were similar in the region of 6000 to 7500 Å. The action spectrum for anthocyanin formation in apple skin has a principal maximum near 6500 Å, a subsidiary maximum near 6000 Å and weak action throughout the visible region. The photoreceptor is probably a flavoprotein similar to acyl coenzyme A dehydrogenase.

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EFFECT OF 6-(SUBSTITUTED)PURINES AND GIBBERELLIN ON THE RATE OF SEED GERMINATION[1]

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Stimulation of lettuce seed germination by some 6-(substituted)aminopurines (3, 5) as well as 6-(substituted)thiopurines (4) has recently been reported. Further, gibberellin has also recently been demonstrated to increase the rate of lettuce seed germination to about the same extent as do the purine derivatives (2). In certain cases, these compounds appear to overcome the light requirements for germination which has been shown to be necessary for certain varieties of seed (1), and in addition, they also exert an effect which augments that of light (5).

In order to study the relationship of the germination effects observed upon lettuce seeds pre-treated with 6-(substituted)purines and gibberellin, the ef-

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fects of combinations of these structurally dissimilar compounds were determined in the present investigation. The results indicate that gibberellin and 6-(substituted)purines are synergistic in increasing the rate of germination of lettuce seed pre-soaked in solutions containing these compounds. Also, the effects of gibberellin and the purine derivatives alone and in combination upon the germination of another variety of lettuce seed and on carpet grass and clover seed have been studied.

Materials and Methods

Lettuce seeds (Early Curled Simpson and Oak Leaf) were pre-soaked for 8 hours, and carpet grass seeds were pre-soaked 12 hours, prior to germination on filter paper wet with the corresponding solutions in Petri dishes; whereas the clover seeds (White Dutch) were not pre-soaked, but placed directly in the Petri dishes on filter paper wet with the corresponding test solutions. The pre-soaking period was carried out in the dark at 25° C, and the seeds were protected from light activation by carrying out subsequent mechanical operations in the presence of blue light (fluorescent light filtered through four layers of du Pont 300 MSC dark blue cellophane). The seeds were germinated either in the dark at 30° C or in the light at 30° C, as indicated in the tables, and 100 or more seeds were used in each assay. When an intermediate time period is reported prior to the terminal count, this count was made in the presence of blue light.

In an effort to determine if there is a specific time sequence of seed activation initiated by these purine compounds during the pre-soaking period, lettuce seeds were treated with a 10 μg/ml solution of 6-benzylaminopurine, and with water separately, for varying time intervals over a combined period of 8 hours. When the seeds were not in contact with the purine solution they were placed in water. Starting with either water or the purine solution, seeds were pre-soaked for one hour in the initial solution, followed by 7 hours in the alternate solution; two hours in the initial solution, followed by 6 hours in the alternate solution, etc., up to 7 hours in the initial solution followed by one hour in the alternate solution, and finally 8 hours in the initial solution. Thus, the seeds were exposed to the active purine solution both prior to and following soaking in water at one hour intervals over a total period of 8 hours. The seeds were then allowed to germinate on filter paper wet with water in covered Petri dishes at 30° C in the dark. It made no significant difference in the eventual germination percentage whether the seeds were initially treated with the purine derivative for 1, 2, or up to 8 hours; or whether the seeds were initially soaked in water and then in the purine solution for any of the 7 time periods, except that the seeds pre-soaked in the purine solution had a higher germination percentage (about 10 % higher) than did corresponding seeds initially pre-soaked in water and then placed in the purine solution.

The synergism was shown experimentally by pre-soaking the seeds in a solution containing the indicated concentration of purine and gibberellin [2] for 8 hours, followed by germination on filter paper in covered Petri dishes at 30° C in the dark. A similar experiment was conducted using indoleacetic acid with some of the more active purine derivatives in an effort to determine whether or not auxin possessed a synergistic effect in combination with 6-(substiuted)purine compounds on stimulation of seed germination.

