

If the amount of Benlate applied to the seed is taken into consideration one would expect very little to be present in the seeds of the mature plant. It has been shown, however, that Benlate is absorbed and translocated in corn tissue (3) so the possibility, however remote, of tissue residues in sweet corn did exist.

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EFFECT OF GROWTH REGULATORS ON STORAGE LIFE OF ONION SEED¹

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Abstract. The objective of this research was to improve the storage life of onion seed with growth regulators. Seed of 3 onion cultivars were treated with two concn of abscisic acid (ABA), gibberellic acid (GA), or kinetin (Kn) dissolved in dichloromethane (DCM). The seeds were stored in sealed jars at 2 temp (10 and 35°C) and at 2 seed moisture levels (10 and 20%). Germination percentage, rate, and ATP content were measured at 0, 3, and 9 months. GA and Kn had little effect on viability of 'Fiesta' and 'Premier' under any storage condition. However, germination in 'Elite', which lost the most viability and vigor, was improved during storage by treatment with Kn. Abscisic acid lowered germination and vigor in all cultivars. Onion seed lost viability and vigor quickly under adverse storage conditions of high temp and moisture. With optimum conditions, increased storage time from 3 to 9 months had no effect on onion seed germination and vigor. Seed ATP was higher in seed treated with growth regulators and did not always relate to germination and other vigor measurements.

Onion seed has been regarded as having a short storage life, generally from 6 to 24 months under normal conditions (9). Rocha (15) found that onion seeds with 13 to 15% moisture were nonviable after 3.5 months storage at 21°C. However, seeds with moisture contents of 6.5 to 9.2% lost no viability after 7.5 months of sealed storage.

Growth regulators have an effect on seed germination. Abscisic acid (ABA) is one of the principal inhibitors involved in seed and bud dormancy in many species (2, 17, 18). Gibberellic acid (GA) promotes seed germination in a number of species. GA stimulates *de novo* synthesis of α -amylase in barley aleurone layers, a prerequisite to complete starch breakdown and germination (12). GA can substitute for red light to overcome dormancy in lettuce (6). Cytokinins are believed to promote seed germination when certain growth inhibitors are present (7). Kinetin (Kn), a synthetic cytokinin, overcame thermodormancy in lettuce

seed (14). Kn pretreatment improved germination of new and old lettuce seed at 30°C (10).

Organic solvents, such as dichloromethane (DCM), have been used to incorporate chemicals into seeds without initiating germination. Meyer and Mayer (11) showed that the growth inhibitor, coumarin, can be applied to lettuce seeds with organic solvents, such as DCM. They proposed that the solvent helped the chemical penetrate deeply into the seed. Light-requiring lettuce seeds, treated with GA dissolved in acetone or DCM, had nearly 100% germination in the dark even after prolonged storage, and those treated with ABA failed to germinate at all (8). Several chemicals, such as fungicides, insecticides, and antibiotics, were applied to dry seeds of various species via organic solvents and preserved seed quality as determined by the germinating capability of the seed or ATP content (20).

The experiment that follows was designed to determine the effect of the growth regulators Kn, GA, and ABA on extending the storage life of onion seed stored under various conditions for 9 months.

Materials and Methods

Onion seeds (*Allium cepa* L. cvs. Fiesta, Elite, and Premier) were obtained from Stokes Seeds Ltd. The seed was produced in 1974 and was not treated with pesticides.

Seeds were soaked for one hour in dichloromethane containing abscisic acid (.25 and .5 mM), gibberellic acid (.5 and 1.0 mM), or kinetin (.5 and 1.0 mM). The seeds were then drained and dried in an oven at 35°C for one hour. Weighed amounts of seed were placed in small mason jars and seed moisture contents were equilibrated to 10 and 20% (15). Duplicated treatments were placed in incubators at either 10 or 35°C. Treatments were sampled at 0, 3, and 9 months for ATP content, germination percent, and germination rate.

ATP content was determined on 25 seeds from each sample according to the procedure of Ching and Danielson (3). All samples were imbibed with distilled water for 4 hours, then crushed and extracted with 10 ml of boiling distilled water for 10 minutes. After cooling, 5 ml of extract were diluted with 5 ml of buffer containing 0.05 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) and 0.05 M magnesium acetate.

