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Full Length Research Paper

Effects of Storage Conditions, Storage Periods and Dormancy-Breaking Treatments on the Viability and Germination of *Citrullus colocynthis* (L.) Schrad Seeds

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Citrullus colocynthis* (L.) Schrad. seeds are difficult to germinate successfully, especially those from freshly harvested fruits. Germination of seeds under natural field conditions is highly variable, erratic and very poor. Seeds of *C. colocynthis* (L.) Schrad have strong dormancy. Therefore, in the current study investigated the effects of eight different pretreatments to break seed dormancy on the germination percentage, the viability percentage, and the germination rate. In addition, the effects of three different storage conditions and four different storage periods in gene bank on the seed parameters were investigated to understand seeds storage behavior. Results showed significant differences between dormancy pretreatments, storage conditions andstorage periods in terms of their effects onthe germination percentage, standard germination percentage, viability percentage, dormancy percentage, and germination rate of *C. colocynthis* seeds. Results indicated that treated seeds with 1%H_2O_2 for 24h and mechanical scarification were the most effective treatments for improvement of seed germination in *C. colocynthis*. It is indicated that the optimum conditions and storage periods for seed storage were -22^{o}c for 12 months which resulted the highest germination percentage. These results reflect the adaptive strategy of germination in *C. colocynthis.

Keywords:*Citrullus colocynthis,* Seed Conservation, Dormancy,Gene Bank, bitter gourd, handal.

INTRODUCTION

Citrullus colocynthis (L.) Schrad is a viny desert plant that grows in arid, sandy soils and is particularly common on sand dunes in the Arabian Peninsula. Previous studies have highlighted its use as a medicinal plant, particularly in traditional medicine and in the treatment of chest diseases, constipation, rheumatic diseases and tumour diseases. It is also used as a source of biofuel and oilseeds. Thus, there is significant interest in expanding the cultivation of this plant throughout Arabian Peninsula. However,*C. colocynthis* (L.) Schrad. also known as the desertgourd,

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bitter gourd, handal, and Shary (in Arabic), is a member of the Cucurbitaceae family known for its high seed protein (35%) and oil (50%) content.*C. colocynthis* (L.) Schrad. occupies the huge area extending from the west coast of northern Africa eastward through the Sahara, Egypt, Arabian Peninsula, Persia, Beluchistan and through India, as far as the Coromandel coast and Ceylon, touching northward the Mediterranean and Caspian seas. The seeds of *C. colocynthis* are well-known for their high protein (8.25%) and oil content (24.86 – 26.9%) in whole seeds (Gurudeeban et al., 2010) and being an important source of both biofuel and oilseed. It is also usedin traditional medicine and clinical studies and being investigated its use as treatment for chest diseases, constipation, rheumatic diseases and tumour diseases.

*C. colocynthis*is able to propagate vegetatively, although it relies mainly on seeds. Similar to many other desert plants,*C. colocynthis* produces thousands of seeds but very few germinate successfully, because its seeds have strong dormancy. In addition, dormancy has been reported by (Abd-El-Hadi, (1994); Sahoo and Kasera (2012); Saberi and Shahriari (2011)..especially fresh *C. colocynthis*seeds, revealing that the germination percentage of seeds extracted from fresh, fully mature, harvested fruits did not exceed 3% germination. Thus, improving the germination efficiency of this species will be vital for its commercial cultivation.

A previous study showed that scratching each seed with sand paper overcame the seed dormancy of *C. colocynthi*s (Saberi and Shahriari, 2011). Therefore, to improve the germination of *Citrullus lanatus* seeds, the latter were subjected to several treatments before germination at 30°C. It is reported that 2% H_2O_2 enhanced the germination of these tetraploid seeds (Jaskani *et al.,* 2005). Storage of seeds as *ex situ* germplasm is an essential step for the long-term conservation of plant genetic resources. Maintaining seed viability for longer period is very essential to preserve the genetic integrity in stored samples. Harrington (1960) also reported that the type of packaging can affect the germination of seeds such as watermelon, stored for 12 months in a cold chamber.Previous studies have shown that the optimal conditions required for storage depend on the ultimate use of the stored seeds and the required storage duration. It is indicated that for storage of base collections which are rarely removed from storage, a temperature of less than -18ºC with 3–7% seed moisture content is recommended for long-term secure conservation (Torres, 2005).Therefore,the objective of this study was to determine the most effective method to break dormancy and to estimatethe optimal storage conditions and length of storage in gene bank for *C. colocynthis* seeds based on the measurement of various viability parameters.

MATERIALS AND METHODS

Seed collection, cleaning and drying

Through year 2012, the Genetic Resources Department, Department of Agricultural Research, Ministry of Municipality and Environment in Qatar collected, surveyed and has some inventory activities for flora in Qatar. Fruits of *C. colocynthis* were collected from the Salwa Road to Rawdat Rashed (Latitude 25.209300° & Longitude 51.226033°). The plants grow in small groups, 20% of the fruits were collected from each individual plant. Fruits were considered fully mature if they were dark yellow or brown and seeds coat color is dark brown. Seeds were extracted from fruits under running water, all the seeds were cleaned according to previous methodologies (Saberi and Shahriari, 2011 and IPGRI, 1994). After the seeds had been cleaned, they were dried in a standard cold drying room, according toprotocols described elsewhere (Rao *et al*., 2006 and AOSA., 1978).

Seed sampling, storage conditions and storage periods

Each treatment had 100 seeds in four replications, each treatment packed into separate bags. Three different storage conditions were selected: (i) storage seeds at room temperature (mean temperature of 20–25ºC; seeds stored in cloth bags); (ii) storage in a refrigerator (mean temperature of +4ºC; 40% relative humidity,“active room – short term”, ,seedsstoredin vacuum--sealed glass jars); and (iii) storage in a freezer (mean temperature of –22ºC, no frost, “base room – long term”, seeds stored in vacuum-sealed aluminum polyethylene bags). After seeds dried, they were storedinfour storage periods: (i) freshly harvested seeds (control un storage seeds), (ii) 6 months, (iii)12 months and (iv)5 years.

Pretreatments to break seed dormancy

One hundred fresh seeds of *C. colocynthis* (L.) Schrad “un stored” were treated in the first of experiment for dormancy pretreatments only, then treatedfor storage periods and conditions treatments before germination test. For each treatment, they were subjected to the following pretreatments before germination: H_2O_2 for 12, 24, or 48 h; H_2SO_4 for 10 min + $CaCl_2$in water; H_2O at 85°C and then kept in normal water for 24 h; H_2O at 70°C and then kept in normal water for 24 h; and a control treatment.

