	+6	84	+18	+15
0	After germinating 2 days	175	+6	..	24.6	+9	..	75	+5	..
2.5		200	+22	+16	27.4	+22	+13	86	+21	+16
1.5		193	+18	+12	26.6	+18	+9	94	+32	+27
1.0		189	+16	+10	27.1	+20	+11	90	+27	+22
0.5		193	+18	+12	25.8	+15	+6	85	+20	+15
0	After germinating 3 days	176	+8	..	24.0	+10	..	76	+7	..
2.5		205	+26	+18	28.3	+25	+15	90	+27	+20
1.5		190	+16	+8	27.6	+23	+13	93	+31	+24
1.0		193	+18	+10	25.5	+14	+4	95	+34	+27
0.5		184	+13	+5	25.1	+11	+1	88	+24	+17

not as great as that achieved by steeping, but nonetheless was significant. This method of treatment is being studied further in order to increase its effectiveness.

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TRANSLOCATION OF HERBICIDES

Fate of 2,2-Dichloropropionic Acid (Dalapon) in the Cotton Plant

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2,2-Dichloropropionic acid (dalapon) is readily absorbed from the soil by the cotton plant and accumulates in the actively growing tissues. As the tissues mature, the dalapon is retranslocated to more actively growing areas. No indications were obtained that any significant amount was metabolized or synthesized into natural products. The herbicide appears to be present in the tissues as a free acid or salt, which can readily be leached from the tissues by simple water extraction.

IN COTTON-GROWING AREAS, 2,2-dichloropropionic acid (dalapon) is used to control established clumps of Johnson grass in cotton fields. Although the herbicide is applied as a directed spray (*7, 8, 10, 12, 13, 17, 18*), there is always the possibility that it will come in contact with the cotton plants either directly or via the soil. Once this happens, it will probably be absorbed and translocated throughout the plant.

In considering the use of a new herbicide it is necessary to determine whether the compound will accumulate in portions of the plant which may be used for feed or food products, or be metabolized into products which would remain as an undesirable residue in the plant.

To investigate the fate of dalapon within the cotton plant it was necessary to develop chemical or radiochemical procedures for determining the herbicide and its possible biological degradation products.

A review of the chemistry of dalapon indicated that the most likely degradation steps would involve dehalogenation, hydration, and decarboxylation reactions with the formation of pyruvic acid, acetic acid, and carbon dioxide. Satisfactory chemical methods are available for the determination of these compounds (*1, 5–7, 12*). Unfortunately, however, all of the compounds occur naturally in plants and it would be impossible to distinguish by chemical means the naturally occurring compounds from the biological degradation products of dalapon.

To ascertain the fate of each portion of the dalapon molecule, radiochemical techniques were employed using labeled dalapon-2-C^{14} (*3*), to obtain residue information on the fate of dalapon in the cotton plant.

Methods

Individual cotton plants (*Gossypium hirsutum* var. Cokers 100 Wilt Resistant) were grown in crocks in a greenhouse. The crocks were approximately $8\frac{1}{2}$ inches in diameter with a capacity of 2 gallons and were filled to within 1 inch of the top with potting soil consisting of an equal mixture of sand, peat moss, and sandy loam. The crocks were arranged in such a manner that the plants could be surface-irrigated and the excess moisture drained through a hole in the bottom into a glass tray.

When the plants had reached the early blossom stage, 12 were selected for uniformity and treated by irrigating each crock with 500 ml. of a dalapon solution containing approximately 71 p.p.m. of dalapon-2-C^{14} having a specific activity of 0.014 mc. per mmole. This solution was neutralized and applied as the sodium salt.

The plants were surface-irrigated daily with 500 ml. of water and the excess was allowed to drain from the bottom of the crock. They were grown 12 weeks in the greenhouse, using artificial lights to supplement the sunlight when necessary. At harvest, many of the bolls had matured, and the cotton was ready for picking.

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