Changes in Biochemical and Isozymes Components of Watermelon seeds during accelerated Ageing Technique

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Abstract: The aims of this work was to study some changes in the total content of storage components of watermelon (*citrullus lanatus*) seeds during accelerated ageing technique and its relation to seeds viability. **Materials and Methods**: Before the experiment, seeds were stored for two years in store house at 25C° in the start experiment, ageing at 50°C with 17% moisture up to 24, 48, 72, and 96 hours respectively. Germination percentage was decreased, a reduction in the total content of storage components such as proteins, carbohydrates, in addition, increasing oils and decreases in the activities of various esterase enzymes under the same condition were observed. **Results:** It was clearly that 50°C with 17% moisture content could be used as a good ageing seed testing condition for watermelon seeds In the present study The treatments watermelon seeds could be identified by Biochemical analysis (Esterase isozyme and Protein) banding pattern.
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Key words: Watermelon, accelerated ageing seed, seed germination.

1. Introduction:

Watermelon (*citrullus lanatus)* is one of important horticultural crops belonging to the family cucurbitaceae mainly propagated by seeds. Ageing tests have been developed to predict emergence performance , seed vigor and comparing seed lost quality **(Yanmaz *et al.*, 1999; Matthews, 1994; McDonald, 1980).** The deterioration of the stored seed is a natural phenomenon and the seeds tend to loose viability even under ideal storage conditions **(Ayyappan *et al.*, 2006; Bhatti and Sata, 1997).**

The seeds with low viability are rejuvenated /multiplied. Frequent multiplication results in genetic drift due to which genetic integrity is impaired, it also involves high risk of out crossing and mechanical mixture during multiplication, so it is very important to prolong the seed longevity. There are number of factors that affect seed longevity in storage **(McDonald, 1999; Powell, 1998)**. Among these factors are temperature and seed moisture content/relative humidity. There are some other factors which can decrease seed longevity, e.g. varietal differences in seed viability.. Therefore, it is important to assess seed vigour and viability during storage. Seed ageing as an important parameter to assess /estimate the seed viability and vigour accelerated ageing which is a good vigour test for various crop seeds **(Anonymous,1983)** and could be used to predict storability of seed lots **(tyagi,1992)**.

Accelerated ageing treatments involve exposing seeds to severe adverse storage conditions, i.e.-raised temperature and high moisture contents for specific periods of time.

The aims of this work was studying the changes in the total content of storage components of watermelon seeds during accelerated ageing technique and its relation to seed viability, in order to reduce the time of storage experiments (which took a long time) to know the extent of changes in content of storage components of seeds.

2. Materials and Methods:

21. Materials

1) Plant material and ageing conditions:-

Watermelon (*citrullus lanatus l.cv giza1*) seeds were collected from plants grown in Moshtohr field at Horticulture Research Institute, 2007.

Seeds were stored for 2 years in store house at 25±-2°C before the experiment started. They were soaked in water for 24 hours at 4°C then air dried for 4 hours. During the experiment, seeds were aged at 50°C and 17% moisture content, stored up to 96 hours (4 days).

Samples of watermelon seeds were collected every 24 hours.

2.2. Methods:

2.2.1. Germination tests:

Germination tests were carried out on four replicates of 25 seeds. The seeds were set to germinate in between moistened paper towels. Seeds were kept at 25°C±2 for 14 days. The numbers of germinated seeds were counted daily up to 14 days in watermelon seeds. At the final count the number of normal and abnormal seedling. Then survival curves were constructed from these results, germination percentage, were recorded.

