Effect of Chilling Imbibition on Seed Germination

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Abstract

Tomato (*Lycopersicon esculentum* Mill) and cucumber (*Cucumis sativus* L.) seeds were imbided with water at 10°c or 4°c for 4, 8, and 16 hours before planting to study germination rate, mean germination time, and final germination percentage as well as electrolyte leakage from chilled seeds. Seeds imbided more water at 10°c compared to that at 4°c. Coefficient velocity of germination and final percent of germination decreased with the decrease of temperature and increasing time of imbibition. Germination percent under laboratory conditions was higher than that under field conditions. Also, it was found that tomato seeds were more sensitive to chilling imbibition. Electrolyte leakage increased as temperature of imbibition increases.

Introduction

Poor germination is a common phenomenon at sub-optimal temperatures, which is of great concern to growers that grow seedlings in late winter and early spring in several regions of the world. Optimum seed germination and seedling emergence occur at relatively high temperatures (20–30°C) for many crops such as tomato, eggplant, bean, watermelon, cucumber, and melon [1]. Tomato and cucumber are warm season crops, therefore, they can not tolerate frosts or low temperature damage [2]. Chilling injury occurs at temperature range of 0-10^{o}c [3,4] and it causes damage to all stages of growth and development in plant life cycle. Chilling imbibition is referred to the occurrence of chilling at the early stage of seed germination. It destroys the ability of seeds to germinate and establish the seedlings [3]. Germination is divided into three phases: imbibition, activation and post-germination growth. The largest effects of cold temperature during germination seem to be associated to the imbibition phase, considered the most sensitive [5]. Emergence percentage decreased under the stressful temperatures because of difficulty in water uptake[6]. Cold temperature during imbibition phase of germination leads to the increase of escape of solutes from the seeds, such as amino acids and carbohydrates, which has been attributed to the disturbance of plasma membrane integrity during imbibition at low temperature. Cellular membrane is considered as the main target for cold damage and the primary cause of other metabolicdisturbances observed within cells[4].

Chilling imbibition has been studied in several crops [7, 8, 9]. In cotton, for instance, it was found that germination percentage was significantly decreased with the increase of chilling period of two varieties [10]. *Pisum* germination rate and seedling growth under low temperature condition were very slow compared to that at room temperature [11, 12]. Maximum percentage of germinationm of *Solanum nigrum* was observed at 27°C while the lowest percentage of seed germination was observed at low temperature (3% after 5 days of incubation at 5°C) [13]. Same results of low germination under low temperatures conditions were reported by others (14, 15). Some investigators have stated that for seeds to be germinated there should be a trigger which stimulated immediately after the hydration of the seeds under normal condition [16, 17]. Because temperature is one of the most effective factor controlling seed germination [18], therefore, any unusual temperature changes will

interrupt the normal series of seed germination and result in dropping the ability of seeds germination. Cucumber and tomatoes are summer crops and they are planted in early spring in order to give early production, therefore, they may exposed to low temperature during germination. The objective of this research was to determine the effect of chilling imbibition at its upper limit (10°c) and lower limit (4°c) on seed germination of tomato and cucumber.

Material and Methods

This experiment was conducted on local varieties of tomato (*Lycopersicon esculentum* Mill) and cucumber (*Cucumis sativus* L.). Seeds of uniform size and free from any decay were chosen. Seeds were weighted and rapped with two layers of Whatman filter paper in Petri dishs. Two groups of Petri dishs were prepared. To the first group, 10-15 ml of deionized water at 10°c was added to each Petri dish and held in a refrigerator set to 10°c for 4, 8, and 16 hours. For the second group, water at 4°c was added to the Petri dish and seeds were held in a refrigerator set to 4°c for the same periods of time. Control treatment was left to imbibe at room temperature. At the end of each period of time, seeds were taken and blotted with filter paper to remove excess of water and weighted again in order to calculate the water imbibed by seeds at each treatment. Then, seeds for each treatment were sown in Petri dishs and left under room temperature for laboratory study. Three replications of 50 seeds each were used for each treatment. Number of seeds germinated was recorded daily. Mean germination time (MGT) was calculated according to [19] as below:

$$MGT = \frac{\Sigma (n_i d_i)}{\Sigma n}$$

Σn the total number of germinated seeds during the germination test, ni is the number of germinated seeds on day di and di is the number of days during the germination period. Coefficient velocity of germination was calculated as follow [20]:

$$C.V = \frac{A1 + A2 + \ldots\ldots\ldots + Ax}{A1T1 + A2T2 + \ldots\ldots\ldots + AxTx} \times 100$$