Viability tests

Seed viability was assessed by germination tests following the approach described by IPGRI (1994). Germination was

Table 1. Effect of storage temperature on the germination percentage, standard germination percentage, viability percentage, dormancy percentage and germination rate of *Citrullus colocynthis* seeds*

Storage temperature	Seed parameter				
	G%	SG%	V%	D%	GR
Room temperature 20–25ºC	43.29 [c]	39.88 [c]	93.52 [b]	48.40 [a]	5.09 [c]
Active room +4ºC; 40% RH	49.61 [b]	49.76 [b]	97.18 [a]	46.74 [a]	6.93 [a]
Base room - 22^{O}c &no frost	52.85 [a]	52.66 [a]	96.88 [a]	42.40 [b]	5.98 [b]

*= Significant at ($p<0.05$) level, Duncan's new multiple range test). G%: germination percentage, SG%: standard germination percentage, V%: viability percentage, D%: dormancy percentage; GR: germination rate.

evaluated before seeds were placed in storage (initial germination) and after storage for 6 months, 12 months and 5 years (final germination). Germination tests were performed according to the methodology of the International Seed Testing Association (ISTA, 1999).

Germination studies

Germination testswere carried out in sterilized sand, 25 treated seeds were germinated in plastic container, then placed in germination incubator with 20°/30°C (12h/12h) following the guidelines of the Association of Official Seed Analysis (AOAC, 1988).The following parameters were measured:

Germination percentage (G% calculated as number of germinated seeds/total number of seeds× 100 according to ISTA, 1999).

Standard germination percentage (SG% calculated as number of normal seedlings/total number of seeds × 100 according to ISTA, 1999).

Viability percentage (V% calculated as number of normal seeds + number of abnormal seeds + number of hard seeds "unterminated seed"/ total number of seeds × 100 according to ISTA, 1999).

Dormancy percentage (D% calculated as hard seeds/ total number of seeds × 100 according toISTA, 1999).

Germination rate (GR was calculated as (n1/d1) + (n2/d2) + (n3/d3) …according to Maguire (1962)where n = the number of germinated seeds and d= number of days).

Tetrazolium chloride test

A tetrazolium chloride (TZ) test was used as a backup to identify viable but dormant seeds that had failed to germinate at the end of each germination test. This was done by evaluating the seeds for a staining pattern under a low-powered binocular microscope; viable tissues stained bright red with TZ, whereas pink and very dark-red stains indicated dead seeds as suggested by Moorem (1973).

Statistical analysis

The experimental design was considered as a split plot with four replicates. Data were statistically analyzed according to Snedecor and Cochran (1967). All data were transformed to meet normality and homogeneity of variance assumptions. Duncan's new multiple range test at $P\leq0.05$ was used to separate the treatment means (Duncan, 1955).

RESULTS

Effect of seed storage conditions on seed parameters

To investigate the effect of gene bank standard storage conditions on *C. colocynthis* seeds germination, seeds were stored at -22 ºC+ no frost, +4 ºC+ 40 % RH and at room temperature of 20–25ºC. The storage conditions used to conservation *C. colocynthis* seeds had a significant effect ($p<0.05$) on the percentage of seeds that germinated (Table 1). Storage at -22 ºC+ no frost resulted in the highest germinationof 52.85%and standard germinationof 52.66%.By contrast, seed stored at room temperatureof 20–25ºC containedhigh percentage of dormant seed of 48.40%, while seeds were stored at +4 ºC resulted in thehighest germination rateof 6.93 and viability percentage of 97.18 %.

Effect of seed storage periods on seed parameters

Seeds of *C. colocynthis* were stored for 0, 6 months, 1 year and 5 years to determine the seed storage behaviors under gene bank standard conditions. There were significant differences between the effects of different storage periods

Table 2. Effect of seed storage period on the germination percentage, standard germination percentage, viability percentage, dormancy percentage and germination rate of *Citrullus colocynthis* seeds*

Storage period	Seed parameter				
	G%	SG%	V%	D%	GR
Fresh (control)	44.99 [c]	40.39 [c]	97.85 [a]	52.28 [a]	5.81 [b]
6 months	51.72 [a]	50.83 [a]	96.52 [ab]	44.18 [b]	6.27 [a]
12 months	50.71 [a]	50.62 [a]	95.75 [b]	43.95 [b]	5.87 [b]
5 years	46.92 [b]	47.79 [b]	93.43 [c]	42.98 [b]	6.05 [ab]

Means followed by the same letter within the same column are not significantly different

*= Significant at ($p<0.05$) level

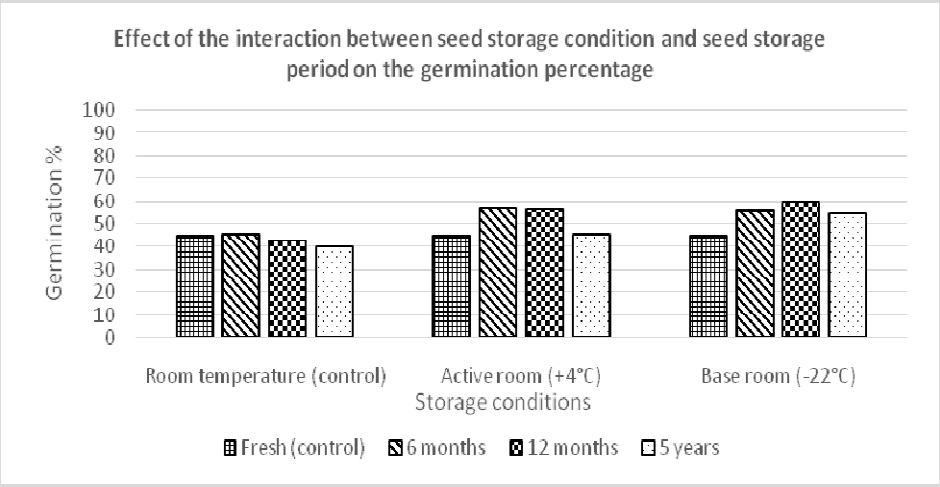

Figure 1. Effect of the interaction between seed storage conditions and seed storage periods on the germination percentage G%

on seed germination and viability (Table 2). Seeds stored for up to 6 months gave the highest germination percentage (51.72%), with a standard germination percentage of 50.83%, whereas these were the lowest for fresh seeds (44.99%).The germination rate was 6.27 when seeds were stored for up to 6 months, and the dormancy percentage was 52.28% with fresh seeds. The viability percentage was the lowestfor seeds stored for 5years (93.43%).

Effect of the interaction between seed storage conditions and seed storage periods

Figure (1) shows the effect of the interaction between storage conditions and storage periods on the seed parameters examined. Storing seeds at–22°C for 1 year resulted in the highest germination percentage of 59.63%, followed by storage in active roomof +4°C for 6 months (56.65%).The percentage germination of seeds stored for 5 years was decreased compared with seeds sortedusing the other storage conditions. Similar results were observed for the standard germination percentage although seeds stored at –22°C for 12 months recorded the highest standard germination percentage (57.69%).The lowest standard germination percentage was recorded for seeds stored at room temperature storage for 5 years (38.30%). Storage at +4°C for 6 months resulted in the highestpercentage of viable seeds (figure 4) and germination rate(figure 5), while seeds stored under +4°Cs howed the same result as the conservation of seeds under different conditions.