2.2.2. Determination of the total carbohydrate:

Total carbohydrates were extracted according to **A.O.A.C. (1990)** 0.1 g of air-dried sample was hydrolyzed with 1 N HCl by refluxing for 6 hrs in a boiling water bath. The obtained solution was filtered, neutralized and the total volume was made up to 100 ml with distilled water. Resulted total reducing sugars was determined calorimetrically using 1 ml of sample with alkaline potassium ferricyanide reagent. The amount of total carbohydrates was determined according to the standard curve of glucose**.**

2.2.3. Determination of total protein:

The dried parts of the plants were used. Total nitrogen in plant was determined based on micro-Kjeldahl method accorded to **Markaham (1942)**, using boric acid modification as described by **Ma and Zuazage (1942),** under steam distillation using Buchii 320 unit, and was calculated as nitrogen percent.

The protein content was calculated as follows: Protein% = Nitrogen% x 6.25.

2.2.4. Determination of total oil:

Seed Oil percentage in dry seeds was determined using Soxhlet apparatus and petroleum ether as a solvent according to **A. O. A. C. (1970).**

2.2.5. Biochemical Markers

2.2.5.1. Protein analysis

SDS-Polyacrylamide gel electrophoresis was performed in 12 % acrylamide slab gels following the system of **Laemmli (1970)** to identify their protein profiles. Young fresh leaves were collected from all studied plants and immediately ground in a mortar using liquid nitrogen. The seeds were grounded to a fine powder using a mortar and pestle, homogenized with 1 M Tris-HCl buffer; pH 6.8 in clean eppendorf tube, left in refrigerator over night, then, centrifuged at 10000 rpm for 10 min. The supernatant of each sample (contains protein extract) was kept in deep-freeze until use for electrophoretic analysis, then boiled for 5 minutes in water bath before loading in the gel.

2.2.5.2. Isozymes Electrophoresis

Native polyacrylamide gel electrophoresis was used to study isozyme variation among the ten populus accessions. The staining solution was composed of 50 ml of 1M Na-Acetate; pH 4.7, 50 ml of Methanol, 50 ml 3,3,5,5 tetra-methylbenzidine (TMBZ) and 2 ml of 30% H_2O_2 while for esterase (Est.) the staining solution composed of 50 ml of 100 mM Na – Phosphate; pH 6.0, 25 mg of α – Naphthyl Acetate and 50 ml of fast blue RR salt according to **Scandalios (1964).**

4. Results:

4.1. Effects of an Accelerated Ageing Technique on chemical Components of Watermelon seeds on total protein ,total carbohydrates, and total oil contents are illustrated in table (1) and figures (1, 2 and 3)

Table (1): Effect of Ageing on total protein (%), total carbohydrate (%) and total oil (%) in watermelon seeds.

Treatment	Total protein (%)	Total carbohydrate (%)	Total oil (%)
Untreated treatment	43.75 a	4.06 a	49.34 c
50 °C with 17% moisture for 24 hours	41.15 b	3.75 b	50.6 b
50 °C with 17% moisture for 48 hours	40.35 b	3.52 c	51.4 b
50 °C with 17% moisture for72 hours	38.9 c	3.34 c	52.4 a
50 °C with 17% moisture for 96 hours	37.75 d	3.08 d	53 a

Values in the same column followed by the same letter(s) do not significantly differ from each other according to Duncan's multiple range tests at 5% level.

Data in table 1 and Fig (1) showed high significant differences in watermelon seeds between untreated treatment seeds and accelerated ageing treatment seeds total protein was significantly decreased in ageing seeds for 50 C° with 17% moisture for (4 days) from 43.75% to 37.75%.

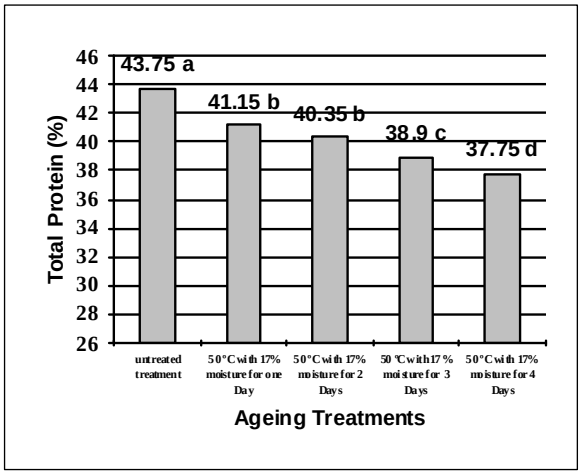


Fig 1: Changes in total protein of watermelon seeds before and during accelerated ageing technique.

