USE OF ACTIVATED CHARCOAL TO ENHANCE THE GERMINATION OF BOTANICAL SEEDS OF POTATO[1]

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Abstract

The effect of activated charcoal (AC) on the germination of botanical potato seeds was tested by applying AC to seeds in petri dishes which had been pretreated with gibberellic acid (GA). A diverse sample of accessions including cultivar and cultivated species germplasm, cultivated species hybrids, wild species, and wild species known for their slow germination was tested. The time required from hydration to 75% germination was significantly less for all types of seeds tested when AC was present. Cultivated species hybrids reached 75% germination an average of four days sooner, wild species two days sooner, and slow germinating wild species 18 days sooner when AC was present. Only slow germinating wild species' seeds germinated with significantly more uniformity in the presence of AC.

Resumen

Se investigó el efecto del carbón activado (AC) sobre la germinación de las semillas botánicas de la papa, mediante la aplicación de AC a las semillas, en platos de petri, las cuales habían sido tratadas previamente con ácido giberélico (GA). Se probó una muestra diversificada de nuevas introducciones, incluyendo el germiplasma de cultivares y especies cultivadas, especies silvestres, y especies silvestres conocidas por su lenta germinación. El tiempo requerido desde la hidratación hasta el 75% de germinación fue significativamente menor para todos los tipos de semillas probadas cuando se encontraba presente el AC. Los híbridos de especies cultivadas alcanzaron en promedio el 75% de germinación cuatro días antes, las especies silvestres 2 días antes, y las especies silvestres de germinación lenta 18 días antes, cuando se encontraba presente el AC. Solamente las semillas de las especies silvestres de germinación lenta germinaron, significativamente, más uniformemente en presencia de AC.

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Introduction

Various treatments have been tested for their efficacy in breaking dormancy in botanical seeds of potato. Among these, the use of KNO_3 and K_3PO_4 has been recommended (1). Steir (9) tested ethyl chlorhydrin, ethanol, and hydrogen peroxide and found them to be ineffective. He indicated that dormancy was dependent on the age of the seeds and when they had been removed from the berries. Simmonds (6) reported that cysteine and gibberellic acid (GA) were useful for increasing germination. Lam (3, 4) found that light and high temperatures were inhibitory. Spicer and Dionne (8) found that a high concentration of GA (2000 ppm) broke dormancy often associated with wild species' seeds. This treatment is widely practiced and is recommended by the Inter-Regional Potato Introduction Project (IR-1) at Sturgeon Bay, Wisconsin.

The ability to further enhance the germination of potato seeds would be beneficial to several areas of potato research and culture. Germination tests are a routine part of germplasm maintenance. Hastened germination would make these tests faster and possibly more accurate, especially for seedlots which germinate poorly even after GA treatment. Faster, more uniform germination would promote uniform seedling size both within and among seedlots, resulting in more uniform transplants. A technique which makes transplants more uniform might also help to avoid inadvertent selection for short dormancy (7) since large seedlings from the first seeds germinated are often the ones chosen for transplanting. Enhanced germination would also be beneficial when true potato seed (TPS) is sown directly into the field for research or cropping purposes.

Activated charcoal (AC) is effective in breaking seed dormancy of some *Trifolium* species (Dr. R.R. Smith, USDA, University of Wisconsin, personal communication), cucumber (5), millet (5), and the weedy grass *Stipa trichotoma* (2). Our investigation was undertaken to determine the potential of AC applied after GA treatment for enhancing potato seed germination.

Materials and Methods

Diverse seedlots tested included inter-cultivar and cultivated species germplasm (Experiment 1), cultivated species and cultivar-species hybrids (Experiment 2), wild species (Experiment 3), and accessions known for their particularly slow germination (Experiment 4). Seeds used in Experiments 1 and 2 were approximately six months old and had been hand extracted from berries about two months post pollination. All other seeds were at least three years old and had been extracted by macerating similarly aged berries in water in a blender, followed by collection of seeds via a series

of sieves. Seeds were soaked for about 18 hours in 1500 ppm GA, surface sterilized in a dilute solution of sodium hypochlorite (0.53%) for about 20 minutes, rinsed in distilled water, and placed on single pieces of Whatman #2 filter paper in 6 cm plastic petri plates.