Effect of dormancy-breaking treatments

Seeds of many land plants fail to germinate and pass through a phase of dormancy that may be caused by several factors and delay the whole life cycle of plants. So,fresh seeds “un stored seeds”of *Citrullus colocynthis were* treated *with v*arious pretreatments to improve

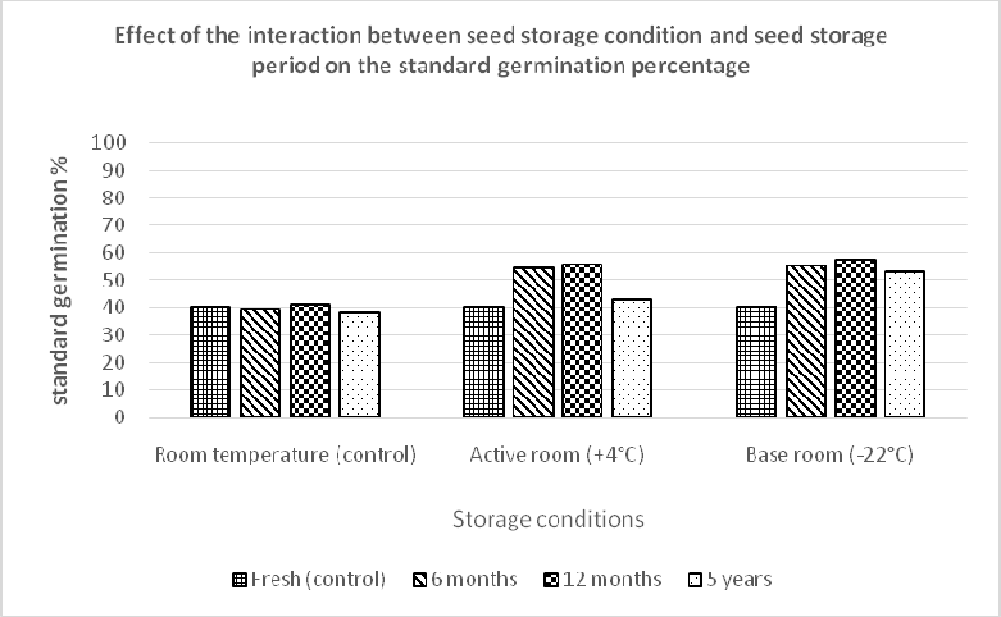

Figure 2Effect of the interaction between seed storage conditions and seed storage periods on the standard germination percentage SG%

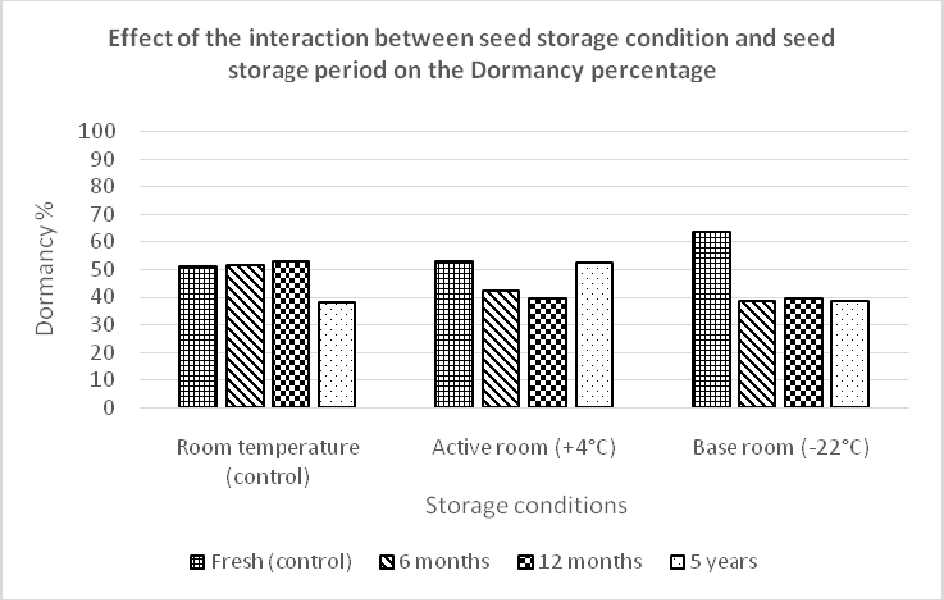

Figure 3. Effect of the interaction between seed storage condition and seed storage period on the dormancy percentage

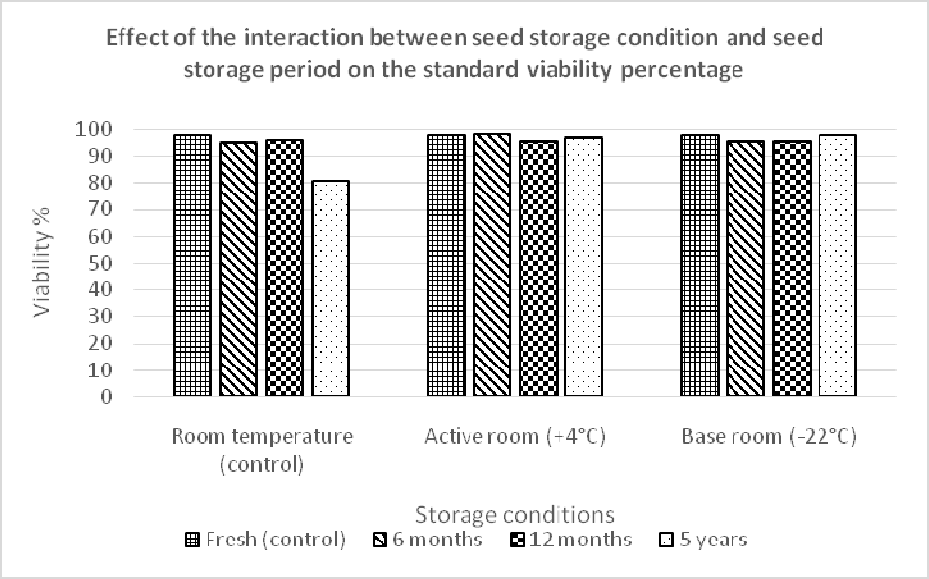

Figure 4. Effect of the interaction between seed storage condition and seed storage period on the viability percentage V%