Where A= number of seedlings emerged at any day
T= day number

Final germination percentage (FGP), the percentage of number of seeds germinated to total seeds planted, was obtained when experiment was terminated. For electrolyte leakage of chilled imbibed seeds, 1g of tomato seeds or 3g of cucumber seeds were taken immediately at the end of each period of chilling at both temperatures, soaked in 10 ml of distilled water at 25c for 24 hours and conductivity was measured [4]. For field study, seeds, after they were treated with chilling temperatures at the desired period of time, were sown in 3-inch diameter pots containing a mixture of sand and loam and kept at lath house. Pots were watered daily and MGT, C.V , and PGA were calculated in the same way for Petri dish experiment.

A completely randomized design was used for both Petri dish and field experiments. Data were analyzed using analysis of variance and means were compared using Duncans new multiple range test at 5% level.

Results and Discussion

Results on water imbibition of seeds held at 10°c and 4°c for various period of time are shown in table(1). Generally, with the increase of time of imbibition, water uptake by seeds was increased. For tomato, the amount of water taken by chilled imbided seeds was less than that for the control. However, the percent of water imbibed by seeds at 4°c for 16 hour was

not significantly different from that of the control. It was hypothesized that imbibition temperature affects seed hydration. Higher seed water content at full hydration was associated with lower imbibition temperatures, apparently as a result of different hydration rates between embryo and endosperm[21]. Bai, [22] indicated that the greater water content of seeds imbibed at 0°c explains the warmer low temperature exothermm for seeds imbibed at lower temperatures than those imbibed at higher temperatures. With regard to cucumber seeds, the percent of water imbibed increased from 95.9% for seeds held for 4 hours at 10°c to 159.8% for seeds held for 16 hours at the same chilling temperature in comparison with 79.8% for the control. This increase was equal to more than double the water content of seeds of the control treatment. At 4°c, seeds have taken less water compared to that at 10°c. This may be due to a relatively warmer temperature (10°c) and the ability of seeds coat to stretch at that temperature. Also, it should be noted that cucumber seeds absorb much more water compared to tomato seeds which indicates differences in the abilities of two tissues to take water.

Germination coefficient velocity was less at 4°c in comparison with that at 10°c for both crops and under both lab and field conditions (Table.2). It was clear that the germination rate was decreased dramatically with the increase of time of imbibition at 4°c rather than at 10°c. Therefore, it seems that there is a time temperature interaction in controlling the rate of germination. Under laboratory conditions, germination rates were 45.80% and 48.00% for the control and they dropped to 30.20% and 28.80% for seeds imbibed for 16 hours at 4°c of tomato and cucumber respectively. Same pattern of germination rate was noted under field conditions. Rate of germination dropped from 44.10% to 28.60% for tomato and from 42.30% to 31.00% for cucumber when seeds imbibed at room temperature and at 4°c for 16 hours respectively. Germination rate, and consequently germination index, is important for crop establishment. They are related to a high seed vigor and this may be the cause of the better performance of crops [23]. This result comes in agreement with the results of [11, 12] who mentioned that *Pisum* germination rate and seedling growth under low temperature condition were very slow compared to that at room temperature. Also, the results of (8) who found that imbibition temperature at 5°c in tomato affected the rate of germination which decline across the different moisture contents.

Mean germination time revealed an opposite pattern to germination coefficient velocity (Table. 3). MGT increased as temperature decreased and time of imbibition increased. This result may indicate an inhibition in the metabolic activities at low temperature (4). Chen, [24] observed that the period of greatest sensitivity to cold corresponds to the first 30 min of imbibition which affect all the consequence events in seed germination and seedling establishment.

