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Plant Germplasm and Extreme Conditions of Outer Space

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Abstract

The extreme temperature fluctuations and the vacuum of the space environment make growing plants in outer space challenging. To simulate the temperature fluctuations and vacuum conditions associated with space environments, dry tomato seeds were placed in a thermal cycle simulator and vacuum simulator chamber of space systems, respectively. A Bradford method was used to determine the total protein content of each group of seeds. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis was used to separate proteins. The seed of the thermal cycle group had the highest protein content (26 to 31 mg/ml), followed by control seeds (8-10 mg/ml) and the vacuum seeds (4-5.6 mg/ml). The molecular weights of the peptides ranged from 8 to 42 kDa. The intensity of the protein bands was significantly different in the thermal cycle group from the other two groups, and vacuum group had the lowest intensity. Water and oil released from seeds in the vacuum environment resulted in a reduction of protein content. In the thermal cycle group, the total protein content and the intensity of the bands were significantly higher than those in the control group, which can be attributed to the degradation of storage proteins involved in seed germination in the control group.

Keywords: SDS-PAGE, Solanum lycopersicum, Space, Spacecraft, Thermal cycle, Vacuum

Nomenclature

KDa	kilodaltons
NASA	National Aeronautics and Space Administration
SDS-PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
LEA	late embryogenesis abundant
SNF4	Non-specific serine/threonine protein kinase
NsLTP-1	Non-specific lipid transfer protein 1 precursor

Introduction

Much basic and applied research is conducted under real and simulated space conditions in support of international space agencies [1-3]. Astronauts will likely have to spend more than one or two years on the Moon or Mars soon. On long-term human missions, food delivery can be costly and challenging [4]. A healthy and varied diet can be provided for astronauts during space missions by growing plants in space and using closed life support systems. Further, plants contribute to the restoration of the atmosphere (by releasing oxygen and fixing carbon dioxide) and to the purification of water (through transpiration) [5].

"plant germplasm" encompasses a broad range of plant material from seeds to pollen to fungus spores. The lifespan of germplasm in a dry state is determined by storage conditions, especially the ambient temperature [6]. Higher plants have evolved seeds as essential components that enable drought tolerance and reproduction after long-term dry storage [7]. Seed aging is a process that can lead to the complete loss of seed viability. This process is triggered by the prolonged storage or controlled deterioration of seeds, which may result from extreme dryness, lack of oxygen, a vacuum environment, or a fluctuation in Earth's temperature or outer space [8, 9]. In the dry state, seeds can lose viability and die due to extreme temperatures or accelerated aging or denaturation of cell structures due to high temperatures [10, 11]. Despite this, seeds of different species of plants exhibit varying aging rates under similar storage conditions [12].
In the dehydrated state, seeds are susceptible to proteomic changes. A proteomics approach has been

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useful for identifying each protein's functions and biological actions and the molecular mechanisms responsible for the aging of dry seeds [13, 14]. A recent study of maize seeds revealed that artificial aging affected the dry seed proteome, with 16 proteins showing increased expression [15].

The tomato (*Solanum lycopersicum L.*) belongs to the Solanaceae family and occupies the second-largest cultivation area in the world after potatoes [16]. Furthermore, tomatoes are one of NASA's candidates for cultivation on Mars and space travel. For example, NASA sent 12.5 million tomato seeds into space during the Challenger space shuttle mission on April 7, 1984. This experiment aimed to determine the effects of the space environment on the seeds, which remained in the earth's orbit for five years. In September 1997, NASA launched 20 pounds of tomato seeds into space using the space shuttle Atlantis. The seeds were placed in deep space conditions for 10 to 14 days. During both of these missions, after the seeds had been returned to the ground and recovered, a national project was conducted with the cooperation of a large number of schools in the United States of America, and the viability, germination percentage, and characteristics of the seedlings were examined [15]

In the context of space missions, it is imperative to determine which species can produce seeds that can withstand the harsh environmental conditions of space. In 1971, for the first time, Stuart Rosa brought hundreds of seeds of tree species into orbit around the moon so that researchers could study their growth on Earth after returning to Earth [17]. As a result of the vacuum and extreme temperature fluctuations in space, space scientists face significant challenges when it comes to the transfer of seeds and the cultivation of plants in space.