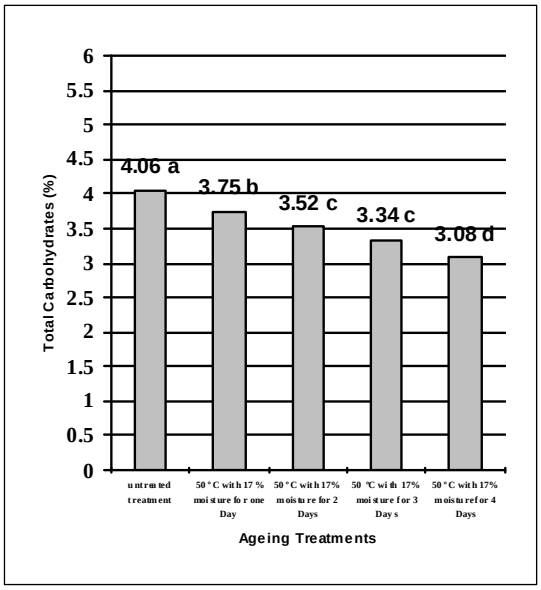


Fig 2: Changes in total Carbohydrate of watermelon seeds before and during accelerated ageing technique.

It is obvious from results shown in fig (3) that significant increase in total oil were detected due to accelerated ageing of watermelon seeds compared with untreated treatment. Total oil (53 %) were obtained from the high level of 50 ºC with 17% moisture for 4 Days while the lowest level (49.34 %) were obtained from untreated treatment.

4.2. Effects of an Accelerated Ageing Technique on Germination percentage of Watermelon Seeds.

Table (2) shows that the germination percentage of watermelon seeds decreased from 60% in untreated treatment seeds to 24% for aged seeds, after 96 hours (4 days).

Treatment	Germination (%)
Untreated treatment	60 a
50 ºC with 17% moisture for 24 hours	48 b
50 ºC with 17% moisture for 48 hours	37 c
50 ºC with 17% moisture for72 hours	30 d
50 ºC with 17% moisture for 96 hours	24 e

4.3. Effects of an Accelerated Ageing Technique on Electrophoretic banding patterns of proteins of Watermelon seeds. "Biochemical markers"

4.3.1. Watermelon seeds protein electrophoresis.

The watermelon seeds protein banding profile which was separated by using SDS-PAGE are illustrated in fig. (4). The total number of bands was 14 with molecular weights ranged from 18.4 KDa to 116 KDa. The highest number of bands was 13, detected in clone no. 1 (control) and clone no. 2 (while the lowest number of bands was 11, identified in *clone* no 4 and clone no. 5.

Fig. (4), is a dendrogram which demonstrated the distance between watermelon seeds under investigation.It showed that treatments of seeds watermelon were separated into two major groups at a distance of 2.5.

The first group included clone no. 4.

The second group involved each clone no. 1 and clone no. 2 was delimited from the first group at a distance of 10.75, while clone no. 3 was delimited, as well, from clone no. 1 and clone no. 2 at a distance of 6.75.

Demonstrative analysis of the presence and absence of bands were assessed with (1) and (0), respectively, are illustrated in Table (3). It is observed that 10 bands were mono-morphic (106.886, 85.898, 75.31, 59.478, 53.786, 44.411, 76.495, 71.709 and 70.015KDa), while 4 bands were polymorphic, giving 28.57% polymorphism. The matrix of similarity index for watermelon seeds is presented in Table (4). The

highest coefficient was 95.2 recorded between *clone no. 4 and clone no. 5* followed by 88.9 recognized between clone no.1 (control) and clone no. 2, followed by 84.2 between clone no.1 (control) and clone no.3 and clone no. 2 and clone no.3 On the other hand, the lowest coefficient value was 60 observed between clone no.1 (control) and clone no.4.