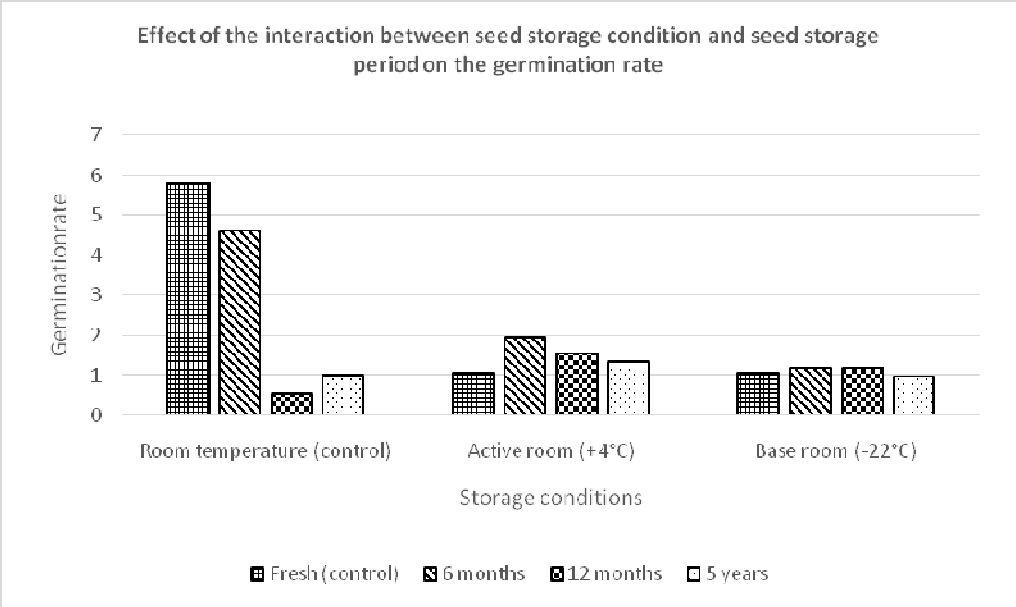

Figure 5 Effect of the interaction between seed storage condition and seed storage period on the germination rate GR

germination. The results in Table (3) show an increase in the germination percentage from 31.65% to 77.77%, an increase in the standard germination percentage from 29.44% to 73.40%, and an increase in the germination rate from 3.369 to 10.67 when seeds were treated with 1% H_2O_2 for 24 h.However, the highest dormancy percentage (63.36%) was recorded in the control (i.e. untreated) seeds, whereas the highest percentage viability was recorded for seeds treated with mechanical scarification, as well as those treated with 1% H_2O_2 for 12 h and 48 h. The latter seeds showed an increase in the germination percentage, standard germination percentage and germination rate relative to the control seeds. Similar results were reported else where (Yang *et al.,* 2005 and Naredo *et al.,* 1998) as reported for watermelon by Jaskani *et al.(* 2005).

Table 3. Effect of dormancy-breaking treatment on the germination percentage (G%), standard germination percentage (SG%), viability percentage (V%), dormancy percentage (D%) and germination rate (GR)of *Citrullus colocynthis* seeds*

Dormancy-breaking treatment	Seed parameter				
	G%	SG%	V%	D%	GR
1% H_2O_2 for 12 h	63.99 ^{b}	62.88 ^{b}	97.58 ^{b}	33.42 ^{cd}	9.55 ^{b}
1% H_2O_2 for 24 h	77.77 ^{a}	73.40 ^{a}	96.68 ^{bc}	23.90 ^{e}	10.67 ^{a}
98% H_2SO_4 + 0.2% $CaCl_2$	64.52 ^{b}	64.40 ^{b}	97.46 ^{b}	32.21 ^{d}	9.04 ^{c}
98% H_2SO_4 + 0.2% $CaCl_2$	24.68 ^{e}	24.50 ^{e}	88.49 ^{e}	62.89 ^{a}	2.26 ^{f}
Mechanical scarification	63.68 ^{b}	63.45 ^{b}	99.00 ^{a}	34.90 ^{c}	6.60 ^{d}
H_2O 85°C for 60 min	36.90 ^{c}	35.20 ^{c}	94.78 ^{cd}	57.21 ^{b}	3.26 ^{e}
H_2O 70°C for 60 min	30.54 ^{d}	28.02 ^{de}	93.78 ^{d}	62.48 ^{a}	3.25 ^{e}
Control	31.65 ^{d}	29.44 ^{d}	96.77 ^{bc}	63.36 ^{a}	3.37 ^{e}

Means followed by the same letter within the same column are not significantly different

*= Significant at ($p<0.05$) level

By contrast, seeds treated with 98% H_2SO_4 showeda decrease in their germination percentage, standard germination percentage, viability percentage and germination rate compared with un treated seeds and other treatments.

Effect of the interaction between seed storage conditions and dormancy-breaking treatments

Table (4) shows the effect of the interaction between seed storage conditions and dormancy-breaking treatments on viability parameters.

Germination and standard germination percentages

The highest germination percentage was recorded in seeds treated with 1% H_2O_2 for 24 h, followed by seeds stored at -22°Cand then seeds stored at +4°C compared with control seeds. Previous studies also reported thatseeds stored at-22°C and treated with 1%H_2O_2 for 24 h showed a germination percentage of 74.25% (Yang *et al.,* 2005) and 74.06%(Naredo *et al.,* 1998).Thus, treating seeds with hydrogen peroxide appears to result in significant increases in seed germination of various species. Similar results were observed in the current study for the standard germination percentage.

Viability percentage

Treating seeds with mechanical scarification and then storing them at room temperature resulted in99.49% of seeds being viable compared with 90.62% in the control group.By contrast, treating seeds with 98% H_2SO_4 decreasedthe percentage of viable seeds compared with control seeds57.59 %

Dormancy percentage

The highest dormancy percentages were recorded for untreated seeds regardless of storage conditions. The optimal pretreatment for breaking seed dormancy was 1% H_2O_2 for 24 h, resulting in the highest germination and standard germination percentages regardless of storage conditions.

Germination rate

Germination rate was a significant response factor in relation to the different storage conditions. There was an increase in the germination rate, addition to controls,in seeds stored at +4°C. Seeds stored at +4°C and treated with 1% H_2O_2 for24 hresulted in the highest germination rate (12.51) whereas the lowest germination rate of 1.93 was recorded for seeds treated with 98% H_2SO_4+ 0.2% $CaCl_2$and stored at room temperature. As a general result, seeds stored at either+4°Cor -22°C showed increased germination rates compared with control seeds.

Table 4. Effect of the interaction between seed storage conditions and dormancy-breaking treatmentson the germination percentage (G%), standard germination percentage (SG%), viability percentage (V%), dormancy percentage (D%) and germination rate (GR)of *Citrullus colocynthis* seeds*