Seed proteins play a critical role in endurance for extreme dryness or drought-like conditions. Therefore, the present study aimed to simulate the stress conditions of the space environment, including thermal cycles and vacuum for seeds to determine effect of these stresses on the total protein content and protein profile of dry tomato seeds.

Materials and methods

The tomato seeds (*Solanum lycopersicum* cv. Superchief), obtained from Pakan Bazar Company of Isfahan, were placed in the thermal cycle simulator to simulate the conditions of temperature fluctuation in space. An aluminum container was prepared and dry tomato seeds were placed inside it so that heat transfer could take place effectively (Fig. 1). Afterward, the seeds were subjected to a thermal cycle involving temperatures ranging from -72 to +80 degrees Celsius with a change rate of 5 degrees per minute. Seeds were exposed to +80°C for 15 minutes and -72°C for 15 minutes. The test lasted one hour and twenty-five minutes (Fig. 2).

Fig. 1. Thermal cycling simulation of the space environment in a simulator and how to insert dry tomato seeds into the simulator. (A) A simulator for simulating the thermal cycle of the space environment. (B) A view of the interior of the thermal cycle simulator chamber illustrating how seeds are placed inside. (C) An aluminum chamber containing seeds.

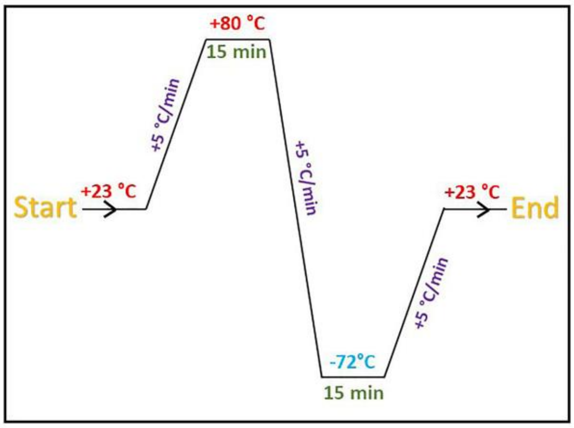

Fig. 2. A Schematic diagram of the temperature cycle applied to seeds.

Dry seeds were placed in the vacuum simulator for space systems (Fig. 3) to simulate space's vacuum conditions. Initially, the air pressure inside the chamber was zero and gradually increased to 10-4 mbar. At the time of applying the vacuum, the ambient temperature was +25°C. The test lasted 4 hours and 23 minutes, and the samples were placed under a vacuum for one hour (Fig. 4A, B). Seeds not exposed to vacuum conditions and stored at room temperature (+23 °C) were considered as control.

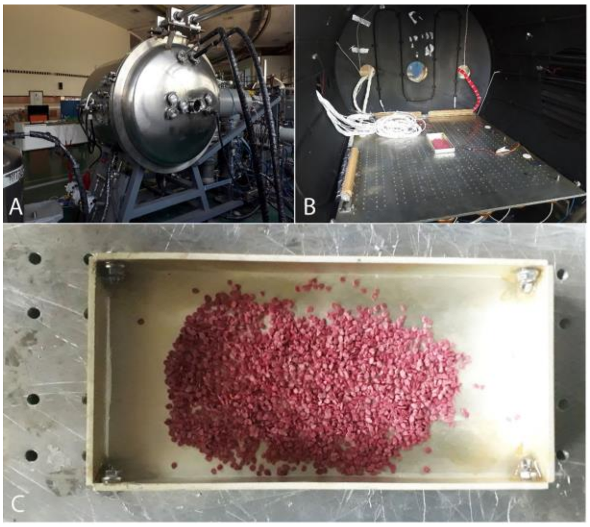

Fig. 3. A vacuum simulator for a space system and how to place dry tomato seeds inside the device. (A) A chamber for simulating the vacuum environment in space. (B) An interior view of the chamber used for simulating a space vacuum environment. C) Dry tomato seeds and how place them in the chamber.