Final germination percentage (FGP) decreased also with the decrease of temperature and the increase of time of imbibition (Table 4). FGP under laboratory conditions was higher than that under field conditions. This may be due to the favorable conditions at laboratory rather than at the field. The lowest percentage was obtained at 4°c for 16 hour of imbibition for both tomato and cucumber under laboratory and field conditions. Therefore, it seems that two factors controlling the percentage of germination; temperature and time of imbibition. Under laboratory conditions, tomato germination percentage dropped from 99.30% to 73.20%, and cucumber germination percentage dropped from 98.20% to 80.60% for seeds imbibed at room temperature and 4°c (16h), respectively. Same trend was found under field conditions. This direction of decreasing percentage of germination with the decrease of temperature and increase of time of imbibition is correspondence to that for the rate of germination. Levitt [4] has attributed the decrease in rate and percentage of germination to the overall decrease in metabolic activities at chilling temperatures.

Also, it was found that imbibition at low temperatures has a harmfull effect on cell membranes which leads to leakage of cell contents and then decrease seed germinability [3]. Pea seeds have showed reduced germination percentage when the

imbibition and germination were carried out under chilling stress on comparison with the seeds germinated under room temperature condition [12].

Poor survival of seeds and seedlings and high leakage of organic and inorganic substances were commonly observed as a result of imbibition at chilling stress [25]. Berrie, [26] indicated that during imbibition under normal temperature, range of dry seeds (less than 20% water content) membranes phospholipids will rearrange themselves from hexagonal to lamellar shape but at chilling temperature, this rearrangement will permit the formation of channels or pores at the membranes and therefore increases the leakage of cell contents and as a result effect all biological functions of the membrane.

It is clear from the results in table 5, that the electrolyte leakage from seeds exposed to 4°c was more than that at 10°c. Chen, [24] has stated that the decrease in exposing temperatures cause leaching of several important cellular contents from the cells due to membrane injury that intensifies the damage to germinating seed. It was also known that imbibition phase was relatively more sensitive to chilling as indicated by the relatively higher damage to membranes [27]. The results show obviously that prolong of exposing time of seeds to chilling temperature caused a continuous increase in leachates. This result comes in agreement with the result of [7] who mentioned that chilling of wheat seeds for 96 h showed maximum electrical conductivity in comparison with that for 1, 2, 6 and 12 h after imbibition. The leakage of solutes from seeds during imbibition is due to reorganization or repair of membrane components and conformational changes occurring in cell membranes [28,29]upon drying of seeds, or may be due to membrane deterioration [30]. It was suggested that a specific structure of the seed coat called semipermeable layer would play a significant role in regulating the electrolyte leakage[8]. However it is worth to mention that tomato seeds were more sensitive to chilling than cucumber as indicated by the differences in the amount of leachates after exposing to chilling temperatures.

From the results presented here, we can conclude that rate and percentage of seed germination were less at 4°c compared to that at 10°c under laboratory and field conditions. Also, these two parameters were more effected under field rather than laboratory conditions. Increase of time of imbibition decreased both rate and percent of seed germination. Tomato seeds were more sensitive to chilling imbibition than cucumber seeds.

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Table(1) Percent of water imbibed by tomato and cucumber seeds at 10°c and 4°c for various periods of time.

Treatment		Water imbibed	
Temperature	Imbibition time (h)	Tomato	Cucumber
control		50.20[z] a	79.80 d
10°c	4	34.50 d	95.90 c
	8	42.10 bc	127.40 b
	16	47.30 ab	159.80 a
4°c	4	37.30 cd	79.80 d
	8	37.30 cd	124.50 b
	16	49.00 a	109.10 bc

Zmean separation within column, by Duncans new multiple range test, at 5% level
- numbers followed by the same letter(s) are not significantly different.

Table(2) Germination coefficient velocity of tomato and cucumber seeds imbibed at 10°c and 4°c for various period of time.