Dormancy-breaking treatments	G%	SG%	V%	D%	GR
Room temperature 20–25ºC					
1% H_2O_2 for 12 h	61.62 ^{d}	58.85 ^{c}	94.86 $^{e-i}$	33.08 ^{f}	8.66 $^{d-f}$
1% H_2O_2 for 24 h	71.81 ^{ab}	68.75 ^{ab}	95.45 $^{c-h}$	24.33 ^{g}	10.13 ^{bc}
1% H_2O_2 for 48 h	60.81 ^{d}	58.45 ^{c}	94.05 $^{f-j}$	28.85 $^{f-g}$	8.12 $^{f-h}$
98% H_2SO_4 + 0.2% $CaCl_2$	22.54 ^{k}	22.94 ^{hi}	89.62 $^{i-k}$	67.85 ^{a}	1.93 ^{m}
Mechanical scarification	51.71 ^{e}	49.25 ^{d}	99.49 ^{a}	48.79 ^{e}	4.71 ^{l}
H_2O 85°C 60 min	33.73 $^{f-h}$	28.73 $^{f-h}$	90.33 $^{h-k}$	46.97 ^{cd}	2.45 ^{m}
H_2O 70°C 60 min	22.28 ^{k}	17.40 ^{l}	88.68 $^{j-k}$	66.42 ^{a}	2.36 ^{m}
Control	25.28 $^{j-k}$	20.68 ^{l}	90.62 $^{h-k}$	62.49 $^{a-c}$	2.37 ^{m}
Active room +4ºC; 40% RH					
1% H_2O_2 for 12 h	62.42 $^{c-d}$	64.28 ^{bc}	98.50 $^{a-f}$	34.31 ^{f}	10.84 ^{b}
1% H_2O_2 for 24 h	74.06 ^{a}	75.04 ^{a}	95.81 $^{b-h}$	24.18 ^{g}	12.51 ^{a}
1% H_2O_2 for 48 h	65.07 $^{b-d}$	65.42 ^{bc}	99.69 $^{a-c}$	33.52 ^{f}	10.61 ^{b}
98% H_2SO_4 + 0.2% $CaCl_2$	25.30 $^{j-k}$	23.55 $^{h-i}$	91.62 $^{g-j}$	62.50 ^{a}	2.22 ^{m}
Mechanical scarification	69.25 $^{a-c}$	70.20 $^{a-b}$	98.09 $^{a-f}$	27.30 $^{f-g}$	7.43 ^{h}
H_2O 85°C 60 min	38.21 $^{f-g}$	38.04 ^{e}	97.63 $^{a-g}$	58.96 $^{b-d}$	3.80 $^{j-k}$
H_2O 70°C 60 min	29.81 $^{h-j}$	20.31 $^{f-h}$	96.44 $^{b-g}$	65.26 ^{ab}	3.63 $^{j-k}$
Control	33.15 $^{g-i}$	32.43 $^{e-g}$	98.85 $^{a-d}$	65.65 ^{a}	4.38 $^{i-j}$
Base room Base room - 22^{O}c & no frost					
1% H_2O_2 for 12 h	59.43 $^{a-d}$	65.42 ^{bc}	98.83 $^{a-d}$	32.91 ^{f}	9.15 $^{d-e}$
1% H_2O_2 for 24 h	74.25 ^{a}	74.34 ^{a}	98.35 $^{a-f}$	23.21 ^{g}	9.37 ^{cd}
1% H_2O_2 for 48 h	67.61 $^{a-d}$	68.01 ^{ab}	98.15 $^{a-f}$	29.50 $^{f-g}$	8.40 $^{e-g}$
98% H_2SO_4 + 0.2% $CaCl_2$	27.04 $^{i-k}$	27.54 $^{g-h}$	83.61 ^{k}	53.00 $^{d-e}$	2.61 $^{l-m}$
Mechanical scarification	68.85 $^{a-c}$	70.85 ^{ab}	99.17 ^{ab}	29.34 $^{f-g}$	7.67 $^{g-h}$
H_2O 85°C 60 min	38.83 $^{f-g}$	39.01 ^{e}	96.44 $^{b-g}$	56.65 $^{c-d}$	3.53 $^{j-k}$
H_2O 70°C 60 min	40.27 ^{f}	38.73 ^{e}	95.12 $^{d-i}$	55.41 $^{c-e}$	3.76 $^{j-k}$
Control	36.90 $^{f-g}$	35.73 ^{ef}	98.85 $^{a-d}$	61.84 $^{a-c}$	3.35 $^{k-l}$

Means followed by the same letter within the same column are not significantly different

*= Significant at ($p<0.05$) level

Effect of the interaction between seed storage periods and dormancy-breaking treatments

There was a significant difference between storage periods and pretreatments for all the seed parameters recorded in this study (Table5).

Germination and standard germination percentages

The highest germination percentage (87.92%) was recordedfor seeds stored for 12 months followed by mechanical scarification and those stored for 6 months followed by the same mechanical treatment (82.73%).Treatment with H_2O_2 for 24 h resulted in the highest germination percentage of fresh seeds. By contrast, storing seeds for 5 years resulted in a decreased germination percentage regardless of dormancy-breaking treatment because of thelong storage period which affected their viability. Similar results were recorded for the standard germination percentage (Table 6).

Viability percentage

Storage of seeds for 6 months, 12 months and 5 years progressively decreased thepercentage of viable seeds (Table5).Storing control (non-treated) seeds for up to 5 years resulted in 88.01% of seeds being viable, which was less than any other pretreatment groups.

Table 5. Effect of the interaction between seed storage period and dormancy-breaking treatment on the germination percentage (G%), standard germination percentage (SG%), viability percentage (V%), dormancy percentage (D%) and germination rate (GR)of *Citrullus colocynthis* seeds*

