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Nota Técnica

Germination of *Punica granatum* seed according to temperature and storage period

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Abstract

The aim of the study was to evaluate the germination of *P. granatum* seeds after storage under different temperatures. The experiment was performed at Universidade Tecnológica Federal do Paraná - UTFPR - Campus Dois Vizinhos. The seeds were stored in sealed glass jars under temperatures of 5°C, 10°C e 25°C, por 0, 30, 60, 90, 120, 150, 180, 210 and 240 days. After each period, the seeds were sowed in Gerbox® boxes kept at 25°C. A completely randomized design was used, in a factorial 3 x 9 (temperature x period of storage), with four repetitions of 100 seeds each. After 60 days of implementation, percentage of germinated seeds, GSI and AGT, were evaluated. The data was transformed and submitted to variance analysis ($p \leq 0,05$) and Duncan's test. Seeds of *P. granatum* can be stored at the temperature of 5°C for 240 days.

Key words: punicaceae, pomegranate, seeds.

Germinação de sementes de *Punica granatum* de acordo com temperatura e período de armazenamento

Resumo

O objetivo do trabalho foi avaliar a germinação das sementes de *P. granatum* após o armazenamento em diferentes temperaturas. O experimento foi realizado na UTFPR - Câmpus Dois Vizinhos. As sementes foram armazenadas em frascos de vidro lacrados. Logo, procedeu-se o armazenamento nas temperaturas de 5°C, 10°C e 25°C, por 0, 30, 60, 90, 120, 150, 180, 210 e 240 dias. Decorrido cada período procedeu-se semeadura em caixas Gerbox® mantidas a 25°C. O delineamento utilizado foi DIC, em fatorial 3 x 9 (temperatura de armazenamento x tempo), com quatro repetições de 100 sementes cada. Após 60 dias da implantação do experimento avaliou-se o percentual de sementes germinadas, IVG e TMG. Os dados das variáveis foram transformados e submetidos à análise de variância ($p \leq 0,05$) e ao teste de Duncan. As sementes de *P. granatum* podem ser armazenadas a temperatura de 5°C por período de 240 dias.

Palavras-chave: punicaceae, romã, semente.

Germinación de semillas de *Punica granatum* de acuerdo con temperatura y período de almacenamiento

Resumen

El objetivo del trabajo fue evaluar la germinación de las semillas de P. granatum después del almacenamiento en diferentes temperaturas. El experimento fue realizado en la UTFPR - Cámpus Dois Vizinhos. Las semillas fueron almacenadas en frascos de vidrio sellados. Por lo tanto, se procedió al almacenamiento a temperaturas de 5 ° C, 10 ° C y 25 ° C, por 0, 30, 60, 90, 120, 150, 180, 210 y 240 días. Después de cada período se procedió a la siembra en cajas Gerbox mantenidas a 25 ° C. El delineamiento utilizado fue DIC, en factorial 3 x 9 (temperatura de almacenamiento x tiempo), con cuatro repeticiones de 100 semillas cada una. Después de 60 días de la implantación del experimento se evaluó el porcentaje de semillas germinadas,

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índice de velocidad de germinación (IVG) y tiempo medio de germinación (TMG). Los datos de las variables fueron transformados y sometidos al análisis de varianza (p≤0,05) y prueba de Duncan. Las semillas de P. granatum se pueden almacenar a una temperatura de 5 ° C durante un período de 240 días.

Palabras clave: punicaceae, román, semilla.

Introduction

Punica granatum L., commonly known as pomegranate (JARDINI & FILHO 2007), belongs to the Punicaceae family, being a woody and branched shrub native to the region from Iran to the Himalayas, Northwest India. Its cultivation is wide in all the Mediterranean region of Asia, America, Africa and Europe (LORENZI & SOUZA 2001, FERREIRA 2009).

It is cultivated on a large scale in regions of tropical and subtropical climates, being used both in fruit growing, in ornamentation and also as a medicinal plant (JARDINI & FILHO 2007).

Among the medicinal properties attributed to this plant, phytochemical analysis shows the presence of alkaloids and gallic tannins in the bark of the stem and fruits. In the leaves and seeds the presence of fatty acids, mainly, Punic acid (SOUSA et al. 1991) was verified. Due to the diverse functional properties and nutraceuticals, the fruit has gained great prominence worldwide (SUMNER et al. 2005). According to GARCIA (1992), the species has antibacterial and anti-inflammatory properties, MENEZES et al. (2008) reported it as astringent, hemostatic, antidiabetic, anthelmintic, antidiarrheal, antiseptic and antiviral. Despite this, it is still very little used in commercial orchards in Brazil, which makes it an alternative for use by producers eager for news.

To solve this situation and make it attractive, first is necessary to carry out the marketing with the population of the advantages that its consumption brings to health and the creation of commercial orchards.

The germination of these seeds is uneven and low in percentage, although there are no studies on storage forms that maintain their physiological quality (PIOTTO et al. 2003).