The ATP assay was previously described (19). Freeze-dried firefly extract containing luciferin-luciferase was reconstituted by adding 5 ml of ice-cold distilled water which resulted in an enzyme preparation containing 0.05 M potassium arsenate and 0.02 M magnesium sulfate, pH 7.4. Light

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emission from the ATP-enzyme preparation was recorded using an Aminco Chem-Glo Photometer. The ATP concentration was determined by comparison to a standard curve.

Germination and seedling vigor measurements were obtained by placing 10 seeds on moist paper towels. The towels were rolled up and placed in an incubator at 25°C. Germination counts were made after 3, 5 and 7 days. Germination rate was calculated according to the formula of Shmueli and Goldberg (16). Radicle length and seedling fresh and dry weights were measured after 10 days on the 9 month sample only.

Results

ABA significantly reduced germination percent and rate in all 3 cultivars (Table 1). Of the 3 cultivars used, 'Fiesta' usually had the highest germination percent, germination rate, and ATP content, but these indices were not improved by chemical treatment. Although 'Elite' produced more ATP than 'Premier', its germination percentage and rate were lower. GA improved germination percent and rate of both 'Elite' and 'Premier' after 3 months storage. In 'Elite', where vigor appeared to decrease at the fastest rate, application of Kn significantly increased the germination

percentage, germination rate, and ATP content after 3 and 9 months storage. Averaged over all cultivars, the seed did not lose viability or vigor between the 3 and 9 month sampling times. This indicated that the weakest seed died off rapidly, within 3 months, and that the more vigorous seed remained viable.

All seeds stored at 10% moisture had greater viability and vigor than seeds with 20% moisture (Table 2). None of the growth regulator treatments improved germination percentage and rate of 'Fiesta' and 'Premier' at either seed moisture level. However, Kn improved germination percentage and rate of 'Elite' stored at 10% moisture. At 20% seed moisture, the viability was reduced greatly and the growth regulators did not improve germination significantly. ABA decreased germination percentage and rate of all cultivars at both moisture levels. ABA did not prevent all seeds from germinating and the ATP levels in both viable and nonviable seeds remained relatively high. Application of GA increased ATP in all cultivars. Kn also increased ATP content of all seed except 'Elite'. ATP content was 2 to 3 times higher in seeds stored at 10% moisture than those stored at 20%.

Seed stored at 10°C had greater germination and vigor than those stored at 35°C (Table 3). The Kn and GA growth regulator treatments did not improve germination in

Table 1. Effects of storage time and growth regulators on germination and seedling vigor of 3 cultivars of onion seed.*

Time (months)	Treatment	Germination (%)			Germination Rate Index			ATP (nmoles/seed)		
		Elite	Fiesta	Premier	Elite	Fiesta	Premier	Elite	Fiesta	Premier
0		100	97	77	4.3	3.7	3.1	29.0	68.3	15.1
3	Control	40c [†]	63a	51bc	1.2c	3.1a	2.3b	6.2f	6.8f	2.8f
	DCM	46b	58b	49cd	1.6b	2.6b	2.2bc	9.0d	9.3e	2.5f
	Kn(.5mM)	51a	48d	53b	2.0a	2.2cd	2.4ab	11.3b	17.1a	7.9c
	Kn(1.0mM)	53a	53c	46d	1.9a	2.4bc	2.0c	7.0e	10.9d	4.5e
	GA(.5mM)	50a	56bc	58a	1.9a	2.5b	2.6a	18.9a	16.8a	11.1b
	GA(1.0mM)	39c	46d	49cd	1.5b	2.1d	2.3b	10.2c	12.7c	10.8b
	ABA(.25mM)	19d	34f	39e	0.6d	1.3e	1.5d	10.8bc	14.9b	12.7a
	ABA(.5mM)	16d	38e	21f	0.4d	1.3e	0.8e	7.2e	10.2d	6.2d
	9	Control	36bc [†]	60b	56a	1.0e	3.2a	2.4ab	13.1b	14.2b
DCM		33c	71a	40c	1.4d	3.2a	2.2bc	11.1c	11.3d	3.8g
Kn(.5mM)		45a	45d	40c	1.9ab	2.0d	2.0cd	15.9a	14.5ab	16.6a
Kn(1.0mM)		46a	59b	48b	1.7bc	2.8b	2.1cd	10.1d	12.7c	9.6c
GA(.5mM)		34c	60b	39c	2.0a	2.8b	2.5a	13.5b	15.8a	9.6c
GA(1.0mM)		39b	59b	46b	1.6cd	2.8b	2.2bc	7.8e	10.3e	5.6e
ABA(.25mM)		26d	50c	23d	1.0e	2.4c	1.9d	10.5cd	15.2a	11.3b
ABA(.5mM)		18e	35e	25d	0.7f	1.7e	1.2e	5.4f	10.0e	4.6f

