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Priming of Watermelon Seeds for Low-temperature Germination[1]

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Abstract. **Watermelon seeds (*Citrullus lanatus* (Thunb.) Matsum. & Nakal cv. Sugar Baby) require high temperatures to germinate successfully. Under light conditions germination is inhibited at 20°C and lower and in the dark below 15°. The cause of the light inhibition at 20° lies in the embryo proper. Imbibition at 0° (up to 10 days) did not cause any apparent damage to the seeds, which germinated freely upon transfer to optimal temperature. Watermelon seeds could be primed for low-temperature germination by pretreatment at 30° or 35° or by imbibing the seeds in salt solutions prior to sowing. Inorganic osmoticum was superior to organic osmoticum. Differences in the efficacy of the priming existed among the different salt solutions tested. The best treatment was 2% or 3% KNO_3 for 6 days. NH_4NO_3, $NaNO_3$, $Ca(NO_3)_2$ and KCl had similar effectiveness. After priming, seeds could be kept in dry storage for long periods (checked up to 20 weeks). Emergence studies confirm the effectiveness of the priming treatment for low-temperature germination of winter-grown watermelon.**

The origin of the watermelon plant is apparently in Tropical Africa and the plant has evidently been cultivated for many centuries in the Mediterranean Basin. The cultivated watermelon plant is known to require a long period of warm, preferably dry weather for good growth and development (16). As other cucurbitis, watermelon seed requires high temp for successful germination, with a soil temp of 35°C being optimum for its germination (8).

Watermelon cultivation procedures in Israel have changed considerably in the last decade. Sowing dates were advanced to Dec.-Jan. (instead of the usual late-spring dates) and harvesting is started in the first week of May. Germination and emergence of the seedlings in the winter is very slow (up to 1 month) and not uniform compared with the rapid (up to a week) and uniform emergence in the summer cultivation.

Various types of seed pretreatment have been suggested for low-temp germination of thermophillic seeds (7, 9, 12, 15). This study was undertaken to find a successful pretreatment for watermelon seeds which would enable them to germinate at low temp. The treatment should be simple to apply, its effect should be retained in the seeds upon redrying, and during the following dry storage and reimbibition.

Materials and Methods

'Sugar Baby' watermelon seeds, were purchased from Hazera Seed Co., Israel, and tested within 1 year of production.

Germination tests were carried out in thermostatically controlled incubators (±0.2°C) illuminated by a mixed cool white fluorescent (Atlas) and incandescent (Philips Argenta) light source providing 4.5 – 6.0 klx at dish level. Dark treatments were either "continuous" or "interrupted." In the "interrupted dark" treatments the seeds were exposed to 2-5 min of light whenever they were counted in the laboratory. In the "continuous dark" treatments a new set of dishes was counted each time and then discarded. Whenever "dark treatment" is mentioned in the text, the reference is to "interrupted dark." Alternating temp were in cycles of 12 hr each, the higher temp (30°C) in the cycle being between 8 AM and 8 PM.

The involvement of the seed coat in the light inhibition of watermelon seed germination was studied by decoating the dry embryo (fully or around the embryonic axis only – "half"), puncturing the coat with a needle (at the cotyledon or laterally), or splitting the coat laterally.

The osmoticum pretreatments were performed by incubating the seeds in aerated solutions at room temp in the light. After the pretreatments the seed were washed thoroughly and transferred to 15°C in the dark. The polyethylene glycol (PEG-6000, Carbowax-6000) was obtained from BDH Chemicals Ltd., England. The osmoticum solutions were prepared fresh for each experiment.

The relative humidity pretreatments were achieved by equilibrating the dry seeds at 20°C for 1 or 2 weeks above salt solutions which maintain a constant known humidity in the range of 90% to 100% (11).

Germination tests were replicated 4 times with 30 or 40 seeds per replication, in 5 or 7 cm Petri dishes, respectively, on a single layer of Whatman No. 1 filter paper moistened with 2 or 2.5 ml of deionized water. When the germination tests were performed at high temp (25°C and above), the dishes of each treatment were enclosed in a transparent polyethylene bag to prevent evaporation.

Seeds were considered to have germinated when the radicle protruded from the seed coat. For seedling emergence studies the seeds were sown 1 cm deep in a 1 peat moss:1 vermiculite mixture in polystyrene flats. The flats were moistened with water and placed in an unheated greenhouse. Black polyethylene (0.1 ml) was used for mulching. The polyethylene mulch was removed 3 days after sowing, when emergence started. The temp under the mulch at the time of the experiment were in the range of 10° – 26°C, compared with 6° – 18° in the unmulched control flats. Straightening of the hypocotyl hook was considered to constitute seedling emergence. The results presented are from experiments which were repeated at least twice.