Results and Discussion

The stimulatory effect on germination produced by pre-soaking seeds in 6-(substituted)purine solutions is not limited to lettuce seed alone, but has been found to be applicable to other varieties of seed as well. An appreciable increase in rate of germination was observed in the case of carpet grass after pre-soaking in several of the more active purine solutions as indicated in table I; however, the concentration required for activation of this seed is somewhat higher than that required for activation of the lettuce seed. Pre-treatment of carpet grass seeds with 30 μg/ml of 6-pentylamino-, 10 μg/ml of 6-benzylamino- or 6-(2-thenyl)aminopurine for 12 hours, followed by germination in the dark for 7 days, gave 56 %, 52 % and 41 % germination, respectively, as compared to a water control of 12 %. These stimulatory effects are also observed even when the seeds are allowed to germinate in the light at 30° C, wherein the corresponding values for the compounds are 71 %, 70 % and 70 % respectively, as compared to a water control of 35 %. These results are comparable in kind to those observed with White Dutch clover as indicated in table I as well as those previously reported for Early Curled Simpson lettuce seed (5). However, whereas the carpet grass and lettuce seeds after pre-soaking in water showed an appreciable light stimulation when allowed to germinate at 30° C, the clover seed did not. Thus, clover seeds pre-soaked in water gave 33 % germination under the usual test conditions in the dark after 96 hours, and seeds in the light were only 39 % germinated in the same time interval. In contrast, the seeds pre-treated with the more active purine compounds had a significantly higher germination percentage under both sets of experimental conditions; for example, pre-soaking in a 3 μg/ml of 6-benzylaminopurine gave values of 74 % and 71 % after germination in the dark and light at 30° C, respectively. Thus, these compounds appear to activate some system(s) governing germination other than those activated by light.

In an attempt to determine if there is an auxin involvement in the purine induced germination of seed, 10 μg/ml solutions of several of the amino and

[2] The gibberellin was obtained through Dr. J. L. Liverman of Texas A. and M. College and was a commercial sample issued by Merck and Co. under the name "Gibrel."

TABLE I

STIMULATION OF SEED GERMINATION BY SOME 6-(SUBSTITUTED)PURINES *

COMPOUND †† 6-(SUBSTITUTED)-PURINE	CONCENTRATION, μG/ML	CARPET GRASS **		WHITE DUTCH CLOVER †			
		PERCENT OF SEED GERMINATED AFTER: (DAYS)					
		7 (LT)	7 (DK)	2 (LT)	2 (DK)	4 (LT)	4 (DK)
Water blank control	..	35	10	22	12	39	33
Pentylamino-	1	33	18	58	17	64	22
	3	49	21	58	42	76	45
	10	59	40	71	30	70	52
	30	71	56	37	19	60	54
Hexylamino-	1	39	16	44	27	63	33
	3	47	14	61	37	69	52
	10	60	28	64	33	76	50
	30	61	41	25	49	57	64
Benzylamino-	0.3	39	25	61	45	77	64
	1	51	31	65	60	72	68
	3	55	41	65	60	71	74
	10	70	52	51	24	62	33
Kinetin	0.3	39	14	20	14	29	24
	1	49	18	49	52	73	63
	3	50	24	63	36	70	57
	10	64	39	14	12	30	25
Thenylamino-	0.3	36	11	61	48	68	58
	1	41	16	59	56	69	70
	3	60	32	57	43	67	60
	10	67	41	63	49	61	64
3-Pyridylmethyl-amino-	1	39	18	30	46	37	63
	3	45	24	46	48	65	53
	10	55	24	62	52	66	59
	30	68	39	60	60	65	68

* 100 or more seeds used in each assay.

** Pre-soaked in the indicated solution for 12 hours, allowed to germinate in the light (Lt) or dark (Dk) at 30° C.

† Seeds not pre-soaked; placed directly on filter paper containing the indicated solution and allowed to germinate in the light (Lt) or dark (Dk) at 30° C.

†† Several thiopurine analogues were tested and found to be appreciably less active than the corresponding amino compounds; however, some stimulation of germination was observed.

thiopurine derivatives were supplemented with 1 and 10 μg/ml of indoleacetic acid, and the resulting solutions were used for pre-soaking lettuce seeds. The results obtained after germination in the dark at 30° C are presented in table II. No appreciable increased rate of germination was observed; however, in many instances the higher concentrations of auxin appeared to inhibit the action of the purine derivative in stimulating germination of lettuce seeds. Since the active concentration of auxins are sometimes quite specific, the effect of varying amounts of indoleacetic acid in the presence of 6-benzylaminopurine on lettuce seed germination was studied over a much wider range of concentrations. The amount of auxin used for pre-soaking lettuce seed was varied in ten-fold increments from 10^{-6} to 1.0 μg/ml in the presence of 0.01, 0.1 and 1.0 μg/ml of the purine derivative. The auxin alone produced no stimulatory effects, and the combination of the two compounds at all of these concentrations gave values which were comparable to those for seeds pre-soaked in the purine solution alone.

