Plant Physiol. (1973) 52, 79–81

Short Communication

Application of Chemicals in Organic Solvents to Dry Seeds[1,2]

Received for publication February 7, 1973

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ABSTRACT

Various chemicals were applied to dry seeds by means of organic solvents. The gibberellic acid-treated (1 mM) lettuce seeds (*Lactuca sativa* L.) germinated nearly 100% in the dark even after prolonged storage, and those treated with abscisic acid (1 mM or 0.5 mM) failed to germinate in the light. The seedlings emerging from morphactin-treated (1 mM) cucumber seeds (*Cucumis sativus* L.) exhibited profound changes in morphology. Different combinations of hormones applied to lettuce seeds caused a promotion or an inhibition of germination. Germination promotion or inhibition studies showed that the applied chemicals could be removed by washing with an organic solvent or water. Progressively larger amounts of chemicals were removed with increasing periods of washing. Thus the chemical appeared to penetrate the seed to some degree. The potential of the organic solvent method is discussed.

Meyer and Mayer (10) showed that the growth inhibitor, coumarin, can be applied to lettuce seeds in organic solvents and emphasized the significance of the approach to practical problems. This work led to further studies and comments by other workers (1, 13). For example, based on his studies, Anderson (1) has emphasized that coumarin is able to permeate the seed only superficially when applied as a solution in dichloromethane, and Triplett and Haber (13) attempted to show that the solvent itself is injurious when allowed to reach the embryo. No serious attempts have been made to explore the potential of the organic solvent method for applying chemicals. The significance, both physiological and practical, of the proposed method is clearly apparent from the many experiments conducted in this laboratory as a sequel to the original publication. Some of these are briefly described.

RESULTS AND DISCUSSION

Grand Rapids lettuce seeds (which fail to germinate in the dark) to which GA_3 (1 mM) in acetone (or dichloromethane) was applied germinated nearly 100% in the dark when placed in water (Table I). Seeds treated with ABA (1 mM or 0.5 mM) failed to germinate (Table I). This concentration is about 50- to 100-fold more than that required for complete inhibition of seeds treated directly with aqueous solutions of ABA. This suggests that a much higher concentration of chemicals may be required for treating seeds with organic solvent than when seeds are treated directly with aqueous solution of the chemicals. The GA_3-treated seeds germinated readily in the dark even after storage (in paper envelopes at room temperature) for as long as 20 months (Table II). Acetone, the solvent used to apply the hormone, did not alter the sensitivity of the seed. Washing the seed with acetone re-established the requirement for GA_3 for dark germination.

The duration of treatment in acetone solution of GA_3 had no effect on the ability of lettuce seed to germinate in the dark (Table III). The longer treatment time, however, required a longer washing period to reduce germination to a given degree (Table III). This would suggest that the depth of incorporation of a chemical into seed by the organic solvent method may depend on the length of time the seed is in contact with the solvent. The seeds from which GA_3 was removed by acetone washing germinated readily in the dark when the hormone was reintroduced as before. This procedure of introduction of a hormone and washing could be repeated several times without any apparent change in the germinability of the seed or light sensitivity.

In other experiments with lettuce seeds treated with ABA (0.5 or 1.0 mM), it was found that the inhibitor could be removed by washing with acetone or water (Table IV). Water was more effective than acetone in removing the inhibitor when washing was conducted without shaking. There was no significant difference (at $P = 0.05$) between water and acetone washings, however, when the seeds were shaken during washing. The reason for this difference is not known. It may be that an increased supply of oxygen during shaking inactivates the inhibitor, thereby nullifying any differences between acetone and water washing. The role of oxygen in promoting seed germination is well known (2). There was a progressive increase in germination of lettuce seeds pretreated with 1.0 mM ABA with increasing periods of washing by shaking. These results together with the data in Table III strongly indicate that the chemicals are able to penetrate the seed, at least to some extent, when applied in acetone solution. In the experiments reported here, a large quantity (50 ml) of water was used to wash the hormone-treated seeds. This may be necessary to prevent the movement of toxic chemicals to the embryo that may occur when only small volumes of water are used to wash the seed as has been pointed out by Triplett and Haber (13).

In further extension of the hormone application studies with lettuce seeds, we attempted to determine if more than one hormone, in different combinations, can be applied to lettuce

[1] This research was supported in part by a grant from the Herman Frasch Foundation and the American Seed Research Foundation.