*Data averaged over all storage temp and moisture contents.

[†]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 2. Effects of storage moisture content and growth regulators on germination and seedling vigor of 3 cultivars of onion seed.*

Moisture (%)	Treatment [†] (0 time)	Germination (%)			Germination Rate Index			ATP (nmoles/seed)		
		Elite	Fiesta	Premier	Elite	Fiesta	Premier	Elite	Fiesta	Premier
		100	97	77	4.3	3.7	3.1	29.0	68.3	15.1
10	Control	59c [‡]	86a	79a	1.8c	4.3a	3.4a	14.1c	16.1c	8.0c
	DCM	64b	90a	63c	2.6b	4.0b	3.4a	15.5b	16.2c	5.3d
	Kn	77a	66c	63c	3.1a	2.9d	2.8b	16.6b	18.4b	13.4a
	GA	68b	73b	70b	3.0a	3.4c	3.5a	18.4a	19.1a	13.3a
	ABA	32d	56d	44d	1.2d	2.4e	1.9c	12.7d	16.5c	11.7b
20	Control	18ab [‡]	36a	29ab	0.5ab	1.9a	1.4a	5.2bc	4.9b	1.5b
	DCM	15b	39a	26b	0.3bc	1.7a	0.9b	4.6bc	4.4b	1.0b
	Kn	21a	37a	31a	0.7a	1.8a	1.5a	5.6b	9.4a	5.9a
	GA	14b	38a	27ab	0.5ab	1.7a	1.3a	7.6a	8.6a	5.3a
	ABA	8c	23b	16c	0.2c	1.1b	0.8b	4.3c	8.7a	5.8a

*Data averaged over all storage temp and times.

[†]Data averaged over all concn.

[‡]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 3. Effects of storage temperature and growth regulators on germination and seedling vigor of 3 cultivars of onion seed.*

Temperature (°C)	Treatment [†] Control (0 time)	Germination (%)			Germination Rate Index			ATP (nmoles/seed)		
		Elite	Fiesta	Premier	Elite	Fiesta	Premier	Elite	Fiesta	Premier
		100	97	77	4.3	3.7	3.1	29.0	68.3	15.1
10	Control	58a*	84a	65ab	1.8bc	4.3a	3.1ab	12.4c	13.5b	5.8b
	DCM	49b	84a	61b	1.7c	4.0b	2.9b	12.2c	12.2c	3.8c
	Kn	62a	79b	68a	2.2a	3.7c	3.2a	13.6b	18.8a	14.2a
	GA	49b	80ab	65ab	2.0ab	3.8bc	3.0ab	17.0a	19.6a	13.1a
	ABA	34c	58c	43c	1.1d	2.5d	2.0c	11.4c	19.9a	13.7a
35	Control	19c*	39b	43a	0.4c	2.0a	1.7a	6.9b	7.6a	3.7b
	DCM	30b	45a	28bc	1.2b	1.7b	1.4b	7.9ab	8.3a	2.5b
	Kn	36a	24d	26c	1.6a	1.0d	1.1c	8.6a	5.4b	5.1a
	GA	32ab	32c	31b	1.6a	1.4c	1.9a	8.2a	8.1a	5.5a
	ABA	7d	21d	16d	0.2c	1.0d	0.7d	5.6c	5.3b	3.7b

*Data averaged over all storage moisture contents and times.