Inter-cultivar and cultivated species germplasm, cultivated species and cultivar-species hybrid, and wild species seeds were placed on moistened filter paper and sprinkled with finely powdered AC. More uniform application of AC to seeds of slow germinating wild species was made by moistening each plate with about 0.8 ml of 0.5% aqueous AC suspension. Distilled water was added to the plates when necessary during the course of the experiments. The need to add water was avoided in the experiments with slow germinating wild species and cultivated species and cultivar-species hybrid seed by sealing the plates with laboratory film. All tests were conducted at 18-22 C (room temperature) in darkness or in indirect light on a laboratory bench. Seeds were considered germinated when 2 mm or more of the radical had emerged.

Nine seedlots of inter-cultivar and cultivated species germplasm were evaluated until the AC treated seeds had reached maximum germination. One AC treated plate and one control plate, each containing about 50 seeds, were tested for each seedlot. The difference between germination in control and AC treated plates was then analyzed for significant deviation from zero.

Seeds in subsequent experiments were observed until both AC and control treatments had reached maximum germination. Treatments in these seedlots were replicated two to four times with 25 seeds per plate. Wild species seeds were scored daily, slow germinating species seeds every two to three days, and cultivated species hybrid seeds about every four days. Sprouted seeds were removed at each scoring except for slow germinating wild species seeds which were removed weekly. Cumulative percent germination was calculated and converted to percent of the maximum germination observed for that seedlot.

Germination in each plate was assessed by calculating three statistics: 1) rate, 2) uniformity, and 3) final percent. "Rate of germination" was expressed as the number of days from hydration until the first 75% of the seeds had germinated. "Uniformity of germination" was represented by the minimum time interval within which any 75% of the seeds had germinated, irrespective of the length of the post-hydration period. "Final percent germination" was the actual percent germination recorded on the final day of each experiment. Percent data were transformed to the arcsin of their square roots and treatment differences were evaluated by analysis of variance.

Experiments were also conducted to test the effects of surface sterilization with hypochlorite, the use of distilled water versus tap water, the method of AC application, and the effect of AC on seeds without GA treatment.

Results

The nine inter-cultivar and cultivated species germplasm seedlots (Experiment 1) reached maximum germination in four to six days when treated with AC (Table 1). At this time, the control germination ranged from 1 to 70 percent, and averaged only 29%.

TABLE 1. — *Cumulative germination of seeds of inter-cultivar and cultivated species germplasm at time of maximum germination of activated charcoal (AC) treated seeds (Experiment 1).*

Seedlot	Percent of max. germination[1]			
	+AC	Control	d	day
Gp. Stenotomum x Gp. Phureja -1	100	15	85	6
(") -2	96	61	35	6
Gp. Stenotomum -1	100	12	88	6
(") -2	100	38	62	4
Gp. Phureja x Gp. Stenotomum	100	6	94	4
Gp. Andigena x Norland	95	30	65	5
Butte x New Haig	100	70	30	5
Superior x Kennebec	100	30	70	5
Superior x New Haig	87	1	86	6
Averages	98	29	68***	5

***significant at .001 probability level.
[1]of AC treated (+AC) and control (-AC) seeds at the indicated day (day) with difference (d).

Germination of cultivated species and cultivar-species hybrid seeds (Experiment 2) was also significantly enhanced in the presence of AC (Table 2). When AC was present, the time from hydration to 75% of maximum germination was an average of four days less than that of controls. No significant differences in uniformity or final (18 day) percent germination were detected.

Germination of wild species (Experiment 3) seeds was similar to that of cultivated species and cultivar-species hybrids (Table 2). Control seeds required nine days from hydration to reach 75% of maximum germination while those treated with AC did so in seven days, but the final average germination levels of the two treatments were not significantly different at 26 days. No significant differences between AC and control treatments were detected for uniformity of germination.