[2] Approved by the Director of the New York State Agricultural Experiment Station as Journal Series Paper No. 2016.

Table I. *Germination of Grand Rapids Lettuce Seeds Treated with Various Concentrations of GA_3 and ABA in Acetone Solution*

Seeds were soaked in 20 ml of acetone solution of the chemicals for 24 hr and dried 30 min in a vacuum desiccator. ABA- and GA_3-treated seeds were germinated in continuous light (8 ft-c) and dark, respectively, at 25 C for 30 hr on two layers of filter paper in 2 ml of water. The results are averages of duplicate samples of 100 seeds each.

Concn	Germination	
	GA_3 (dark)	ABA (light)
	%	
1.0 mM	98	0
0.5 mM		6
0.25 mM	90	
0.1 mM	52	87
0.01 mM	14	
0.001 mM	6	
0 (— acetone)	9	98
0 (+ acteone)	12	99

Table II. *Germination in the Dark of Grand Rapids Lettuce Seeds in Aqueous Solution of GA_3 or after Pretreatment of Seeds with GA_3 in Acetone or Dichloromethane*

Seeds were treated with 1 mM GA_3 in 20 ml of acetone or dichloromethane solutions, vacuum dried, and stored at room temperature in paper envelopes. Water, acetone, and dichloromethane controls were included in each case. Seeds were germinated as described in Table I.

Time Stored	Germination (30 hr)					
	Water	Water plus GA_3 (1.0 mM)	Pretreatment in			
			Acetone	Acetone plus GA_3 (1.0 mM)	Dichloro-methane	Dichloro-methane plus GA_3 (1.0 mM)
	%					
0 day	8	98	14	97	12	93
10 days	8	98	18	98	8	99
30 days	16	99	15	99	22	100
2 months	14	93	16	98	10	97
20 months	12	89	5	94	5	92

Table III. *Dark Germination of Lettuce Seeds following GA_3 Treatment in Acetone and Washing*

Following acetone (50 ml) washing, seeds were rinsed with 10 ml of water, vacuum dried for 30 min, and germinated as described in Table I.

Washing Time	Germination (30 hr)		
	Acetone (24 hr)	Acetone plus GA_3 (24 hr)	Acetone plus GA_3 (5 min)
	%		
0 min	12	99	95
5 min	16	96	99
30 min	14	91	94
1 hr	11	81	87
5 hr	14	72	50
10 hr	9	64	28
24 hr	15	53	24
48 hr	14	27	

seeds. It is well known that treatment of several types of seeds with a number of hormonal combinations involving ABA, GA_3, or kinetin, at certain concentrations, would lead to either germination or inhibition (9). When the lettuce seeds were treated in acetone with ABA or ABA plus kinetin or ABA plus GA_3, the seeds failed to germinate in the dark (Table V). When GA_3 or a combination of GA_3 plus kinetin or a combination of GA_3 plus kinetin plus ABA was applied, the seeds germinated readily in the dark (Table V). The combinations causing inhibition or promotion of lettuce seed germination were already known to us from studies with seeds treated with aqueous solutions of hormones (8). These studies suggest that a number of chemicals with different biological properties can be applied to a seed at the same time.

Other chemicals were also applied to lettuce and other seeds in acetone. For example, cycloheximide completely inhibited lettuce seed germination at 2.5 mM, while 6-methylpurine at 5.0 mM gave 81% germination. The antibiotic actinomycin D applied in acetone at 0.01 M had no effect on lettuce seed germination. Mature seeds presumably contain stored messenger RNA which functions in germination (3, 5). Actinomycin D at 0.01 M or at lower concentration is toxic to bacteria, fungi, and other microorganisms (6, 12). Thus, we may have a means

Table IV. *Germination in Light of Lettuce Seed Treated with ABA (5 min)*

Seeds were washed in 50 ml of acetone or water (100 seeds/250 ml beaker) with or without shaking. Shaking was conducted on a metabolic shaker. Seeds were rinsed and germinated as described in Table III.