[†]Data averaged over all concentrations.

*Mean separation in columns by Duncan's multiple range test, 5% level.

'Fiesta' and 'Premier' but Kn and GA did improve the germination and vigor of 'Elite' at 35°C. ATP content was generally higher in seed treated with Kn or GA as compared to untreated seed.

Additional seedling vigor measurements made after 9 months included radicle length and fresh and dry weights (Table 4). 'Fiesta' had the greatest average radicle length and seedling fresh weight. Seedling dry weights were equal for all 3 cultivars. GA treatment at either concn significantly increased average radicle length above those of untreated seed, whereas ABA inhibited radicle lengths. Only ABA reduced the fresh weights of the seedlings, whereas Kn and ABA increased the seedling dry weights slightly. Low storage temp (10°C) and low seed moisture content (10%) maintained better viability and seedling vigor after 9 months storage.

Discussion

Barton (1) observed that onion seed stored at laboratory temp and high moisture content (16 to 22%) exhibited a drastic drop in germination after one year. Onion seed

Table 4. Effects of growth regulators, cultivars, temperature, and seed moisture content on germination and seedling vigor of onion seed stored for 9 months.

Cultivars	Germination (%)	GRI	ATP (nmoles/seed)	RLA (cm)	Fresh wt. (mg)	Dry wt. (mg)
Elite	35c*	1.4c	10.9b	1.00c	18.3b	2.3a
Fiesta	55a	2.6a	13.0a	1.85a	22.8a	2.5a
Premier	41b	2.1b	8.5c	1.28b	18.2b	2.5a
Treatment						
Control	51a*	2.2ab	11.3c	1.74b	22.0a	2.1de
DCM	48a	2.3a	8.7d	1.61bc	20.1a	1.9e
Kn(.5mM)	51a	2.2ab	10.8c	1.35d	22.2a	2.6abc
Kn(1.0mM)	43b	2.0bc	15.7a	1.57c	22.3a	2.7abc
GA(.5mM)	48a	2.2ab	7.9e	2.03a	21.0a	2.3cde
GA(1.0mM)	44b	2.4a	13.0b	1.93a	21.3a	2.5bcd
ABA(.25mM)	26d	1.2d	6.7f	0.36e	12.2c	3.0a
ABA(.5mM)	36c	1.8c	12.3b	0.42e	16.6b	2.8ab
Temp. (°C)						
10	62a*	2.8a	13.4a	2.10a	22.0a	2.5a
35	29b	1.3b	6.1b	0.65b	13.5b	2.0b
Moisture (%)						
10	66a*	2.9a	14.4a	1.83a	19.3a	2.3a
20	25b	1.1b	5.5b	0.92b	19.4a	2.4a

*Mean separation in columns by Duncan's multiple range test, 5% level.

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stored at 6 to 22% moisture for one year at 5°C had 80% germination. In the present study, germination and vigor of all seed changed from the 0 to 3 month period, but not from the 3 to 9 month sampling time. Viability was reduced by as much as 50% after the first 3 months of storage. Seed stored at 10% moisture had up to 3 times greater germination and vigor than seed stored at 20%. A similar pattern persisted between the low (10°C) and high (35°C) temp treatments. Thus, storage life may be prolonged at 10°C and 10% moisture, but complete loss of viability may occur at 35°C and 20% moisture.

Certain plant growth regulators increase germination. Puls and Lambeth (13) noted increased germination rate of 10-year-old tomato seeds by soaking them in a combination of GA, Kn, and potassium nitrate. However, total germination percent was not improved. In the present experiment, none of the growth regulators tested, GA, Kn, or ABA, increased germination of 'Fiesta' or 'Premier' onion seed. Germination percentage and rate were, however, significantly increased in 'Elite' by Kn treatment. 'Elite' lost viability and vigor when stored under adverse conditions faster than either of the other 2 cultivars examined. Treatment with ABA reduced germinability and vigor of all cultivars under all conditions. This decrease with ABA treatment agrees with previous work. Halloin (4) inhibited the germination of cottonseed with ABA, while Khan *et al.* (8) obtained no germination of light-treated lettuce seed after soaking in ABA.