Dormancy-breaking treatment	G%	SG%	V%	D%	G.R
Fresh (control) 0storage					
1% H_2O_2 for 12 h	67.36 $^{b-d}$	70.60 $^{b-e}$	99.35 $^{a-d}$	24.82 $^{g-l}$	2.47 ^{a}
1% H_2O_2 for 24 h	76.96 $^{b-d}$	44.80 $^{j-k}$	94.42 $^{c-j}$	44.96 $^{b-d}$	1.23 ^{c}
98% H_2SO_4 + 0.2% $CaCl_2$	64.06 ^{cd}	49.90 $^{i-j}$	100 ^{a}	47.83 ^{bc}	1.62 ^{b}
98% H_2SO_4 + 0.2% $CaCl_2$	24.02 $^{l-m}$	23.72 ^{m}	100 ^{a}	65.18 ^{a}	0.81 $^{e-j}$
Mechanical scarification	44.77 $^{e-f}$	49.90 $^{i-j}$	100 ^{a}	50.10 ^{b}	1.12 $^{c-f}$
H_2O 85°C 60 min	33.33 $^{h-k}$	27.13 $^{l-m}$	98.72 $^{a-g}$	65.32 ^{a}	0.73 $^{h-j}$
H_2O 70°C 60 min	23.08 $^{l-m}$	28.21 $^{l-m}$	100 ^{a}	71.73 ^{a}	0.65 ^{j}
Control	28.13 $^{i-m}$	37.07 ^{kl}	100 ^{a}	62.93 ^{a}	0.72 $^{i-j}$
6 months					
1% H_2O_2 for 12 h	74.91 $^{b-d}$	71.73 $^{b-e}$	99.35 $^{a-d}$	22.52 $^{i-l}$	1.22 ^{c}
1% H_2O_2 for 24 h	72.71 $^{c-e}$	70.03 $^{b-e}$	98.52 $^{a-g}$	23.34 $^{h-l}$	1.19 ^{cd}
98% H_2SO_4 + 0.2% $CaCl_2$	60.40 $^{f-k}$	55.31 $^{f-j}$	99.39 $^{a-d}$	37.15 $^{b-g}$	1.08 $^{c-g}$
98% H_2SO_4 + 0.2% $CaCl_2$	62.10 $^{e-j}$	59.32 $^{e-i}$	96.01 $^{b-i}$	30.34 $^{e-k}$	1.14 $^{d-e}$
Mechanical scarification	82.73 ^{ab}	78.56 ^{ab}	98.82 $^{a-f}$	11.72 ^{mn}	1.48 ^{b}
H_2O 85°C 60 min	55.99 $^{h-m}$	52.54 $^{h-j}$	98.82 $^{a-f}$	40.50 ^{be}	1.04 $^{c-g}$
H_2O 70°C 60 min	62.63 $^{e-j}$	60.13 $^{e-i}$	99.72 ^{ab}	35.70 $^{c-h}$	1.15 $^{d-e}$
Control	47.51 $^{l-n}$	44.14 $^{j-k}$	99.71 ^{ab}	50.80 ^{b}	0.91 $^{e-j}$
12 months					
1% H_2O_2 for 12 h	76.12 $^{b-d}$	73.18 $^{b-d}$	99.15 $^{a-e}$	19.32 $^{k-m}$	0.96 $^{c-i}$
1% H_2O_2 for 24 h	77.70 ^{bc}	74.23 ^{bc}	98.81 $^{a-f}$	16.63 $^{l-n}$	1.19 ^{cd}
98% H_2SO_4 + 0.2% $CaCl_2$	66.12 $^{d-i}$	61.91 $^{d-i}$	99.15 $^{a-e}$	30.41 $^{e-k}$	0.96 $^{c-i}$
98% H_2SO_4 + 0.2% $CaCl_2$	64.20 $^{e-j}$	59.21 $^{e-i}$	95.76 $^{b-j}$	30.50 $^{e-k}$	1.11 $^{c-f}$
Mechanical scarification	87.92 ^{a}	85.32 ^{a}	99.71 ^{ab}	9.82 ^{n}	1.48 ^{b}
H_2O 85°C 60 min	55.97 $^{h-m}$	54.23 $^{g-j}$	97.81 $^{a-g}$	38.91 $^{b-f}$	0.91 $^{e-j}$
H_2O 70°C 60 min	63.41 $^{e-j}$	62.63 $^{d-h}$	99.57 $^{a-c}$	35.41 $^{c-i}$	1.09 $^{c-f}$
Control	46.72 $^{m-o}$	44.83 $^{j-k}$	99.57 $^{a-c}$	50.73 ^{b}	0.86 $^{f-j}$
5 years					
1% H_2O_2 for 12 h	64.32 $^{e-j}$	60.17 $^{e-i}$	94.33 $^{d-j}$	20.62 $^{j-m}$	0.77 $^{h-j}$
1% H_2O_2 for 24 h	71.41 $^{c-f}$	69.72 ^{be}	92.64 $^{g-j}$	20.92 $^{j-m}$	1.11 $^{c-f}$
98% H_2SO_4 + 0.2% $CaCl_2$	58.43 $^{g-l}$	52.52 $^{h-j}$	96.02 $^{b-i}$	34.42 $^{c-i}$	1.00 $^{c-h}$
98% H_2SO_4 + 0.2% $CaCl_2$	54.12 $^{j-m}$	46.43 $^{j-k}$	87.34 ^{j}	33.10 $^{d-j}$	0.94 $^{d-i}$
Mechanical scarification	68.61 $^{c-g}$	65.42 $^{c-g}$	88.13 $^{i-j}$	15.23 $^{l-n}$	1.13 $^{c-f}$
H_2O 85°C 60 min	48.31 $^{l-n}$	44.16 $^{j-k}$	93.89 $^{e-j}$	40.56 $^{b-e}$	0.81 $^{g-j}$
H_2O 70°C 60 min	63.43 $^{e-j}$	59.23 $^{e-i}$	93.30 $^{f-j}$	26.14 $^{f-l}$	1.08 $^{c-g}$
Control	40.72 $^{n-p}$	37.30 $^{k-l}$	88.01 $^{i-j}$	41.23 $^{b-e}$	0.717 $^{i-j}$

Means followed by the same letter within the same column are not significantly different

*= Significant at ($p<0.05$) level

Dormancy percentage

The general trend was that all dormancy pretreatments across the different storage periods decreasedthe percentage of dormant seeds relative to controls. For example, treating seeds with mechanical scarification following storage for 12 months resulted in 9.82% of seeds being dormant compared with 50.73%for control seeds. This decrease in dormancy was particularly obvious following treatment with 1% H_2O_2 for 24 h.

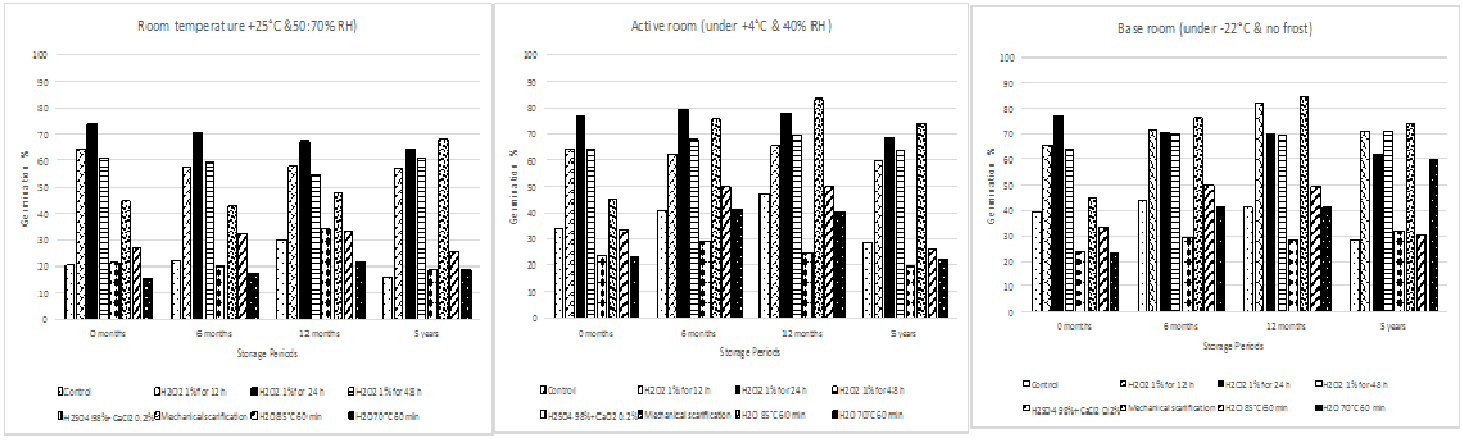

Figure 6. Effect of the interaction between seed storage conditions, seed storage periods and dormancy-breaking treatments on the germination percentage G%