Studies on the physiological quality of seeds are of most importance, due to many degenerative changes which may occur, of physiological, chemical and physical origin, which occur after maturity, and are closely linked to the reduction or even loss of seed vigor. In this way, storage overlaps as an essential practice to conserve the physiological quality of the seed (AZEVEDO et al. 2003), and to reduce the speed of deterioration, since the improvement of the quality is not possible, even in ideal conditions (VILLELA e PEREZ, 2004).

Thus, seed storage becomes a safe and economical way of conserving plant genetic diversity, being efficient when it maintains the physiological potential for as long as possible, which depends on the behaviour of the seed along storage (COSTA 2009) and the condition in which it is.

The most relevant factors for conservation of seed viability are the environmental storage conditions, such as temperature and water content. To maintain the quality of orthodox seeds, low relative humidity and low temperature are required, thus the metabolic activity of the embryo reduces, consequently decreasing the deterioration (CARVALHO & NAKAGAWA 2012, LIMA et al. 2014, SMANIOTTO et al. 2014).

Due to the lack of information about this species, the objective of this work was to evaluate the germinative process of *P. granatum* seeds after storage at different temperatures.

Materials and methods

The experiment was carried out at the Laboratory of Plant Physiology, at the Universidade Tecnológica Federal do Paraná - Campus Dois Vizinhos.

Seeds of mature fruits of *P. granatum* were used, from the orchard of the respective institution. The seeds were extracted manually, then lime was added to facilitate the removal of the aril by friction in a fine mesh sieve, with successive washing in running water. The seeds were then kept in the shade for 24 hours at room temperature to remove the excess of moisture.

After this process, the seeds were separated into batches of 100 units and stored in sealed glass jars of approximately 20mL. Therefore, incubated at temperatures of 5ºC, 10ºC and 25ºC. Storage periods were 0, 30, 60, 90, 120, 150, 180, 210 and 240 days.

After each period, seeds were sowed in Gerbox® boxes with lid, containing Germtest paper moistened as a substrate. For the germination tests, the boxes were placed in a B.O.D. at 25ºC.

The design was completely randomized, in factorial 3 x 9 (storage temperature x time), with four

replicates of 100 seeds each.

The counting was carried out daily, being considered as germinated the seeds that had visible radicle. After 60 days of the implantation of the experiment the percentage of germinated seeds, germination speed index (GSI) and average germination time (AGT) were evaluated. The data of the evaluated variables were previously submitted to the normality test of Lilliefors, confirming the need to transform all data. The transformation was carried out using √x+1 for GSI and AGT and sine arc √x 1 for germination. They were then submitted to analysis of variance ($p \leq 0.05$) and Duncan's test for comparison of means using SANEST® software.

Results and discussion

According to the analysis, there was a significant interaction between temperature and time in all variables analysed (Tables 1, 2 and 3).

For germination, the highest averages were obtained at 30, 60 and 210 days using a storage temperature of 5°C. At 10°C at 60, 90 and 210 days, and at 25°C at 60 and 90 days, with the latter reaching 50% germination (Table 1).

It should be noted in these data from Table 1, that in both storage temperatures, the seeds, despite varying their germination, were still viable, which may be associated with the use of the sealed glass jars to preserve them, avoiding exchanges with the environment.

The moisture content of the seeds stored in permeable packages is influenced more by the atmospheric conditions of the storage site, different from the impermeable ones that do not allow water vapour exchanges of the seeds with the medium in which they are stored and of the semipermeable ones that provide better resistance (SILVA et al. 2010). However, it is not possible to determine the effect of water permeability on the permeability of the soil.

The packaging that presents greater permeability and consequently greater exchange of humidity of the environment with the seeds, propitiates greater activity of microorganisms, insects and entails greater consumption of reserves, reducing the quality of the seeds (CONDÉ e GARCIA, 1984). However, the waterproof packaging maintains the physiological quality of the seeds for longer storage periods, since it reduces the availability of oxygen reducing the loss of dry matter mass, as well as insect proliferation (BAUDET, 2003).

Comparing the germinations obtained in each period, the highest averages were found at 30, 150 and 240 days at 5°C, and at 180 and 210 days, this did not differ statistically from 10°C. However, at 120 days there was similarity in superiority at 5°C and 25°C and at 90 days at 10°C and 25°C. At 60 days, both temperatures did not differ statistically from each other (Table 1).

Table 1: Germination of *P. granatum* seeds stored under different temperatures and storage time.

	Temperatures			
Time	**5°C**	**10°C**	**25°C**	
0	20,99 d	A 20,99 bcd	A 20,99 c	A
30	41,09 abc	A 27,24 bc	B 27,21 b	B
60	48,48 a	A 39,99 a	A 50,23 a	A
90	17,99 d	B 46,49 a	A 50,74 a	A
120	34,05 bc	A 17,21 d	B 29,79 b	A
150	35,86 bc	A 18,43 cd	B 18,16 c	B
180	30,89 c	A 28,23 b	A 7,94 d	B
210	43,18 ab	A 44,68 a	A 21,99 bc	B
240	36,07 bc	A 26,34 bc	B 15,46 c	C
CV (%)		12,27		

*Averages with different letters, lowercase in the column and uppercase in the row, differ significantly at a 5% level of probability by Duncan's test.

