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SCIENTIFIC COMMUNICATION

PRE-GERMINATIVE TREATMENTS IN POMEGRANATE SEEDS (*Punica granatum* L.): EFFECT ON PHYSIOLOGICAL QUALITY[1]

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ABSTRACT - This study aimed to evaluate the efficiency of different removal methods of sarcotesta and their effects on the physiological quality of the seeds. We applied the following treatments: T1 - friction in coarse sand on a sieve; T2 and T3 - immersion in sulfuric acid (98%) for 30 minutes and washing in water, with and without a vernalization for 48 hours in a refrigerator at 4 °C, respectively; T4 and T5 - natural fermentation for 72 hours, with seeds submitted and not submitted to drying in shade for seven days; and T6 - seeds with intact sarcotesta as control. The experiment was carried out in a completely randomized design. For the analysis of the viability and vigor, we used the germination test, germination on first count, germination speed index, accelerated aging, emergence test, emergence speed index, number of leaves, plant height, stem diameter, and root length. The data were submitted to analysis of variance and Tukey test. Seeds submitted to fermentation showed the highest germination and emergence values. However, it was not statistically different from the control, probably due to the sensitivity to desiccation or seed dormancy, which was shown in the accelerated aging test.
Index terms: Pomegranate Tree, Sarcotesta, Dormancy, Physiology.

TRATAMENTOS PRÉ-GERMINATIVOS EM SEMENTES DE ROMÃ (*Punica granatum* L.): EFEITO SOBRE A QUALIDADE FISIOLÓGICA

RESUMO - Objetivou-se avaliar a eficiência de diferentes métodos de remoção da sarcotesta de sementes de romã e seus efeitos sobre a qualidade fisiológica. Foram empregados os seguintes tratamentos: T1 - fricção em areia grossa sobre peneira; T2 e T3 - imersão em ácido sulfúrico (98%) por 30 minutos e lavagem em água corrente, com e sem vernalização durante 48 horas em geladeira a 4ºC, respectivamente; T4 e T5 - fermentação natural por 72 horas, com sementes submetidas e não submetidas à secagem à sombra, durante sete dias respectivamente, e T6 - sementes com sarcotesta intacta, como testemunha. Utilizou-se o delineamento inteiramente casualizado, com dados submetidos à análise de variância, e as médias comparadas pelo teste Tukey a 5% de probabilidade. Observou-se superioridade nos valores de germinação e emergência para os tratamentos submetidos à fermentação, associando isto a possível sensibilidade à dessecação da semente e dormência, comprovado pelo teste de envelhecimento acelerado.
Termos para indexação: Romãzeira. Sarcotesta. Dormência. Fisiologia.

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The pomegranate (Punica granatum L.) belongs to the Lythraceae family and is a shrub or small tree that can reach a height of up to six meters (LIMA et al., 2006). It has several medicinal applications for being rich in vitamin E (α-tocopherol), vitamin C (ascorbic acid) and β-carotene (NODA et al., 2002), and is widely used in the food, cosmetics and plant industry and as ornamental plant for presenting beautiful flowers and pleasant appearance.

The propagation of the pomegranate can be done by seeds, since it is an economical and practical method, but the germination is considered slow, uneven and irregular, due to the presence of the sarcotesta that surrounds the seeds and present in their constitution phenolic compounds, anthocyanins and tannins (NODA et al., 2002), which act as inhibitory agents and/or delay seeds germination (TOKUHISA et al., 2008). However, several pre-germination treatments can be used to eliminate sarcotesta in seeds, such as mechanical (abrasion, crushing, smashing, etc.), chemical (use of acids, bases and salts) or natural methods (outdoor drying) (SILVA, 2000). However, the use of these treatments may compromise the seeds physiological quality.

The physiological quality of the seed is associated with its vigor. Carvalho and Nakagawa (2012) describe the seeds physiological vigor as the conjunction of storage potential, germination speed, resistance to adverse factors and emergence capacity. Therefore, the physiological quality in seeds is the sum of those properties that determine the potential level of activity and performance of a seed. The aim of this study was to evaluate the efficiency of different methods of removal of the pomegranate seeds sarcotesta and their effects on the physiological quality.