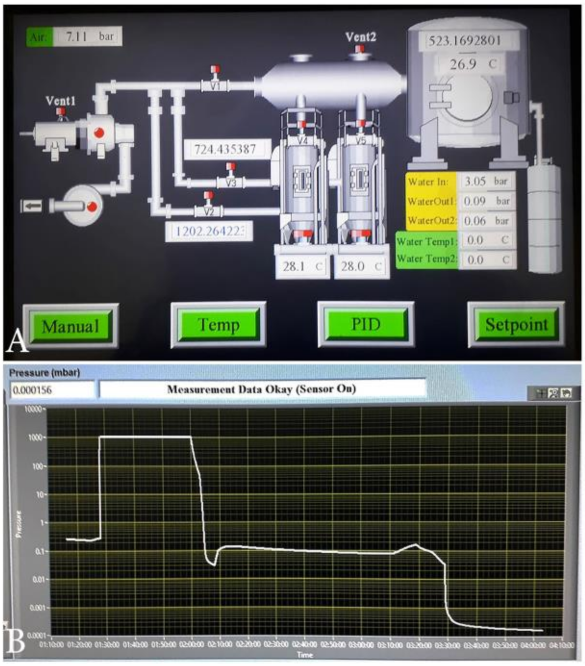

Fig. 4. (A) A diagram illustrating the mechanical construction of a space simulation vacuum chamber (B) and the vacuum simulation process up to 10-4 mbar.

The extraction of soluble and insoluble proteins from dry tomato seeds was performed in three groups (vacuum, thermal cycle, and control) using the method described by Miskoska-Milevska et al. [18]. Dry ground seeds were mixed with 0.0625 mol TRIS-HCl (pH=6.4), 2% (w/v) SDS, 5% (v/v) mercaptoethanol, 10% (w/v), and glycerol in a ratio of 1:2.5. The mixture was homogenized by vigorous vortexing and allowed to stand at room temperature for two hours. It was then denatured at 95°C in a water bath for two minutes. Following these steps, sonication was performed six times on ice and the mixture was centrifuged at 1000 to 2000 rpm. Supernatants were used for electrophoresis analysis. The Bradford method quantified the seeds' protein content [19]. In this method, the total protein concentration of a sample is determined. An SDS-PAGE analysis was used to determine the qualitative content of tomato seed proteins. As a result, SDS-PAGE was performed on a 15% acrylamide gel according to Laemmli's method (19). The intensity of protein bands was calculated using Image J software (Version 1.410).

Results

A Bradford test revealed that seeds treated with thermal cycle have the highest protein content (26-31 mg/ml), while seeds treated with vacuum have the lowest protein content (4-5.6 mg/ml). However, the total protein content of seeds in the control group ranged from 8 to 10 mg per ml. Fig. 5 presents the storage protein profiles for tomato seeds in the control group, under simulated space thermal cycle treatment and vacuum treatment in space. As a result of the extraction method utilized in this study and the use of 15% separating gel, eight distinct protein bands in all three seed groups were identified. Peptides were detected in the molecular weight range of 8 to 42 kDa. The intensity comparison of protein bands between three groups showed significant differences (Fig.6). The bands with the highest intensity were found in the thermal cycle, control, and vacuum, respectively.