Results and Discussion

Watermelon seeds germinated fully under a wide range of constant temp (15°-35°C), provided the imbibed seeds were kept in darkness. When the imbibition in dark was interrupted by daily short irradiations, the germination at 15° was slower and the final germination percentage lower. Continuous light completely inhibited the germination of watermelon seeds at 20° or lower (Fig. 1). In a few instances some germination took place at 20° in light but it was always greatly inhibited. The rate and the final germination percentages in the range of

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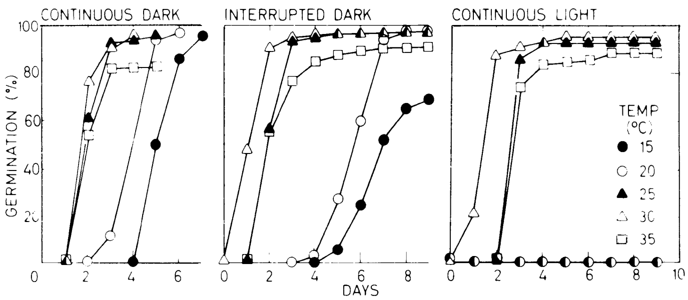

Fig. 1. Effect of temp on the germination of watermelon seeds under various light regimes.

20^o to 35^o were similar in all experiments performed. Irrespective of the light treatment, no germination of watermelon seed occurred at constant temp below 15^o. In some experiments the germination of watermelon seeds at 15^o in "interrupted light" regime reached over 90% in 10 days, whereas in others the germination was nil. It is assumed that one reason for this great variance could be temp changes of $\pm 1^o$ which occurred during the imbibition period.

It was concluded that the optimum constant temp for the germination of watermelon seeds is 30^oC (Fig. 1). At 35^o the germination percentage was somewhat lower, but the few seeds (10-20%) that did not germinate had not undergone thermodormancy and were able to germinate readily upon transfer to 25^o. The same holds true for seeds which were imbibed at suboptimal temp (5^o-15^o) for 10 days. They germinated fully within 2 days after being transferred to 25^o in the dark, irrespective of whether the preimbibition had taken place in the light or dark. Hence, lack of germination at low temp (5^o-15^o) did not induce any dormancy or cause any harm to the imbibed seeds. Moreover, because of the high rate of germination at 25^o, no increase in the rate of germination could be detected when compared with the appropriate control seeds. Therefore, it can be concluded that the block to germination imposed by low temp and/or light takes place at the very early stages of the sequential germination processes.

In several thermophilic seeds it has been shown that imbibition at low temp brought about "chilling injuries" and as the temp were lowered and their duration prolonged, the effect was more pronounced and irreversible (3, 15). A 0^oC chilling pretreatment did not hinder the germination processes in watermelon seeds at optimal temp (Table 1). When the pretreatment

Table 1. The effect of dark imbibition at 0^oC on subsequent germination of watermelon seeds at optimal temp.

Pretreatment		% germination at 30/20[z]			
		Day			
Days at 0^oC	Medium[y]	1	2	3	5
0	H_2O	0	88 ± 3	96 ± 2	98 ± 1
	KNO_3	1 ± 1	98 ± 1	98 ± 1	99 ± 1
2	H_2O	0	84 ± 2	92 ± 2	95 ± 2
	KNO_3	17 ± 2	94 ± 2	98 ± 2	98 ± 1
4	H_2O	0	82 ± 4	94 ± 2	98 ± 1
	KNO_3	48 ± 11	96 ± 1	99 ± 1	100
6	H_2O	0	84 ± 2	93 ± 1	96 ± 1
	KNO_3	52 ± 5	96 ± 2	98 ± 1	98 ± 1
8	H_2O	0	77 ± 2	91 ± 3	94 ± 3
	KNO_3	43 ± 1	97 ± 1	99 ± 1	99 ± 1
10	H_2O	0	71 ± 2	95 ±1	98 ± 1
	KNO_3	33 ± 5	94 ± 2	99 ± 1	99 ± 1

[z]Daily alternation 30^oC/12 hr and 20^o/12 hr.

[y]Distilled water or 2% KNO_3. The germination at optimal temp was in distilled water.

Table 2. The effect of removal, puncturing or splitting of the seed coat on subsequent germination of watermelon seed.

	% germination at 20^oC	
Treatment[z]	Light	Dark
Control	0	100
Decoated	0	100
Half-decoated	0	100
"Punctured" cotyledon	20	90
"Punctured" lateral	20	100
"Lateral-split"	10	90

[z]Each treatment consisted of 10 seeds.

at 0^o was performed in 2% KNO_3 solution, germination at optimal temps was advanced.