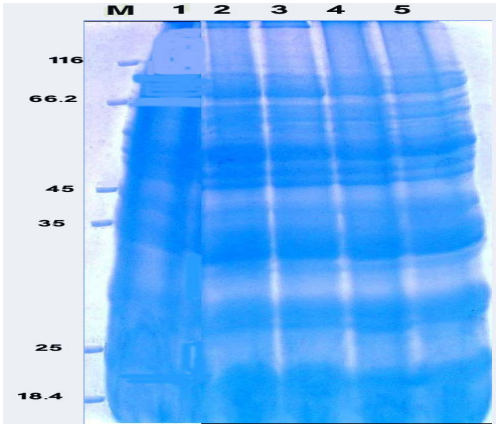


Fig. (3): SDS-protein banding patterns of seeds proteins
Clone no 1- untreated treatments
Clone no 2- ageing at 50C° with 17% moisture for one day
Clone no 3- ageing at 50C° with 17% moisture for two day
Clone no 4- ageing at 50C° with 17% moisture for three day
Clone no 5- ageing at 50C° with 17% moisture for four day

Table (3) Data matrix illustrating the presence or absence of bands in the seeds protein electrophoresis *banding patterns*.

No. bands	MW	Untreated treatment	Ageing at one day	Ageing at two day	Ageing at three day	Ageing at four day
1	106.886	1	1	1	1	1
2	85.898	1	1	1	1	1
3	75.31	1	1	1	1	1
4	68.765	0	0	0	1	1
5	60.64	1	1	0	0	0
6	59.478	1	1	1	1	1
7	53.786	1	1	1	1	1
8	44.411	1	1	1	1	1
9	33.483	1	1	1	0	0
10	76.495	1	1	1	1	1
11	76.444	1	1	1	1	1
12	71.709	1	1	1	1	1
13	70.015	1	1	1	1	1
14	18.064	1	1	1	0	0

Table(4) Proximity Matrix of protein results

	Untreated treatment	Ageing at one day	Ageing at two day	Ageing at three day	Ageing at four day
Untreated treatment		0.889	0.842	0.600	0.632
Ageing at one day	0.889		0.842	0.700	0.737
Ageing at two day	0.842	0.842		0.762	0.700
Ageing at three day	0.600	0.700	0.762		0.952
Ageing at four day	0.632	0.737	0.700	0.952	