Seeds obtained from physiologically mature fruits of the "Molar" variety, harvested in an organic orchard at Tamanduá Farm in January 2014, in the municipality of Sousa - PB, were used.

A completely randomized design was used, with four replicates of 25 seeds for all variables. The data were submitted to analysis of variance and the averages were compared by Tukey test at 5% probability, using the SISVAR 5.1 statistical program.

The seeds were extracted from the fruits with the help of a knife and later homogenized and separated into six lots. For each lot a single pre-germinative treatment was applied, consisting of: T1 - friction in coarse sand on a sieve; T2 and T3 - immersion in sulfuric acid (98%) for 30 minutes and washing in water, with and without a vernalization for 48 hours in a refrigerator at 4 °C, respectively; T4 and T5 - natural fermentation for 72 hours, with seeds submitted and not submitted to drying in shade for seven days; and T6 - seeds with intact sarcotesta as control. In order to evaluate the effect of the pre-germinative treatments of the seeds, the germination test was used, using four replicates of 25 seeds, and the test was carried out according to the Rules for Seed Analysis (RSA) predicted for this species (BRASIL, 2009). In conjunction with the germination test, the first germination count and the germination speed index as described by Maguire (1962) were carried out. The accelerated aging test was also carried out by gerbox method at 45ºC for 72 hours (KRZYZANOWSKI et al., 1999), with determination of the total germination.

The emergence test in greenhouse was used, with four replicates of 25 seeds sown at one centimeter of deep in tubes filled with commercial substrate. The percentage of emerged seedlings, stem diameter, plant height, number of leaves and length of the main root and the shoot were determined using a digital caliper at 60 days after sowing.

When the seeds were submitted to Treatment 5, we obtained the best results for: germination, first germination count and accelerated aging when compared to the other pre-germinative treatments (Table 1), although the germination did not differ from the treatment in which the seeds were maintained with intact sarcotesta (T6). These results contradict the inhibitory effect caused by substances such as sugars, phenolic compounds, anthocyanins and tannins present in the sarcotesta that surround the pomegranate seeds, which can considerably reduce the germination (NODA et al., 2002). However, Henderson and Nitsch (1962) highlight that these constituents can act as activators or as inhibitors of the enzymatic system, favoring or not the auxin activity, consequently influencing the plant growth.

As for the treatments 2 and 3, in which the seeds were immersed in sulfuric acid (98%) for 30 minutes, provided deleterious effects to the physiological quality, leading to seed death (Table 1). Possibly, this occurred due to temperature elevation or to some physiological damage in the internal structure of the seeds, with embryo compromise, different from what was observed by Gokturk et al. (2012) that obtained significant values in all the treatments used.

In the seeds that were submitted to the treatment 4, a significant reduction in germination (Table 1) was verified when compared to those of the fermentation treatment without drying (T5). Zhu et al. (2014) report that certain periods of dehydration in seeds may inhibit the germination and the establishment of plants. Similar behavior was observed by Lopes et al. (2001) that, evaluating pomegranate seeds submitted to drying after fermentation, found a reduction in the physiological quality and attributed this to the possible sensitivity of the seeds to drying.

According to Brito et al. (2013), the fermentation process promotes temperature rise from 2 to 3ºC, heating the seeds, promoting not only the degradation of the sarcotesta by the action of the microorganisms present in the process, but also can contribute to the elimination of possible dormancies caused by the impermeability of the tegument.

The pomegranate seeds submitted to the different pre-germinative treatments and later exposed to the accelerated aging test were physiologically benefited by the increase of the germination percentage, with emphasis on the T5 treatment, which presented a germination percentage of 93%, significantly higher than the other treatments (Table 1). Although the accelerated aging test provokes high rates of respiration, consumption of reserves and acceleration of metabolic processes that lead to seed quality reduction (PONTES et al., 2006), deterioration and death (PIÑA-RODRIGUES et al. 2004), the conditions of high temperatures and the humidity of the test can also promote the overcoming of dormancy.