Scarification treatments did not affect the dark germination at 20^oC nor did they overcome the light inhibition (Table 2). When the "light-inhibited" seeds were transferred after 10 days at 20^o to 25^o (dark), they germinated fully in 2 days. It can be assumed that the cause of the light inhibition lies in the embryo itself and that the treatments did not interfere with normal germination in the dark.

Watermelon seeds imbibed in optimal conditions (30^oC in the dark) for various periods of time (up to 24 hr) germinated freely after being transferred to low temps and darkness. A minimal period of 18 hr of preimbibition at 30^o is needed to bring about germination at 10^o. After 24 hr of high-temp preimbibition, over 90% of the seeds germinated at 10^o but two-thirds of the seeds already had germinated within the period of 18 to 24 hr at 30^o (Fig. 2a). Pretreatment periods of less than 18 hr were ineffective for subsequent germination at 10^o. On the other hand, germination at 15^o was enhanced by imbibition of 6 hr at 30^o, and after 18 hr of pretreatment over 80% of the seeds germinated within 24 hr (Fig. 2b). Pretreatment at 25^o and 35^o gave similar results, the only significant difference being that 18 and even 24 hr at 25^o were not enough to bring about any germination at 10^o.

Until "fluid-drilling" (2, 6) becomes feasible in agricultural practices, the pretreated seeds have to be dried to suit common field-sowing practices. Hence it would be crucial that the dry seeds retain their ability to germinate at low temperatures when reimbibed. In other words, the seeds should be able to "remember" and "store" whatever changes took place during the pretreatment period.

When seeds of watermelon were imbibed at 30^oC for 18 hr and thereafter dried (at room temp in the light) for periods of up to 8 days and reimbibed at 15^o, they germinated far ahead of the control seeds (Table 3). Although there were differences in the percentage of undried and dried primed seeds which

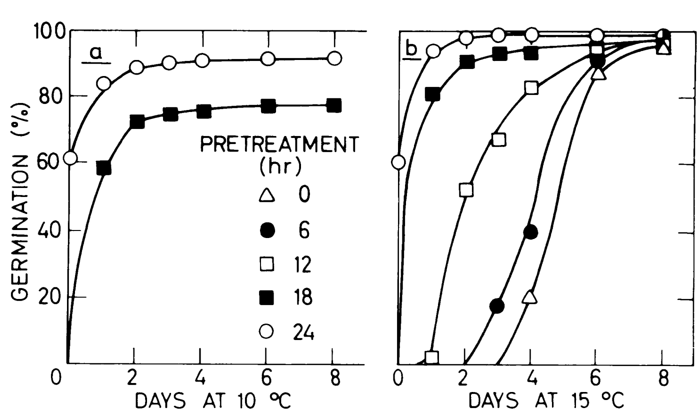

Fig. 2. The effect of priming watermelon seeds at 30^oC in darkness for various durations on subsequent germination at suboptimal temp (10^o and 15^oC).

Table 3. The effect of drying of "optimal-temperature-primed"[z] watermelon seeds for various periods, on the subsequent germination at suboptimal temperature (15°C in darkness).

Duration of dry storage (days)	% germination at 15°C		
	Day		
	1	3	4
0	76 ± 5[y]	88 ± 3	90 ± 3
1	63 ± 4	73 ± 6	81 ± 6
2	65 ± 2	79 ± 3	90 ± 3
4	58 ± 5	78 ± 4	81 ± 3
8	29 ± 2	83 ± 3	85 ± 2
Unprimed seeds	0	0	18 ± 3

[z]Priming at 30°C in darkness for 18 hr.
[y]±SE.

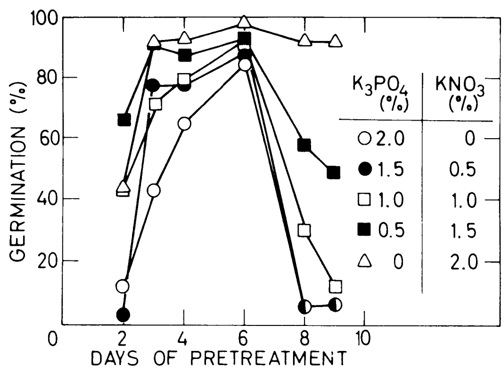

Fig. 3. The effect of duration of different salt pretreatments of watermelon seeds on their subsequent dark germination at 15°C for 10 days (No germination of untreated seeds occurred within 10 days at 15°C).

germinated after 24 hr, these differences disappeared during the following days. It can be concluded that the effects of pretreatment under optimal germination conditions remain unaffected after 8 days in the dry stage.