Since gibberellin (2), as well as purine derivatives, have been observed to stimulate seed germination, it was decided to examine the effect of pre-soaking seeds in a combination of these two structurally dissimilar compounds. Such a combination proved to be synergistic in stimulating seed germination as indicated in table III. Pre-soaking Early Curled Simpson lettuce seeds in gibberellin solutions at 30 and 100 μg/ml gave 11 and 15 % germination, respectively, while pre-soaking the seed in 1 and 10 μg/ml of 6-benzylaminopurine resulted in 4 and 23 % germination, respectively. A combination of gibberellin and the purine derivatives yielded, in every case, a marked synergistic stimulation of rate of lettuce seed germination. For example, a mixture of 1 μg/ml of 6-benzylaminopurine and 30 μg/ml of gibberellin gave 71 % germination. The thiopurine analogs were less effective than the corresponding amino-

TABLE II

EFFECT OF 6-(SUBSTITUTED)PURINES AND AUXIN ON THE GERMINATION OF LETTUCE SEED *

6-(SUBSTITUTED)-PURINE, 10 μG/ML	INDOLEACETIC ACID CONC, μG/ML	PERCENT OF SEED GERMINATED AFTER: (HOURS)	
		48	96
None	0	2	19
	1	2	17
	10	0	3
Phenylethylamino-	0	74	81
	1	58	74
	10	27	51
Phenylethylthio-	0	22	60
	1	27	65
	10	7	55
Butylamino-	0	83	92
	1	63	81
	10	39	63
Butylthio-	0	9	40
	1	14	39
	10	4	31
Heptylamino-	0	66	76
	1	44	71
	10	19	66
Heptylthio-	0	3	15
	1	4	19
	10	0	16
2-Thenylamino-	0	68	83
	1	54	73
	10	34	57

* In each assay 100 or more Early Curled Simpson lettuce seeds were pre-soaked for 8 hours in the dark at 25° C and germinated on wet filter paper in the dark at 30° C.

purines; however, a stimulation was observed when the seeds were pre-soaked in a mixture of the thiopurine analogs and gibberellin over that of those seeds soaked in either of the compounds alone. A similar synergistic stimulation of germination was also observed with 6-benzylaminopurine and gibberellin using another variety of lettuce seed as indicated in table IV.

The observed stimulation of germination of carpet grass and clover seeds after pre-soaking in the purine solutions was extended to an examination for a possible synergistic effect using gibberellin and the 6-(substituted)purine derivatives with these seeds. Some increase in stimulation of germination was observed with carpet grass seeds pre-soaked in mixtures of gibberellin and 6-benzylaminopurine, as indicated in table IV. In contrast, no appreciable effect was noted, under the test conditions used, for a stimulation of germination of clover seed over that of the stimulatory effect of the purine derivatives alone.

The structural feature of the purine derivative initiating this activity does not appear to be solely the purine nucleus, since adenine, at similar concentrations, gave no stimulatory effects either alone or in the presence of gibberellin. The fact that both the thio- and amino- analogs affect the rate of germination of lettuce seed indicates that structural specificity is not very exacting. However, it appears that the purine structure itself may be necessary to produce an active analog since the corresponding 4-(substituted)-pyrazolo(3,4-d)pyridine derivatives of some of the more active purine analogs were essentially inactive in this type assay system (6).

The synergistic effect of the purine derivatives and gibberellin indicate that these substances differ in their mode of action but are probably closely related in their functions. It appears likely that their effects are exerted in sequence in certain plant systems.

TABLE III

SYNERGISTIC GERMINATION EFFECTS OF GIBBERELLIN AND SOME 6-(SUBSTITUTED)PURINES *

6-(Substituted)-purine	Conc, μg/ml	Gibberellin, μg/ml: 0	30	100
		Percent of seed germinated after 24 hours **		
None	..	1	11	15
6-*n*-Hexylamino-	1	8	69	54
	10	17	80	82
6-*n*-Hexylthio-	1	1	21	27
	10	12	40	53
6-Benzylamino-	1	9	71	69
	10	23	85	93
6-Benzylthio-	1	0	24	21
	10	4	39	56
Kinetin	1	15	69	89
	10	29	85	81
Adenine	1	2	6	16
	10	1	6	7

* Early Curled Simpson lettuce seeds were pre-soaked for 8 hours in the indicated solutions, and allowed to germinate in the dark at 30° C.

** 100 or more seeds were used in each test.

TABLE IV

ACTIVITY OF GIBBERELLIN AND 6-BENZYLAMINOPURINE ON THE RATE OF GERMINATION OF SOME SEED

6-Benzylamino-purine conc, μg/ml	Time interval of germination, days	Gibberellin concentration, μg/ml: 0	30	100	300
		Percent of seed germinated in time interval			
Carpet grass *					
0	5	13	13	18	18
0	7	18	19	24	21
1	5	15	18	23	27
1	7	21	26	34	34
10	5	27	41	47	51
10	7	37	45	50	57
White Dutch clover **					
0	1	7	5	12	8
0	2	20	14	23	22
1	1	36	29	36	34
1	2	61	55	56	55
10	1	35	36	31	36
10	2	57	57	40	55
Oak Leaf lettuce ***					
0	1	3	15	41	36
1	1	36	63	77	88
10	1	58	81	84	89

* Pre-soaked for 12 hours in the indicated solution in the dark; germinated in the dark at 30° C.