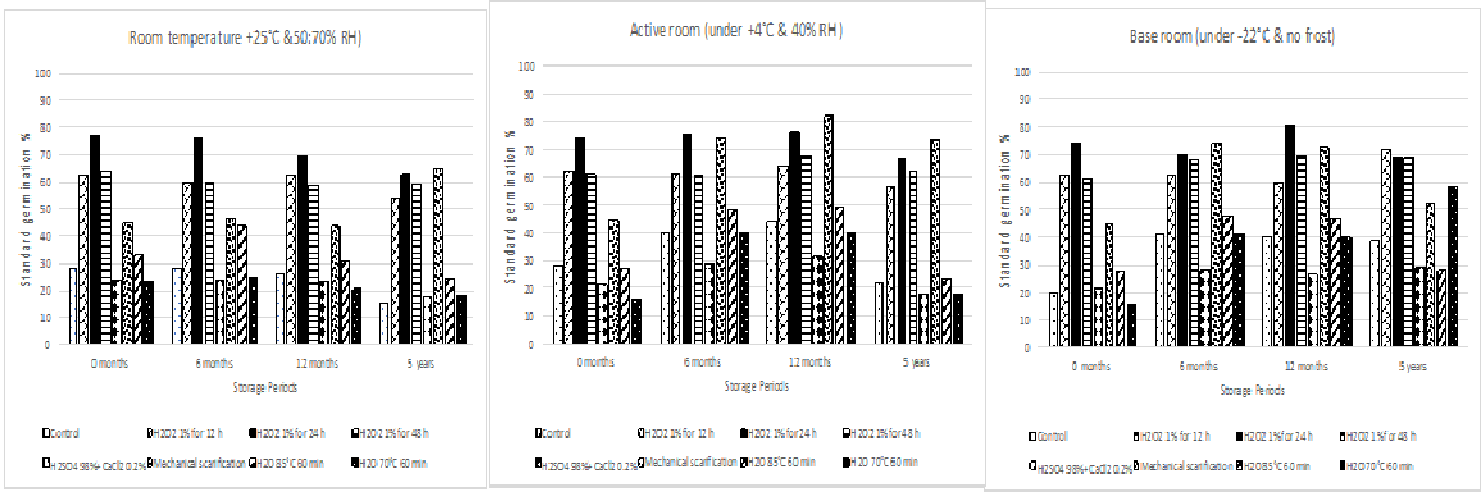

Figure 7 Effect of the interaction between seed storage condition, seed storage period and dormancy-breaking treatment on the standard germination percentage SG%

Germination rate

Treating seeds with mechanical scarification after storing for 6 or 12 months resulted in higher germination ratescompared with those stored for 5 years, fresh seeds or control seeds. Thus, storage for 6or 12 months can increase the germination rate while storing seeds for 5 years can decrease it regardless of the dormancy pretreatment.

Effect of the interaction between seed storage conditions, seed storage periods and dormancy-breaking treatments

The data presented in Figure (6) show the effect of the interaction between seed storage conditions, seed storage periods and dormancy-breaking treatments on the germination percentage. Storage of seeds at -22°C for 12 months followed by mechanical scarification resulted in the highest germination percentage (84.78%), while seeds at the same storage conditions and storage periods but treated with 1% H_2O_2 for 24 h resulted in germination percentage of 81.95%. The germination percentage was recorded as 83.33% for seeds stored for 12 months at +4°C and then treated with mechanical scarification. By contrast, storage of seeds at either -22°Cor +4°Cfor up to 5 years decreased the germination percentage, although the lowest germination percentage was recorded for seeds stored at room temperature regardless of pretreatments.

The data presented in Figure (7) show the effect of the interaction between seed storage conditions, seed storage periods and dormancy-breaking treatments on standard

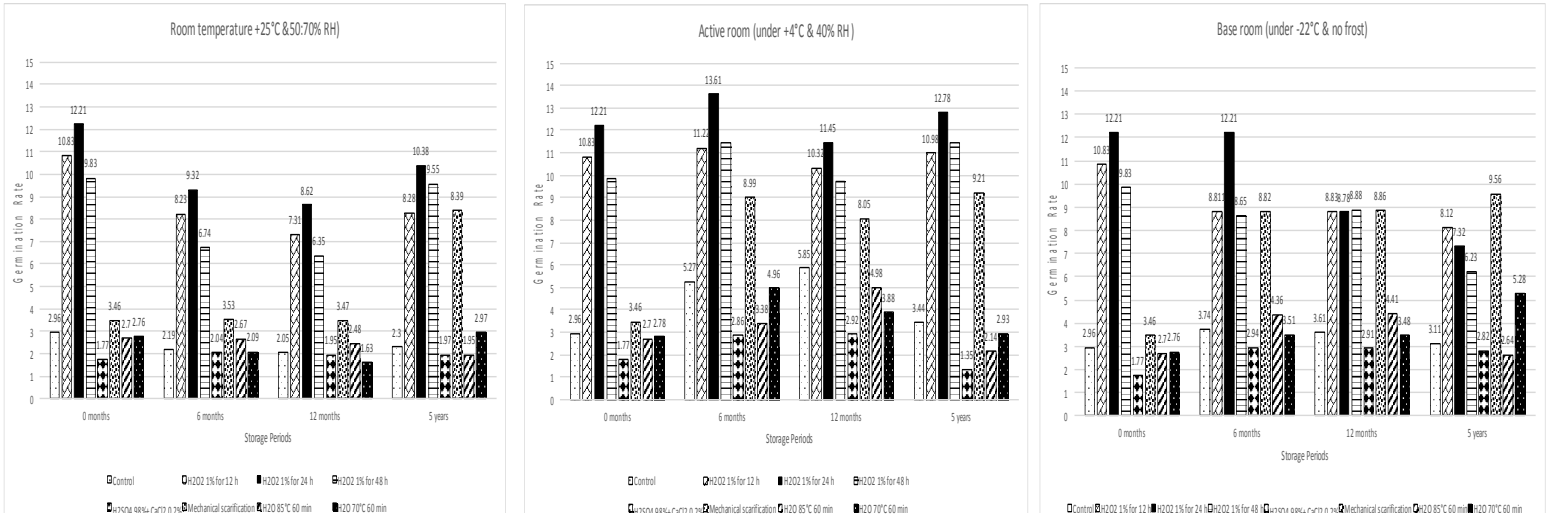

Figure 8 Effect of the interaction between seed storage condition, seed storage period and dormancy-breaking treatment on the Germination Rate GR