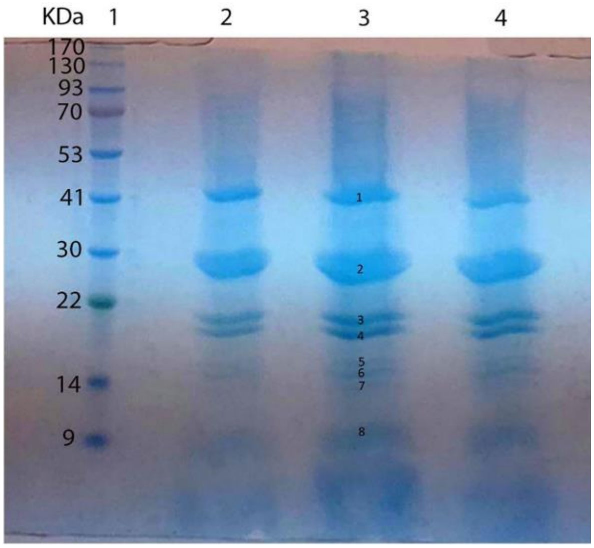

Fig. 5. Comparison of protein profiles of tomato (*Solanum lycopersicum*) seed storage proteins in 3 groups (vacuum, thermal cycle, control). Lane 1: Molecular weight marker. Lane 2: Coomassie-stained SDS-PAGE of tomato seed extract in simulated vacuum treatment. Lane 3: Coomassie-stained SDS-PAGE of tomato seed extract in simulated thermal cycle treatment. Lane 4: Coomassie-stained SDS-PAGE of tomato seed extract in the control group.

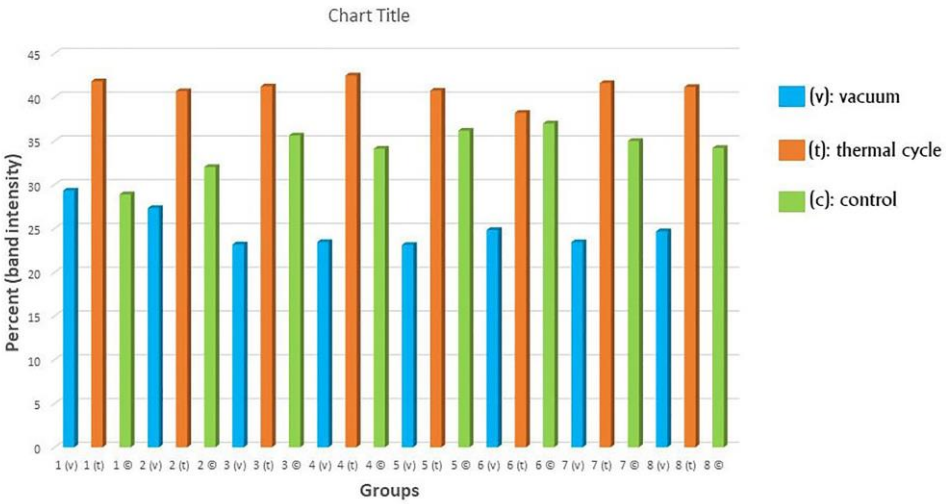

Fig. 6. Densitometry analysis of protein levels expressed in band intensity. Bands 1 to 8 were compared in all three groups (vacuum, thermal cycle, control).

Discussion

Plant growth in space is challenged by the vacuum or atmosphere prevailing on other planets, which is unsuitable for plant growth and hinders survival and seed germination. A lack of oxygen and extreme dryness are two characteristics that can be produced naturally in the vacuum of space and artificially in plant seed banks on Earth. The extreme temperature fluctuations of the space environment are another major challenge for plant biologists. Temperature in outer space can range from very cold, hundreds of degrees below zero, to very hot, hundreds of degrees above zero (especially when the spacecraft is near the Sun). Shifts in the expression of proteins are among the first reactions of seed to stress. In this regard, the seeds have a glassy cytoplasm in the dry state, and the molecular mobility and the speed of chemical reactions are greatly reduced. Still, the molecules are not completely limited in their movement. Accelerated aging, deterioration, temperature, humidity and ultra-drying can affect molecular mobility in dry seeds and cause molecules to diffuse at a low rate. This can cause structural changes in proteins. In the present study, the ultra-drying (vacuum) and the temperature fluctuations of the space environment were simulated, and the effect of these two parameters on total protein content and protein profile of dry tomato seeds was compared by the Bradford method and SDS-PAGE electrophoresis, respectively. The results of determining the protein profile of tomato seed using the SDS-PAGE method were consistent with the total protein content. Based on SDS-PAGE analysis of dry tomato seeds, the results largely agree with previous studies by other researchers [18, 20, 21]. Anyway, there are slight differences in protein profiles between studies, which can be attributed to several factors, including the method of preparing and optimizing the protein extract, the ratio of separating gel to proteins, and the variety of plants used in the study [22]. Legumin, Putative vicilin, LEA (late embryogenesis abundant), a Non-specifc serine/threonine protein kinase (SNF4), 9, Non-specific lipid transfer protein 1 precursor (NsLTP-1), Non specific lipid transfer protein 2 (NsLTP-2), Profilin and a pathogenesis-related protein are among the important proteins that are in the molecular weight range of 8 to 42 kDa [23-25]. Regarding the effect of vacuum conditions, there is evidence that seeds undergo changes in their biological structure when placed in the outer space vacuum. As an example, water and oil molecules leave the seeds. As a result of these changes, seeds may have a different biochemical composition and proteome, ultimately affecting their survivability and germination ability [9]. Consequently, it appears that the removal of water and oil from the seeds caused a decrease in the content of water-soluble and fat-soluble proteins, leading to a reduction in the total protein content of seeds as well as a decrease in the intensity of protein bands in the vacuum group as compared to those in the control group. In general, the degradation of proteins occurs in the aging or deterioration of seeds, resulting in a decrease in the intensity of protein bands or their disappearance [26]. In this regard, several other researchers have also reported the degradation of seed proteins in terms of the reduction of bands and their intensity with increasing seed age or deterioration