The greatest enhancement of germination due to AC occurred with slow germinating wild species seed (Experiment 4) (Table 2). The time from hydration to 75% germination was an average of 18 days less when AC was present. These seeds also germinated more uniformly, since the minimum time interval within which any 75% of the seeds germinated was an average of 17 days less with AC. The final germination scored at 48 days was significantly greater for AC treated seeds.

TABLE 2. — *Comparison of germination with and without activated charcoal for cultivated species and cultivar-species hybrids, wild species, and slow germinating wild species seeds.*[1]

Seedlot/accession	Rate[2]		Uniformity[3]		Final % germination	
	+AC	Control	+AC	Control	+AC	Control
Cultivated Species and Cultivar-Species Hybrids (Experiment 2)						
Gp. Stenotomum x Gp. Tuberosum haploid	6	12	6	9	98	89
Kennebec x Gp. Andigena	4	7	4	4	83	83
Butte x *S. gourlayi*	6	7	6	7	88	97
Kennebec x *S. sucrense*	9	14	8	9	75	71
Butte x *S. sucrense*	5	10	5	6	99	89
Averages	6.0	10.0	5.8	7.0	88.6	85.8
Average difference	4.0**		1.2ns		2.8ns	
Wild Species (Experiment 3)						
S. acaule PI 175395	6	6	2	4	98	100
S. acaule PI 175395	8	9	5	3	96	100
S. acaule PI 230529	9	9	6	5	100	100
S. commersonii PI 243503	6	9	2	4	100	100
S. demissum PI 161365	7	12	4	4	42	48
S. weberbaueri PI 442703	8	10	6	6	98	96
Averages	7.3	9.1	4.2	4.3	89.0	90.7
Average difference	1.8*		0.1ns		-1.7ns	
Slow Germinating Wild Species (Experiment 4)						
S. morelliforme PI 275221	9	12	6	11	98	99
S. morelliforme PI 275222	11	20	5	9	100	96
S. multiinterruptum PI 498266	6	40	3	37	93	73
S. multiinterruptum PI 498267	5	29	3	26	99	94
S. chomatophyllum PI 243341	8	41	5	38	93	77
S. chacoense PI 473827	5	11	3	7	100	93
Averages	7.3	25.5	4.2	21.3	97.2	88.7
Average difference	18.2*		17.1*		8.5**	

[1]Rate, uniformity, and maximum difference calculations are based on maximum germination: the final average percent germination of the highest germinating treatment for each seedlot.
[2]Days elapsed from hydration to 75% germination for AC (+AC) and Control treatments.
[3]Minimum number of days within which any 75% of AC (+AC) and Control treated seeds germinated.
*significant at .05 probability level.
**significant at .01 probability level.
ns = not significant.

The response of seeds to AC was similar whether treated with a 0.5% AC suspension or by the sprinkling method, surface sterilized or unsterilized. A small but significant improvement due to AC was detected in cultivated species and cultivar-species hybrid and slow germinating seedlots which had not been treated with GA. Similarly, GA always improved germination of seeds which had not been treated with AC.

Discussion

The enhancement of seed germination can be accomplished by one or more of the following means: 1) decreasing the time between hydration and germination, 2) promoting uniformity of germination, i.e., more seeds germinating within a given time interval irrespective of the length of the post-hydration period, or 3) increasing final percent germination. The corresponding statistics used in this study were 1) rate of germination, 2) uniformity of germination, and 3) final percent germination.

The cumulative germination curves of cultivated species and cultivar-species hybrid (Experiment 2), and wild species (Experiment 3) seeds shown in Figures 1 and 2, respectively, indicate that most of the enhancement by AC in these seedlots is due to a more favorable rate of germination. This is evidenced by the similar shapes of the AC and control germination curves but with the AC curve responding two to four days sooner.