Treatment		Germination coefficient velocity			
Temperature	Imbibition time (h)	Tomato		Cucumber	
		L	F	L	F
control		45.80[z] a	44.10a	48.00a	42.30c
10°c	4	33.30d	42.00ab	46.00a	47.10b
	8	42.00b	39.30c	40.00b	52.10a
	16	40.10b	40.90bc	36.00cd	47.50b
4°c	4	34.90cd	39.20c	38.30bc	42.40c
	8	36.00c	31.20e	35.10d	35.00d
	16	30.20e	28.60f	28.80e	31.00e

Zmean separation within column, by Duncans new multiple range test, at 5% level
L Laboratory condition F Field condition
- numbers followed by the same letter(s) are not significantly different.

Table(3) Mean germination time of tomato and cucumber seeds imbibed at 10°c and 4°c for various period of time.

Treatment		Mean germination time			
Temperature	Imbibition time (h)	Tomato		Cucumber	
		L	F	L	F
control		6.72[z] d	7.85d	6.05d	6.75e
10°c	4	7.12c	7.90d	6.00d	6.98de
	8	7.10c	8.40c	6.78c	7.43cd
	16	6.90c	8.95b	7.85ab	7.89bc
4°c	4	7.12c	8.53bc	7.55b	7.80bc
	8	7.85b	9.11b	7.50b	8.30b
	16	8.55a	10.09a	8.21a	8.95a

Zmean separation within column, by Duncans new multiple range test, at 5% level
L Laboratory condition F Field condition

- numbers followed by the same letter(s) are not significantly different.

Table(4) Final germination percentage (FGP) of tomato and imbibed at 10°c and 4°c for various periods of time. cucumber

Treatment		Final germination percentage (FGP)			
Temperature	Imbibition time (h)	Tomato		Cucumber	
		L	F	L	F
control		99.30 [z] a	86.30 a	98.20 a	90.10 a
10°c	4	93.30 a	86.20 a	92.40 a	89.10 a
	8	90.00 ab	82.20 ab	95.10 a	87.20 a
	16	88.90 b	77.20 b	88.70 b	83.40 ab
4°c	4	95.20 a	81.70 ab	92.50 a	86.70 a
	8	98.00 a	75.40 b	79.60 c	77.50 b
	16	73.20 c	68.50 c	80.60 c	70.60 c

Zmean separation within column, by Duncans new multiple range test, at 5% level

L Laboratory condition F Field condition

- numbers followed by the same letter(s) are not significantly differe

Table(5) Electrolyte leakage from seeds of tomato and cucumber chilled at 10°c and 4°c for various period of time.

Treatment		Electrolyte leakage	
Temperature	Imbibition time (h)	Tomato	Cucumber
control		22.20 [z] f	18.56 e
10°c	4	27.50 e	21.75 e
	8	33.45 d	26.66 c
	16	44.80 b	34.95 b
4°c	4	39.33 c	27.80 c
	8	46.70 b	37.65 b
	16	57.55 a	45.45 a

Zmean separation within column, by Duncans new multiple range test, at 5% level

- numbers followed by the same letter(s) are not significantly different.

تأثير التشرب بدرجات الحرارة المنخفضة في نسبة انبات البذور

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الخلاصة

شبعت بذور الطماطة (*Lycopersicon esculentum* Mill) والخيار(*Cucumis sativus* L.) بالماء بدرجة حرارة 4c او 10c لمدد 4 و 8 او 16 ساعة قبل زراعتها تحت ظروف المختبر في اطباق بتري او في الظلة الخشبية. قيست سرعة الانبات ،و معدل عدد ايام الانبات ،و نسبة الانبات وكذلك كمية المواد الالكتروليتية الناضحة من البذور بعد تعريضها للتشبع بدرجات الحرارة المنخفضة.اوضحت النتائج ان البذور قد تشبعت بكمية ماء اكبر بدرجة 10c مقارنة بدرجة 4c . وان بذور الطماطم كانت اقل قابلية لاخذ الماء من بذور الخيار. كما ان نسبة وسرعة الانبات قلت مع خفض درجة حرارة التشرب وزيادة مدة التشرب في حين زادت كمية المواد الالكترولية الناضحة من البذور مع خفض الحرارة وزيادة مدة التشرب. نسبة الانبات تحت ظروف المختبر كانت اعلى مقارنة بتلك تحت ظروف الحقل. كذلك فان بذور الطماطم كانت اكثر حساسية للتشرب بدرجات الحرارة المنخفضة.