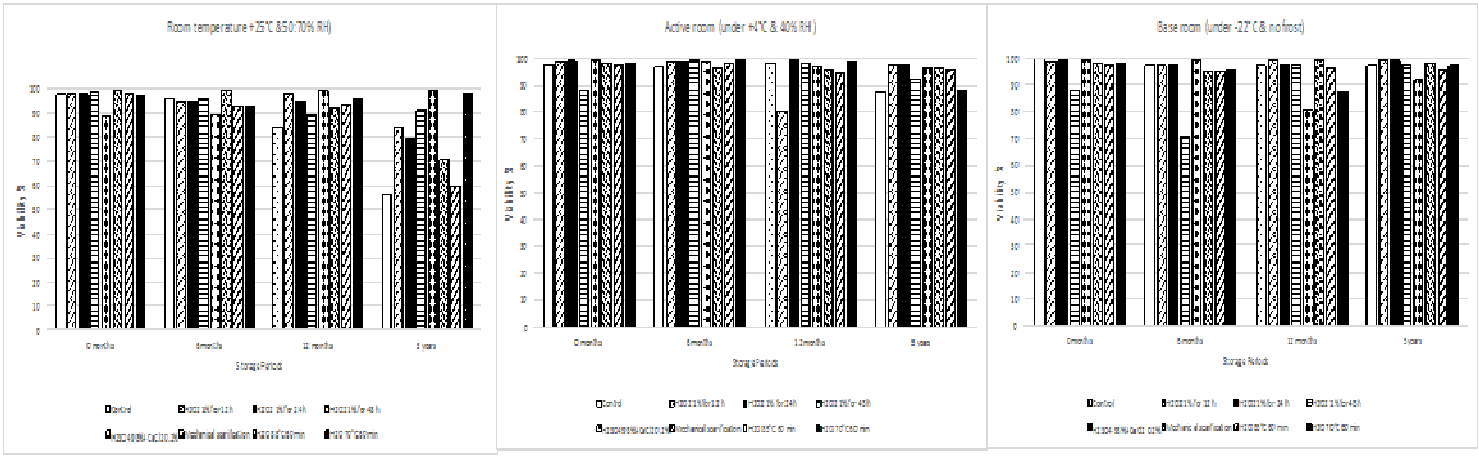

Figure 9 Effect of the interaction between seed storage condition, seed storage period and dormancy-breaking treatment on the viability percentage V%

germination percentage. Treating fresh “un stored” seeds with 1% H_2O_2 for 24 hresulted in standard germination percentage of 76.94%at room temperature, whereas storage of seeds for 6 months, 12 months and 5 years followed by the same pretreatment decreased the standard germination percentage. Storing seeds at room temperature without any treatment decreased the standard germination percentage. Storage of seeds at +4°C for12 months followed by mechanical scarification resulted in the highest standard germination percentage of 81.77% at this temperature; when treated with 1% H_2O_2 for 24 h, the standard germination percentage was 76.20%. At -22°C for 12 months, seeds treated with1% H_2O_2 for 24 h resulted in the highest standard germination percentage of 80.49% within this treatment group.

The data presented in Figure (9) show the effect of the interaction between seed storage conditions, seed storage periods and dormancy-breaking treatments on viability percentage of *Citrullus colocynthis* seeds

Germination rate

The data indicate that the germination rate was affected by storage conditions, storage periods and dormancy pretreatments. The optimal conditionsfor rapid germination were +4°C,a storage time of 6 months and treatment with 1% H_2O_2 for 24 h (germination rate of 13.61), while storing seeds at room temperature followed by treatment with 1% H_2O_2 for 24 h resulted in a germination rate of 12.21, as didstorage at –22°C for 6 months followed by treatment with 1% H_2O_2 for 24 h.Thus, cooler storage conditions are more optimal for an increased germination rate, followed by treatment with 1% H_2O_2 for 24 h or mechanical scarification, regardless of storage period.

Effect of seed storage conditions and storage temperatures on the percentage viability of *Citrullus colocynthis* seedsas assessed byTZ

The results of the TZ test revealed that the percentage of viable seeds increased with decreasing storage temperatures (Figure 10); that is; room temperature < +4°C < –22°C.

The percentage viability was the highest in the freshly harvested (i.e. control) seeds (97%; Figure 11). The percentage viability of seeds stored for 6 months was 91.70%, while that of seeds stored for 12 months was

93%. By contrast, the percentage viability of seeds stored for the maximum of 5 years was 88%.

DISCUSSION

Citrullus colocynthis seeds germinate under effective pretreatments mainly on seed coat like mechanical scarification, H_2O_2, KNo_3 and hot water. It could be said that this plant *Citrullus colocynthis* seeds dormancy presumably mechanical dormancy from seed coat. Results indicated that mechanical resistance of seed coat against seed emergence cause *Citrullus colocynthis* seeds dormancy.

Citrullus colocynthis seeds need to postharvest periods for six to 12 months to germinate with high percentage. These results are similar to those obtained for watermelon seeds in a previous study in which immature seeds harvested 28 days after flowering lost their ability to germinate after only 4–5 years of storage. However, mature seeds (collected 42–49 days after flowering) retained their full germination potential even after 10 years of storage, the same trend obtained by Nerson and Paris (2001). These authors found fresh *Citrullus colocynthis* and one-year-old stored seed germination were 6.67% and 66.67%, respectively.

The percentage of viable seeds was affected by different combinations of storage periods, storage conditions and dormancy pretreatments. Storing seeds at room temperature gradually reduced the viability of seeds, with the highest viability recorded with fresh seedswhile lower seed viability was recorded in seeds stored for 5 years (e.g. 98.14% versus 87.97% of seeds viable when stored for 5 years at +4°C versus room temperature). Storage at -22°C for 5 years appeared to be the most optimal for maintaining viability. These results are in agreement with those reported by Paul and Sen (1987). Similar results were reported by Roos and Davidson (1992). These authors investigated the viability of the seeds of various species stored at the National Seed Storage Laboratory and concluded that the seeds of most species examined lost at least 30% viability. Onions, peppers, and watermelons showed the greatest loss of viability (50%, 58% and 51%, respectively), while peas showed the least value (7%).

Fresh harvested seeds of *Citrullus colocynthis failed to germinate* according to the results obtained by Saberi and Shahriari (2011) who reported that many seeds fail to germinate after processing and placement in favorable growing conditions, such seeds known to be dormant. In some dormant seeds, morphological changes must take place before germination can start. For others, parts of the seed must undergo physiological changes before germination can occur. The present results determined that scratching is the most suitable method for dominance on seed dormancy of *Citrullus colocynthis* species.

Successful seed germination of *Citrullus colocynthis* under mechanical scarification treatments confirming mechanical resistance of shell against sprouts for emergence. Like this condition may observe in mention plants: *Parkia biglobosa* (Aliero, 2004), *Tamarindus indica* (Mohammad and Amusa, 2003), *Ulex europaeus* (Sxitus et al., 2003), *Medicago* (Uzen and Aydin, 2004), *Ferula gummos* and *Teucrium polium* (Nadjafi et al., 2006). Allan *et al*. (2004) concluded that germination was increased by using dormancy-breaking treatments.

CONCLUSION

Storing seeds under room temperature (control) were saved seeds viability up to one year. Storing seed under +4 ^oC (active) or -22 ^oC (base) were saved the viability of seeds to 5 years. Physical dormancy was a major hurdle for completed and rapid germination seeds of *Citrullus colocynthis*. Pre-treated seeds of *C. Colocynthis* with 1% H_2O_2 for 24 hours enhancing germination. Also, storage seeds of *C. Colocynthis* in base room of -22 ^oC at 12 months and treated seeds with mechanically scarification, gave the highest germination percentage.

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