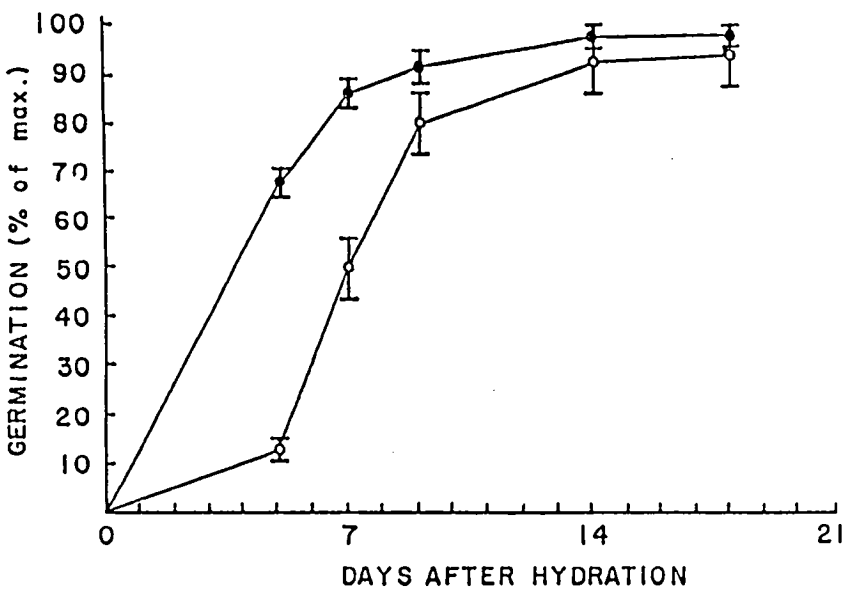

FIG. 1. Average germination of AC treated (●) and control (○) cultivated species and cultivar-species hybrid seeds (Experiment 2) over time.

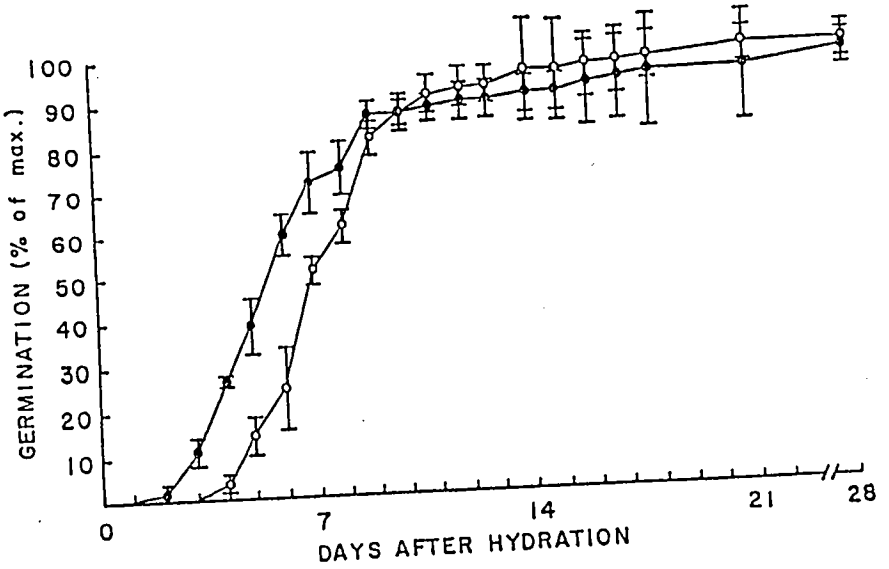

FIG. 2. Average germination of AC treated (●) and control (○) wild species seed (Experiment 3) over time.

Slow germinating species seeds (Experiment 4) had both a faster rate of germination and more uniform germination and had a higher final percent germination with AC than without it. Germination curves of AC treated seeds are similar regardless of the seed type tested (Figures 1, 2, 3). Thus, the major benefit of AC application in slow germinating seedlots resulted from causing slow, non-uniform germination to become more normal.

The significant difference in final percent germination seen in the slow germinating seedlots may not be related to the mechanism by which AC enhances germination. Regardless of which treatments are administered, one might expect maximum germination to be lower among slow germinating seedlots due to the greater chance of seeds dying during long periods in the hydrated condition. Thus any negative aspects of the germination environment would be more influential on slow germinating seeds.