The ATP content of the onion seed increased when GA, Kn, or ABA were used, especially at the lower concn. This suggested that those compounds may have had some type of biochemical effect on the seed. Puls and Lambeth (13) believed that the combination of GA; Kn, and potassium nitrate accelerated germination by correcting enzymatic and substrate deficiencies at the initial stages of germination. However, ABA inhibited RNA synthesis in bean axis (21) and reduced α -amylase in barley aleurone (5). In the present study, the increase in ATP did not coincide with increases in germinability in any seed except 'Elite', and this occurred only with Kn treatments.

Combinations of GA and Kn at different concn were not investigated. Possibly, the optimum mixture of these 2 compounds might further increase the effectiveness of growth hormones on seed preservation.

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EFFECT OF PRESOWING SEED TREATMENTS ON GERMINATION OF LETTUCE SEED AT HIGH TEMPERATURE¹

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Abstract. The potential use of presowing treatments to prevent high temperature induced dormancy in lettuce (*Lactuca sativa* L.) seed was evaluated. Seeds of the cultivar 'Minetto' were soaked in water or 1% sodium phosphate (Na_2HPO_4) soln at 2 temp (15 and 25°C) for 1, 3, 6 or 12 hours. Germination tests were done immediately after each treatment at 35°C on moist filter paper and after redrying the seed. When the seeds were redried, germination tests were done at 20°C and 35°C in petri dishes, and, in soil at 35°C. At 20°C seed germination was high (92 to 97%) regardless of treatment. However, at 35°C germination occurred only in presoaked seeds. Redrying the seed was necessary in order to obtain maximum germination. For the best soak treatments, soaking at 15°C was more effective than at 25°C. Sodium phosphate was only more effective than water when seeds were soaked at 15°C. The optimum duration of soak was 3 and 6 hours in petri dishes and 6 hours in soil.

In many plant species the optimum germination temp range coincides with the optimum temp range for plant growth (7). In lettuce, plant growth can continue above the upper temp for optimum growth (15 to 25°C), however, germination may be completely inhibited at temp only slightly above the optimum germination temp range (3). For most lettuce cultivars, the "cut-off point" for normal germination is about 27 to 30°C. According to Sharpless (14), the germination of even the most heat-tolerant cultivars is seriously reduced when the temp is held constant at

30°C during inhibition and is completely inhibited at 35°C. Evenari (2) described this phenomenon as "heat dormancy". McCoy and Harrington (10) suggested that older seeds were desirable for warm weather planting because they were less susceptible to high temp dormancy. However, vigor of seedlings from older seeds was reduced.

A number of growth regulators affect lettuce seed germination. Reynolds and Thompson (11) reported that the high temp "cut-off point" may be shifted upwards by applying kinetin or downwards by applying abscisic acid. Kinetin was not as effective in the absence of light (12). Heydecker and Joshua (5) reported good results by soaking the seeds for 15 min in a 70 to 100 mg/l⁻¹ solution of kinetin dissolved in dichloromethane. This system permitted kinetin to be absorbed by the seeds without initiation of germination. Treated seeds germinated at considerably higher temp than did untreated seeds up to one year after treatment. The use of dichloromethane, however, is dangerous since the chemical is flammable and the fumes are toxic when inhaled. Ells (1) and Koehler (8) used fairly strong salt solutions to "vigorize" seeds. More recently, seed treatments have been successfully used on a number of species, whereby seeds are brought to the brink of germination in water or an osmotic medium. This procedure called "seed priming" (4, 9, 13) allows seeds to germinate more rapidly under unfavorable environmental conditions. In the present work, the effect of presoak treatments in water or sodium phosphate solutions at different temp for various durations was evaluated to determine if high temp dormancy in lettuce seed could be circumvented.

Materials and Methods

Non-photosensitive lettuce seeds (*Lactuca sativa* L. cv. Minetto) were soaked in water or 1% sodium phosphate (Na_2HPO_3) soln at 15 and 25°C for 1, 3, 6 and 12 hours. Each treatment consisted of 0.5 g of seeds and was replicated twice. The expt was arranged as a split plot design with soak the main plot and temp and time the subplot treat-

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