However, the percentage of germination remained low throughout the study. LOPES et al. (2001), working with pomegranate seeds, analyzed five fermentation periods in relation to the two conditions (with and without drying), concluding that they had characteristics of dormancy or drying sensitivity characteristics of recalcitrant seeds, which may have influenced these values obtained in the present study (Table 1).

The temperature variations considerably compromise germination speed, percentage and uniformity, and it is important to identify the most efficient combination between germination percentage and germination speed (MARCOS FILHO, 2005).

The germination speed index did not show significant differences from zero at 60 days in the three temperature conditions (Table 2). In the other periods, considering the 120, 150, 180, 210 and 240 days, the highest IVGs were with seeds kept at 5°C, which was statistically similar in the last two periods with the condition of 10°C (Table 2). Already at 90 days the highest averages were with seeds kept at 10°C and 25°C.

The higher superiority obtained at 5°C may be due to the lower metabolic activity provided to the

seed, thus favouring the conservation of the reserves, which are useful to allow greater vigour.

The average germination time of the pomegranate seeds stored at 5°C was lower at 0, 60, 90, 180 and 240 days, at 10°C at 0, 30, 60, 120, 180 and 240 days and at 5°C the highest AGT was obtained at 60 and 180 days, with 10°C at 60, 90 and 180 days, and at 25°C only at 60 days (Table 2). The results obtained with GSI at 25ºC partially confirm the hypothesis related to the possible higher amount of reserve in seeds kept at the lowest temperature, since it was only possible to maintain the highest vigour until 60 days, since from this period the same Consume their reserves to maintain the seeds allowing the least vigour.

Table 2: Germination of *P. granatum* seeds stored under different temperatures and storage time.

	Temperatures			
Time	**5°C**	**10°C**	**25°C**	
0	1,28 b	A 1,28 bcd	A 1,28 b	A
30	1,43 b	A 1,23 cd	A 1,27 b	A
60	2,44 a	A 1,96 a	A 2,31 a	A
90	0,72 c	B 1,72 abc	A 1,46 b	A
120	1,27 b	A 0,64 e	B 0,59 cd	B
150	1,18 b	A 0,57 e	B 0,40 cd	B
180	2,22 a	A 1,65 abc	B 0,27 d	C
210	1,35 b	A 1,84 ab	A 0,62 c	B
240	1,28 b	A 1,11 d	A 0,44 cd	B
CV (%)		10,02		

*Averages with different letters, lowercase in the column and uppercase in the row, differ significantly at a 5% level of probability by Duncan's test.

During storage, temperature and relative humidity are decisive in the process of loss of seed viability, also influencing the quality of the product and by-products (MALAKER et al. 2008).

According to a study conducted by ARAÚJO NETO et al. (2005) the storage of *Acacia polyphylla* DC seeds. In an environment without temperature control and air humidity was inefficient to maintain their physiological quality. However, when stored in impermeable packaging and in a cold room, the seeds remained viable for two years. Similar results were obtained by BARBEDO et al. (2002) with *Caesalpinia echinata* Lam seeds that lost viability when stored under normal ambient conditions (22 ± 7°C) in less than three months. On the other hand, under low temperature (cold chamber at 7 ± 1°C) the viability was maintained for up to 18 months, with germination higher than 80%.

In those maintained at 25°C, the results only stood out at 0 and 30 days (Table 3). Comparing storage temperatures, it can be seen that the use of the 5 and 10°C conditions showed the best averages, since they maintained less germination time up to 240 days, except for 180 days at 10°C (Table 3). This may be related to the need of pomegranate to the stratification to break its dormancy, thus allowing the shorter times for germination, as already verified by RAWAT et al. (2010).

Table 3: Average germination time (AGT) of *P. granatum* seeds stored under different temperatures and storage time.

	Temperatures			
Time	**5°C**	**10°C**	**25°C**	
0	21,54 ab	A 21,54 a	A 21,54 a	A
30	26,79 bc	A 25,47 ab	A 23,26 a	A
60	23,11 abc	A 25,23 ab	A 26,67 b	A
90	23,58 abc	A 28,49 bc	A 36,36 cd	B
120	27,71 c	A 25,67 ab	A 43,21 d	B
150	30,71 d	A 33,36 c	A 45,58 d	B
180	14,65 a	A 21,68 a	B 31,89 bc	C
210	32,61 d	A 32,78 c	A 46,68 d	B
240	23,67 abc	A 24,31 ab	A 35,45 cd	B
CV (%)		6,76		

*Averages with different letters, lowercase in the column and uppercase in the row, differ significantly at a 5% level of probability by Duncan's test.

Conclusion

The seeds of *P. granatum* can be stored at the temperature of 5oC under the period of 240 days. However, further studies are needed to increase the number of germinated seeds.

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