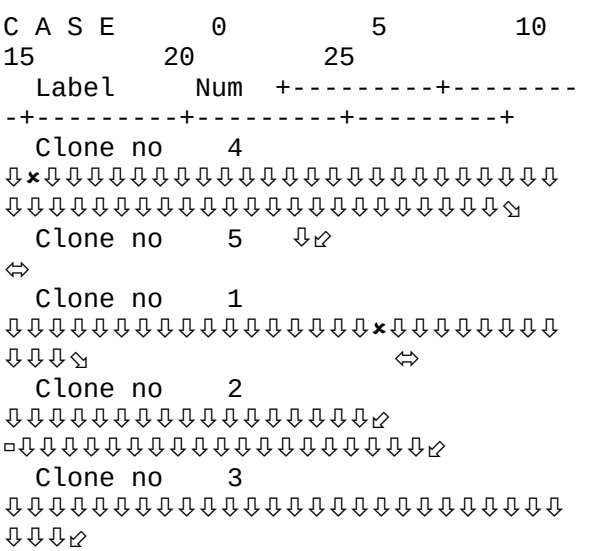


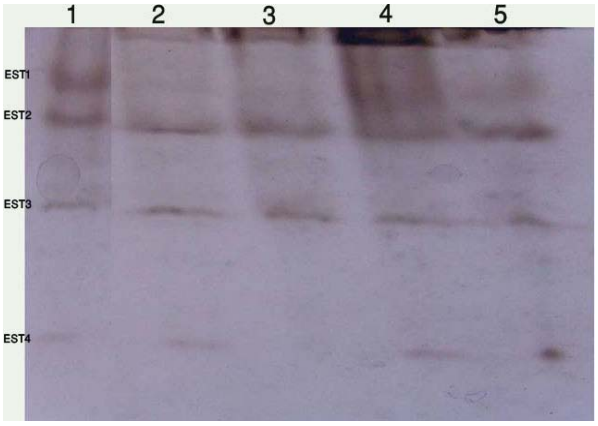
Fig (4) Dendrogram for the seeds treatment and untreated treatment of the protein data using UPGMA and Similarity matrices computed according to Dice coefficients.

Based on the similarity matrix developed by analyzing only the common bands between the different accessions representing each seeds a dendrogram (Fig. 5) was constructed. The obtained dendrogram was divided into two main clusters; one cluster included. clone no. 4 and 5, while the other one included 1, 2, and 3, the cluster no. 2 divided to two subcluster one of them included clone no. 3 while the other subcluster included clone 1 and 2 .the highest relationships was between clone no. 4 (ageing at 50C° with 17% moisture for three day) and clone no. 5 (ageing at 50 C° with 17% moisture for four day). It was found. The highest variation between clone no 1(untreated treatment) and clone no 4, 5 that indicate to the effects of accelerated ageing on banding patterns of proteins of watermelon seeds.

4.3.2. Esterase banding patterns:

Esterase banding patterns was illustrated in Fig. (6) and dendogram in fig (7).

Fig (6) represents esterase electrophoretic banding patterns among examined seeds, a total of 4 bands were identified in this study, which were presented in some seeds treatment and absent in some others. The analysis of data showed 3 bands were polymorphic with 75 % polymorphisms.



Clone no 1- untreated treatments
Clone no 2- ageing at 50C° with 17% moisture for one day
Clone no 3- ageing at 50C° with 17% moisture for two day
Clone no 4- ageing at 50C° with 17% moisture for three day
Clone no 5- ageing at 50C° with 17% moisture for four day

Fig. (6) Electrophoretic profiles of the esterase enzyme system

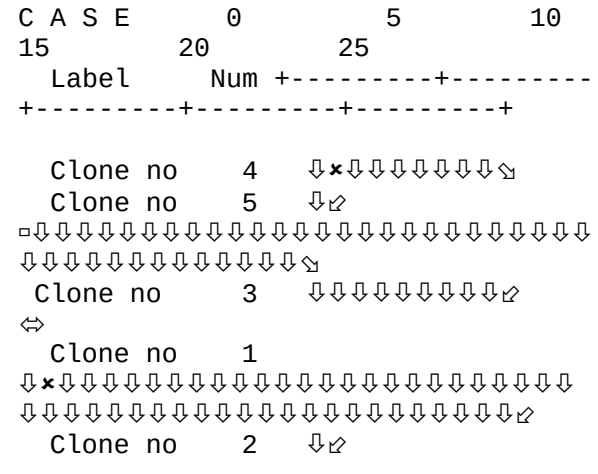


Fig. (7) Dendrogram for seeds treatment and untreated treatment of the esterase data using UPGMA and Similarity matrices computed according to Dice coefficients.

Estrase isoenzyme polymorphism detected in seeds watermelon (Fig.6), was represented by four zones of estrase activity Est.1, Est.2, Est.3 and Est.4 (in the order of increasing mobility from cathodal end). Results of seeds watermelon treatments showed that Est.1 was appeared in all treatments, results of zone Est.2 , Est.3 showed that new isoenzyme bands appeared and other disappeared, the highest close was between clone 1 (control) and clone 2, Est.3 showed decrease in its band between clone 3 and clone 4 with control.