** Seeds placed directly on filter paper wet with the indicated solution and allowed to germinate in the dark at 30° C.

*** Seeds pre-soaked for 8 hours in the indicated solutions in the dark; germinated in the dark at 30° C.

SUMMARY

The previously observed stimulation of the rate of seed germination by pre-soaking lettuce seeds in 6-(substituted)purine solutions has been extended to a comparable stimulation effect using carpet grass and clover seeds. Further, a combination of gibberellin and 6-(substituted)purines has been found to act synergistically in stimulating the rate of germination of lettuce and carpet grass seeds under certain experimental conditions. These results indicate different modes of action of gibberellin and 6-(substituted)purines and suggest that these substances are closely related in their biochemical functions, which are perhaps in sequence.

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THE FIXATION OF $C^{14}O_2$ INTO TARTARIC AND MALIC ACIDS OF EXCISED GRAPE LEAVES[1,2]

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Tartaric acid is the major organic acid in the leaves and fruits of the grape (*Vitis vinifera*) (2, 3, 9, 10), and together with malic acid, constitute 80 to 90 % of the total organic acid fraction. Although the data are not conclusive, there is evidence that both tartaric and malic acids are synthesized only in the leaves, and are translocated to the ripening fruits during the late summer (9). The malic acid content decreases rapidly both in leaves and in fruits under certain conditions, but the tartaric acid content remains relatively constant. Leaves picked in the early morning or night showed no significant change in the amounts of malic or tartaric acids, suggesting that there is little or no diurnal variation in these organic acids (9).

No specific enzymatic reactions involving (+)-tartaric acid are known in higher plants, although bacteria and fungi can oxidize or dehydrate all three isomers (13, 15, 16, 20, 21, 28). Extracts from higher plants and animals, on the other hand, can oxidize (−)- and meso-tartaric acid, and there is preliminary evidence that seed tissues of higher plants can also dehydrate (−)- and meso-tartaric acid forming oxaloacetic acid (23, Stafford, unpublished data). The formation of meso-tartaric acid has been demonstrated in several fungi, bacteria, and a yeast (15).

The present study was undertaken to gain information concerning the metabolic origin of tartaric acid, using in vivo techniques and $C^{14}O_2$ as a source of label. The results indicate that tartaric acid is formed in a secondary process from the primary carbohydrate products of photosynthesis. Organic acids formed by CO_2 fixation, such as malic acid, do not appear to be likely precursors.

MATERIALS AND METHODS

The 2nd or 3rd leaves from the growing tips of the fruiting vines of the Mission variety of grape were picked at about 9:00 AM, and the petioles were immediately recut under water. In each experiment, three leaves were used. These were placed in a beaker of water under a micro bell jar (325 ml capacity) fitted with tubulation and a ground glass bottomed plate. A vial containing dry $BaC^{14}O_3$ was connected to the tubulation with a short piece of rubber tubing and $C^{14}O_2$ was released by injecting a few milliliters of 50 % lactic acid into the vial via the rubber tube. The jars were then either placed in the dark (experiment 2) or in the light (experiments 1, 3, and 4) under two 20-watt daylight fluorescent tubes at a distance of about 10 cm at room temperature (about 25° C). After three hours in the light, the remaining $C^{14}O_2$ in experiment 4 was removed by aspirating through a *N* NaOH trap and was replaced with $C^{14}O_2$-free air. This bell jar was then placed in the dark for the remaining 21 hours.

At the end of the experimental period, all bell jars were aspirated, the leaves weighed, plunged into boiling alcohol for two minutes and frozen in a dry ice-alcohol mixture prior to homogenization (in a Virtis homogenizer). After centrifugation of the homogenate and decantation of the supernatant, the residue was resuspended in water and decationized with Dowex 50 (H^+ form) batchwise to insure the solubilization of any remaining potassium acid tartrate. These decationized washings were added to the original supernatant, the combined extract was concentrated in vacuo at 40° C to remove the alcohol, and was centrifuged at high speed. Finally, the supernatant was filtered through a Dowex 50 column (H^+) (1.5 × 7 cm) to remove any remaining cations. The effluent, which was composed primarily of sugars and organic acids was added to a Dowex 1 column (formate, 200 to 400 mesh, 1.2 × 8 cm). After washing the column with 2 or 3 column volumes of distilled water, a 3.0 M formic acid gradient was started (17) and the eluted acids were collected in 100 successive 4-ml fractions. A 0.1- to 0.2-ml aliquot from each tube was used to estimate the activity of the fraction. This was accomplished by plating the aliquot on a steel planchet and measuring the activity under a thin-window, gas flow counter in the Geiger

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