[27-29]. The decrease in the content of soluble proteins in quiescent dry seeds has already been reported during accelerated aging and long-term storage for several seeds [30-32]. This decrease in soluble protein content in seeds is correlated to the loss of seed vigor and viability [13, 33-35]. However, the present work is the first study documenting total protein content reduction and protein bands' intensity under vacuum conditions. Although increased expression of proteins in a dry

system is scarce, the present study showed a significant increase (2.5 to 3 times) in total protein content and an increase in the intensity of protein bands of the seeds of the thermal cycle group compared to the control group. In agreement with the results of the present study, the increased expression of 16 proteins in dry maize seeds after treatments associated with artificial aging has been reported. In this regard, an increase in proteases and breakdown of storage proteins were caused by artificial aging, which disrupted metabolism and energy supply, ultimately causing seed deterioration [15]. Although thermal treatment of seeds affects the extractability of proteins and can also lead to changes in the secondary structure of proteins as well as an increase the presence of proteins associated with the cell wall (Yu 2005; Gall et al, 2005), It seems that in the present study, some storage proteins were degraded in the control group, while those proteins remained unchanged in the thermal cycle group. This is probably due to the role of those proteins in the process of seed germination. In this regard, Legumes and vicilins are among the most important and abundant storage proteins in tomato seeds [25]. Legumin proteins act as nitrogen donors for seedling growth during germination [36]. Besides their defensive role, vicilins are the main source of nutrition during seed germination [37]. 14-3-3 proteins also play diverse roles during seed germination [38]. One of the roles of nsLTP is involvement in plant development processes, namely embryogenesis and seed germination [39, 40]. Some members of profilins have essential roles during plant development [41].Pathogenesis-Related Proteins play important roles in seed germination [42]. Therefore, it seems that the degradation of these proteins in the control seeds provides the necessary energy for seed germination. When seeds deteriorate, they lose quality, viability, and vigor, become more sensitive to stresses and lose their germination ability [43, 44]. It is therefore imperative to explore plant species and varieties whose seeds are able to maintain their viability under harsh conditions of the space environment, such as vacuum and extreme temperature cycles.

Conclusion

The present study investigated the effect of the stresses of the space environment such as thermal cycles and vacuum environment on plant germplasm i.e. dry seeds. The quantitative and qualitative content of storage proteins of tomato seeds was studied under the simulated thermal cycle of space and a simulated vacuum of space. When it comes to space missions, it is critical to know which species can produce seeds that can withstand the extreme environmental conditions of space. Besides bio-space applications and producing species capable of withstanding high temperatures, understanding the mechanisms of tolerance to high temperatures in dry seeds can also be helpful for climate change research, since these species contain unknown genes that can be applied to agriculture or forest management.

Conflicts of interest

The author declare that she has no conflict of interest.

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