Washing Time	Germination (30 hr)				
	No shaking		Shaking		
	Acetone	Acetone plus ABA (0.5 mM)	Acetone	Acetone plus ABA (0.5 mM)	Acetone plus ABA (1.0 mM)
	%				
Acetone					
0 min	98	0	98	0	0
5 min	100	11	100	75	0
20 min	100	33	98	85	0
1 hr	98	28	98	93	17
6 hr	100	26	97	87	75
Water					
5 min	99	57	99	98	0
20 min	97	44	99	80	0
1 hr	100	80	98	92	15
6 hr	100	83	100	97	78

Table V. *Dark Germination of Lettuce Seeds Treated in Acetone with Various Combinations of Hormones*

The seeds were treated and germinated as described in Table I, except that germination period was 48 hr.

Hormonal Combinations	Germination
	%
GA_3 (0.25 mM)	91
Kinetin (0.5 mM)	17
ABA (0.2 mM)	2
ABA (0.2 mM) + kinetin (0.5 mM)	6
ABA (0.2 mM) + GA_3 (0.25 mM)	3
GA_3 (0.25 mM) + kinetin (0.5 mM)	97
ABA (0.2 mM) + kinetin (0.5 mM) + GA_3 (0.25 mM)	76
Acetone control	15

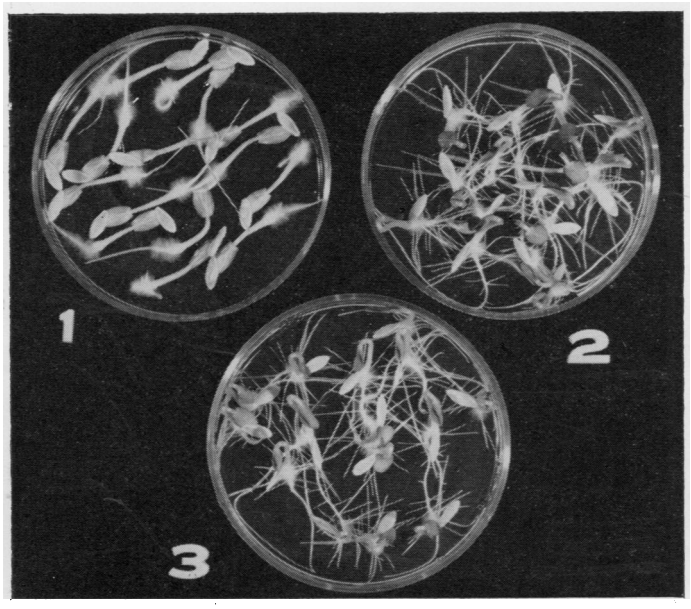

FIG. 1. Cucumber seeds were treated for 5 min with acetone solution of morphactin, IT 3456 (10^{-3} M). The seeds were vacuum dried for 30 min prior to germination for 3 days on 0.75% Bactoagar at 25 C in the dark. 1: Morphactin-treated; 2: acetone control; 3: water control.

here for keeping certain microorganisms from infecting the seeds in storage. A proliferation of microorganisms under conditions of high humidity and temperature in stored seeds is not uncommon. Under conditions of storage, we do not expect the dilution of the applied chemical (in seeds) to any considerable extent. Other chemicals with no apparent effect on germination may be similarly used. Thus, the organic solvent application method is not limited to germination inhibitors or promotors (10).

In our attempt to seek more diversified uses of the solvent-chemical application method, we used a morphactin,[3] the growth-regulating property of which is well known (7, 11). The seedlings emerging from morphactin-treated cucumber seeds exhibited profound changes in morphology (Fig. 1). Among the many altered features, these seedlings had no lateral roots. Some of the morphactin-induced changes persist even in mature plants obtained by transplanting the germinated seedlings. The physiological significance of these changes has not been estimated.

The results described here indicate that many chemicals, such as antibiotics, growth regulators, and chemical protectants against fungi, insects, bacteria, and rodents, can be conveniently applied to seeds, the seeds stored in this fashion for prolonged periods of time, and the chemicals removed or allowed to remain in the seed, depending on the nature of the chemical and the planting requirement of the seed. It appears that superficial retention of chemicals may be more suited to our needs than deep permeation. The ease of removal of a chemical, not deeply infused in seed tissues, may be fundamental to the success of the method described in the original report (10).

[3] Morphactin IT 3456 used in the experiment is methyl-2-chloro-9-hydroxy-fluorene-(9)-carboxylate and was obtained through the courtesy of E. Merck AG, Darmstadt, West Germany.

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