Similarly, one would expect differences in uniformity of germination to be more readily detected in slow germinating seeds. AC improved uniformity scores to about five days regardless of control uniformity. Consequently slow germinating seeds with very poor control uniformity would be most likely to show significant improvements due to AC.

One model which could explain these results would involve two factors which inhibit germination, the first of which is alleviated by GA, the second by AC, with possible interaction effects. In no case was GA found to be inhibitory. The apparently universal effectiveness of GA as a promoter of

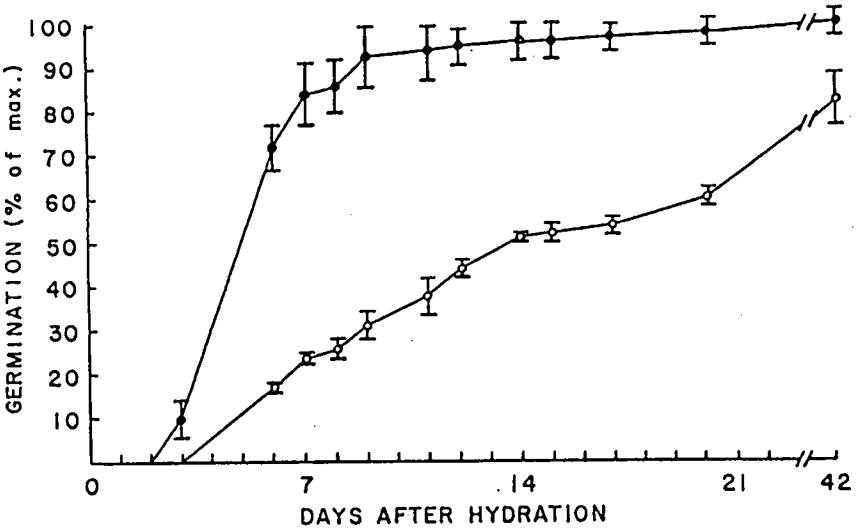

FIG. 3. Average germination of AC treated (●) and control (O) slow germinating *S. multiinterruptum* (PI 498266 and PI 498267), and *S. chomatophilum* (PI 243341) seeds over time.

potato seed germination (J. Smejkal, Potato Introduction Project, Sturgeon Bay, Wisconsin; personal communication) suggests that in most cases GA alleviates the more limiting of the two inhibitions. In some seeds (e.g., the slow germinating seedlots of Experiment 4) however, the second factor may be unusually prominent, and its alleviation by AC results in dramatic improvement in the rate, uniformity and final percent germination.

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Literature Cited

1. Anon. 1980. Seed production research for developing countries. pp. 69-72. *In:* Annual Report 1979. Centro Internacional de la Papa. Lima, Peru.
2. Joubert, D.C. and J.G.C. Small. 1982. Seed germination and dormancy of *Stipa trichotoma* (Nassella Tussock). Part 1. Effect of dehulling, constant temperatures, light, oxygen, activated charcoal and storage. S Afr J Bot 1:142-146.
3. Lam, S. 1966. Interaction of light and gibberellin on potato seed germination. Am Potato J 43:443-449.
4. Lam, S. 1968. Interaction of temperature and gibberellin on potato seed germination. Am J Bot 55:193-198.

5. Lockerman, R.H. and A.R. Putnam. 1981. Growth inhibitors in cucumber plants and seeds. J Am Soc Hortic Sci 106:418-422.

6. Simmonds, N.W. 1963. Experiments on the germination of potato seeds I. Eur Potato J 6:45-60.

7. Simmonds, N.W. 1964. The genetics of seed and tuber dormancy in the cultivated potatoes. Heredity 19:489-504.

8. Spicer, P.B. and L.A. Dionne. 1961. Use of gibberellin to hasten germination of *Solanum* seed. Nature 189:327-328.

9. Steir, H.L. 1937. The effect of certain seed treatments on the germination of recently harvested potato seeds. Proc Am Soc Hortic Sci 35:601-605.