In this sense, observing the humidity data of the pomegranate seeds after the different pre-germinative treatments (Table 1), the degree of humidity of the seeds from treatment 5 showed humidity contents higher than 77%, while the fermentation treatment followed by drying (T4) reached levels below 8%. This behavior seems to show a possible sensitivity to the desiccation of the seeds that some species present, reflecting in the initial events of the germination process, as observed by Lopes et al. (2001).

Table 2 shows that, as for germination, the first germination count and accelerated aging (Table 1), the seeds submitted to the fermentation without drying (T5) showed emergence values and an emergence speed index superior to the other treatments. Possibly, this increase may be associated to the favor in the performance of metabolic activities and water absorption, resulting in a higher percentage of emerged seedlings, corroborating with the results observed by Lopes et al. (2001).

The treatment in which seeds were submitted to fermentation and drying (T4) hindered the emergence and the vegetative development of pomegranate seedlings (Table 2). On the other hand, the leaves number, seedlings height, stem diameter and root length of T5 and T6 treatments showed greater vegetative development.

These results show that there is no influence of the sarcotesta on the germination and vigor of the pomegranate seeds and that perhaps the presence of tegument impermeable to water intake is one of the reasons that lead to delays and inequality in the germination process of these seeds. For Chow and Lin (1991), the sarcotesta can prevent the germination due to the presence of inhibiting compounds, whereas Viggiano et al. (2000) observed dormancy in seeds deprived from sarcotesta.

The natural fermentation process in distilled water and sugar solution (10: 1) for 72 hours, followed by washing in running water without drying, guaranteed the increase in the percentage and speed of germination of pomegranate seeds; The presence of the sarcotesta affected the physiological quality of the pomegranate seeds, without, however, being a determining factor in the germination; pomegranate seeds subjected to fermentation and drying tend to reduce their physiological quality. Treatments with sulfuric acid at high concentrations and time promote the death of the pomegranate seeds when still surrounded by the sarcotesta.

TABLE 1 - Average values for germination (GER), first germination count (FGC), germination speed index (GSI), accelerated aging (AGI), humidity content of submitted and not submitted to accelerated aging (AGI and NAGI, Respectively) of pomegranate seeds (*Punica granatum* L.) submitted to different pre-germinative treatments. Pombal – PB, 2016.

TREAT	GER (%)	FGC (%)	GSI	AGI	HUMIDITY (%)	
					AGI	NAGI
T1	39.00 c	4.00 b	4.00 ab	62.00 c	72.74	70.96
T2	0.00 d	0.00 c	0.00 b	0.00 e	52.07	15.29
T3	0.00 d	0.00 c	0.00 b	0.00 e	57.81	53.78
T4	41.00 bc	6.00 b	7.00 a	51.00 d	26.00	7.89
T5	68.00 a	10.00 a	9.00 a	93.00 a	83.43	77.72
T6	55.00 ab	5.00 b	5.00 ab	75.00 b	77.78	76.15
CV%	20.72	37.52	56.57	8.78		

* Averages followed by the same letter in columns do not differ from each other by Tukey test, 5% probability.

TABLE 2 – Average values for emergence (EMER), emergence speed index (ESI), leaves number (LN), seedling height (HGT), stem diameter (SD) and length of main root (LR) of pomegranate seedlings (*Punica granatum* L) from seeds submitted to different pre-germinative treatments. Pombal – PB, 2016.

TREAT	EMER	ESI	LN	HGT	SD	LR
T1	24.00 bc	0.67 bc	9.84 b	26.75 b	0.94 b	161.83 a
T2	0.00 d	0.00 c	0.00 c	0.00 d	0.00 c	0.00 c
T3	0.00 d	0.00 c	0.00 c	0.00 d	0.00 c	0.00 c
T4	16.00 c	0.35 bc	8.22 b	15.47 c	0.81 b	113.39 b
T5	47.00 a	3.06 a	13.97 a	42.07 a	1.21 a	176.95 a
T6	29.00 b	0.71 b	15.14 a	60.51 a	1.21 a	174.92 a
CV%	20.40	37.70	13.78	21.77	10.36	10.94

* Averages followed by the same letter in columns do not differ from each other by Tukey test, 5% probability.

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