The possibility of "high salt priming" was well established for tomato, onion and other seeds (7, 9, 10). While no germination of the control unprimed seeds occurred within 10 days of imbibition at the low temp all the K_3PO_4/KNO_3 combinations examined induced germination at low temp (Fig. 3). The optimum duration of the pretreatment was found

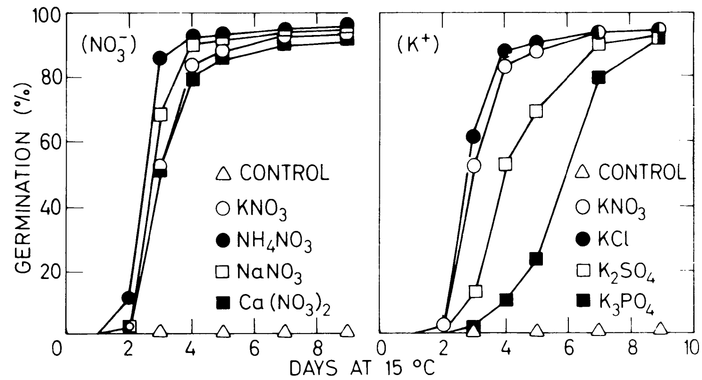

Fig. 5. The effect of priming watermelon seeds with different salt solutions, on the subsequent germination at suboptimal temp in darkness.

to be 6 days and the best salt concn was 2% KNO_3 solution. The superiority of the 2% KNO_3 pretreatment is shown also by its effectiveness in hastening germination and increasing the daily rate of germination. No significant differences were found when the effectiveness of K_3PO_4 was compared with that of K_2HPO_4 and KH_2PO_4.

Watermelon seeds were germinated at optimal temp in KNO_3 solutions and it was found that concn up to 0.08 M did not affect germination behavior. Germination was delayed and its rate reduced at 0.12 M, but the final percentage was reduced only at KNO_3 concn of 0.16 M and higher. No germination occurred at 0.3 M KNO_3 (Fig. 4). Seeds which were imbibed in 0.3 M (3%) KNO_3 for 16 days and then washed, subsequently germinated fully at optimal conditions. When 3% KNO_3 was used as a priming pretreatment for subsequent low-temp germination, the results obtained were similar to those with 2% KNO_3.

The effectiveness of a 6-day pretreatment with solutions of NH_4NO_3 (0.2 M), $NaNO_3$ (0.2 M), Ca $NO_3)_2$ (0.1 M) and KC1 (0.1 M) was very similar to that of KNO_3 (0.2 M). On other other hand, when K_2SO_4 (0.1 M) or K_3PO_4 (0.07 M) was used, the rate of the low-temp germination was slower (Fig. 5). Similar results were obtained when isoosmotic concn of these salts were compared. The differences in the effectiveness of the K_3PO_4 solution can be attributed to its relative high pH (12.0 – 12.2) at the concn tested compared with the other salts (5.4 – 6.5).

The effect of organic osmoticum was different. Pretreatment with isoosmotic solutions of sucrose, mannitol and polyethylene glycol (Carbowax 6000) with osmotic values of 9.0 atm were not as effective as KNO_3 priming. The respective

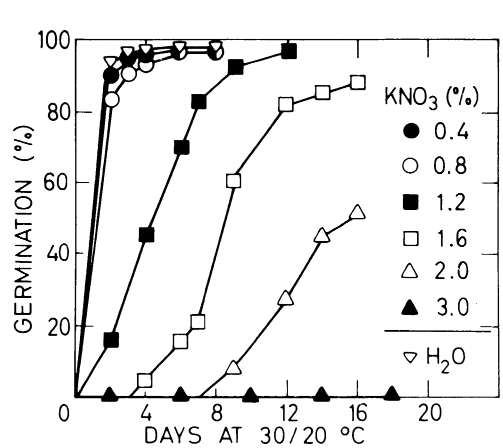

Fig. 4. The effect of various concentrations of KNO_3 on the germination of watermelon seeds at optimal temp regime in darkness.

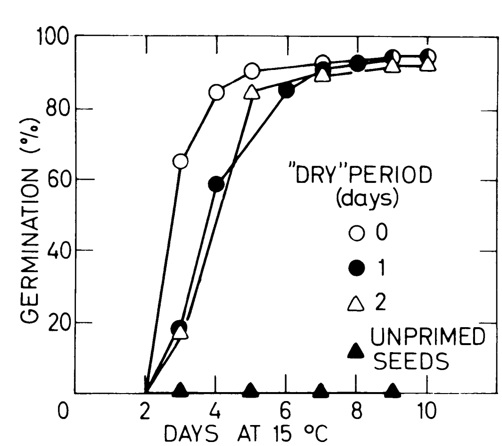

Fig. 6. The effect of drying "high-salt-primed" watermelon seeds on their subsequent germination at suboptimal temp in darkness.