Finally, the results obtained suggest that, the clone no. 1 (control with very close with clone 2 (Ageing at one day).

Based on the similarity matrix developed by analyzing only the common bands between the different accessions representing each seeds treatment and untreated treatment a dendrogram was constructed. The obtained dendrogram was divided into two main clusters; one cluster included. clone no. 1 and 2, while the other one included 4, 5, and 3 , the cluster no. 2 divided to two subcluster one of them included clone no. 3 while he other subcluster included clone 4 and 5. The highest relationship was between clone no. 1 (untreated treatment) and clone no. 2 (ageing at 50c with 17% moisture for one day). It was found. The highest variation between clone no 1(untreated treatment) and clone no 4, 5 that indicate to the effects of accelerated ageing on Esterase banding patterns.

4. Discussion:

The process of deterioration which occurs under these special ageing conditions is assumed to be similar to those which occur during natural ageing **(Delouche and Baskin, 1973; Perl *et al.*, 1978)**. The main difference being the speed at which these changes occur. A reduction in the total content of storage components such as proteins and carbohydrates **(Ayyappan *et al.*, 2006 and Agnieszka. *et al*., 2010)**. Increase in total oil were detected due to accelerated ageing (**Maqsood *et al.*, 2000).**

Results recorded high significant differences in watermelon seeds between untreated treatment seeds and accelerated ageing treatment seeds .Concerning total protein and total carbohydrate values, it was significantly decreased in ageing seeds for 50 C° with 17% moisture for (4 days). These results were in agreement with **Ayyappn *et al.*, 2006)** who found that total protein was decreased to half of initial content in cucumber seeds at 8th day of ageing as previously reported (**Ravikumar *et al.*, 2002)**, while the amount of free amino acids increased

gradually as previously reported (**Coolbear *et al.*, 1984**; **Ravikumar *et al.*, 2002)**. This increase in the amount of the free amino acids may be due to the hydrolysis of proteins during ageing.

Feeney and **Whitaker,(1982)**; **Ayyappn *et al.*, (2006)** who reported that the reduction of sugar content might be due to hydrolysis proved by Amadori and Maillard reactions. Starch. As well as wheat seed did not increase total carbohydrate levels as a result of accelerated ageing, on the contrary the amount of carbohydrates in these seeds slightly decreased **(Agnieszka., *et al* 2010)**.

In contrast, our results showed significant increase in total oil were detected due to accelerated ageing of watermelon seeds compared with untreated treatment. These results were harmony with (**Crowe *et al.* 1989**) who showed that addition of total fatty acids increased fusion of plant vesicles which led to an increase in membrane leakage. (**Copeland** and **McDonald 1995**) reported that continual accumulation of total fatty acids culminates in a reduction of cellular pH and is determined to normal cellular metabolism. Further more, it denatures enzymes resulting in loss of their activity. Individual cotton seeds containing 1% or more of free fatty acid usually will not germinate. The germination percentage of watermelon seeds decreased from 60% in untreated treatment seeds to 24% for aged seeds, within 4 days. The results were in agreement with **Yanmaz *et al.*, 1999** and **Alsadon *et al.* (1995)** found that cucumber seeds showed 82% of germination after storing at 24% moisture content and 45°C for 72 hours. In our experiment, at 17% moisture content and 50°C, cucumber seeds lost viability after the same period. Obviously, little variation in moisture and temperature had great effect on viability as pointed by **(Ellis and Roberts 1980)**.

Enzyme activity of esterase isozyme was parallel with the extent of germination of those seeds, since these activates owes mainly to esterase of endosperm. It was suggested that the endosperm of aged seed retains normal responsibility to the growth of seedling even if it was 2 years old (**Momotani, *et al.*, 1989**).

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