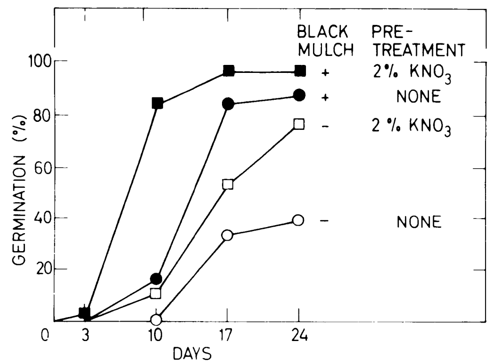

Fig. 7. The effect of KNO_3 pretreatment and temporary black polyethylene mulching on the emergence of watermelon seedlings.

low-temp germination levels at 15°C in darkness obtained were 69%, 50% and 54% vs. 94%.

When "high-salt primed" watermelon seeds were dried for 1 or 2 days and then reimbibed at suboptimal temp, the effectiveness of the pretreatment persisted (Fig. 6) irrespective of the length of the pretreatment (3 or 6 days). The primed seeds can be kept dry for longer periods (tested up to 20 weeks) and still germinate well at low temp. The length of the effective "dry period" is probably affected by the variety, vigor of the primed seeds, or their physiological age.

It is a well-established phenomenon that increasing the initial seed moisture content of unimbibed seeds has a protective effect against chilling injury during low-temp imbibition (4, 13, 15). When watermelon seeds were equilibrated at different relative humidities the seeds' moisture content was increased from an initial 6.2% up to 21.2% in the higher relative humidity treatment.

None of the pretreatments brought about an increased final germination percentage (compared with the control untreated seeds) at 15° or 20°C.

Control untreated seeds emerged slowest in a typical experiment, sown on Dec. 4, 1975 and reached only 40% emergence in 24 days (Fig. 7). Seeds germinated under black polyethylene mulch achieved 84% emergence in 17 days and those pretreated with 2% KNO_3 and sown under black mulch attained similar emergence in only 10 days.

The results correspond to those obtained in previous experiments of this study. The beneficial effect of the black polyethylene mulch is a result of both elevated temp and the elimination of light in the first stages of the germination. The effect of KNO_3 pretreatment approached that of the black mulch and when combined, the best emergence performance was obtained.

Priming of seeds for germination under unfavorable environmental conditions, or for accelerated germination under optimal conditions, is well documented in the literature and various pretreatment procedures have been suggested: Wetting and drying (1), favorable conditions for short periods (5), imbibition in salt solutions (7), imbibition in high-molecular-wt organic osmoticum (10), increasing the initial seed water content (15), and incorporation of growth regulators into the dry seed (14). Except for the last, these pretreatments included the normal start of the metabolic processes of imbibed seed at different activation levels and consisted of a series of irreversible steps toward normal germination.

When different pretreatments were applied to watermelon seeds it was established that elevating the initial seed moisture content was ineffective, and imbibition in aerated sucrose, mannitol or PEG solutions (ca. 9 atm) brought about relatively slow and low germination at suboptimal temp. On the other hand, imbibition in various aerated inorganic salt solutions for 6 days or at optimal temp in water for shorter periods, brought about rapid and full germination at 15°C. The most effective salt treatments were KNO_3, NH_4NO_3, $NaNO_3$, $Ca(NO_3)_2$ and KC1, and the optimal "high temp" pretreatment was 30° or 35°C for 18 hr.

Although the mechanisms of low temp and light inhibition may operate independently, both are responsive to "salt priming." Primed watermelon seeds will germinate successfully at 20°C and partially at 15°, under continuous light (unpublished data). The involvement of the phytochrome system in the priming process in watermelon seeds is now being explored.

In experiments where low-temp germination was achieved through the use of "salt priming," the seedlings developed well up to the stage of "fully expanded cotyledons." Further root and shoot development of the seedlings at low temp was very slow. It seems that even though we have a "tool" for achieveing low-temp germination, it can be used in the field only at slightly below threshold temp. In this case young seedlings will continue their development at a satisfactory rate. With the cultural practices used in Israel, such growing conditions are present in the environment of the watermelon seed sown in January under polyethylene mulch and covered with a low polyethylene tunnel.

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