Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 11, pp.42146-42149, November, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

GERMINATION AND EMERGENCE OF POMEGRANATE SEEDS ON DIFFERENT SUBSTRATES

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ARTICLE INFO

Article History:
Received 17th August, 2016
Received in revised form
10th September, 2016
Accepted 21st October, 2016
Published online 30th November, 2016

Key words:
Punica granatum L.,
Propagation,
Substrate.

ABSTRACT

This study aimed to evaluate the effect of different substrates on germination and emergence of *Punica granatum* L. seeds. The seeds were removed from the fruits and submitted to fermentation and drying. The germination and emergence tests were performed using the following substrates: washed and autoclaved sand, fine texture vermiculite, carbonized rice husk, commercial substrate (Tropstrato HT®), and the mixtures - washed and autoclaved sand + carbonized rice husk and commercial substrate + carbonized rice husk. The germination test was performed in clear plastic boxes at alternated temperature of 20 and 30°C (8h - 16h), in the absence of light, with weekly evaluation up to 42 days after sowing. The characteristics evaluated were percentage of germinated seeds, normal seedlings, abnormal seedlings and hard seeds. The emergence test was conducted at the seedling nursery using styrofoam seed trays, the counting was realized until the 35th day. The characteristics evaluated were emergence percentage and emergence speed index. In both tests, the experimental design was completely randomized with six treatments and four replicates. The data were analyzed by Assistat program and the means were compared by Tukey's test at 5% probability. The commercial substrate and the mixture Tropstrato HT®+ carbonized rice husk, provided higher, faster and more uniform germination. In additing, the Tropstrato HT® and vermiculite substrates, as well as the mixtures promoted greater emergence speed index.

Citation: Maria Aparecida da Cruz, Deived Uilian de Carvalho, Ronan Carlos Colombo, Luiz Henrique Tutida Yokota, Jéssica de Lucena Marinho and Elisete Aparecida Fernandes Osipi, 2016. "Germination and emergence of pomegranate seeds on different substrates", *International Journal of Current Research*, 8, (11), 42146-42149.

INTRODUCTION

The pomegranate fruit (*Punica granatum* L.) is one of the oldest known edible fruits; native from the Iran region, it belongs to the Lythraceae family (Varasteh *et al.,* 2012; Dahham, *et al.,* 2010). It is a shrubby, woody and branched plant (Lorenzi and Souza, 2001) widely cultivated in Spain, Egypt, Russia, India, France, China, Japan and the United States (Khoshnam *et al*., 2007). The edible portion of the fruit, the sarcotesta, corresponds to approximately 55-60% of the total fruit weight, which consists around 75-85% of juice and 15-25% of seeds (Al-Maiman and Ahmad, 2002). The plant has many medicinal properties in different parts, such as flower, fruit and bark, presenting anthocyanins that are used to treat several health problems, predominantly gastrointestinal (Langley, 2000). The pomegranate juice also has antioxidant activity, three times higher than green tea or red wine, being used against ulcers, earaches, dysentery and leprosy (Jadon *et al*., 2012). Besides the medicinal and nutritional properties, the *Punica granatum* fruits are an alternative cultivation, especially in arid regions, due to the drought tolerance (Batista *et al*., 2011). The pomegranate is a rustic plant, which adapts to different climatic conditions, from tropical and subtropical to temperate and Mediterranean climates, and to the great variety of soils, preferring deeper soils. The plant also presents salinity tolerance and waterlogging (Regato and Guerreiro, 2012). The pomegranate propagation is usually realized through the seeds, but may also be carried out vegetatively by cuttings. There is a widely use of rootstocks from seeds for pomegranate propagation (Piotto *et al*., 2003). However, seed germination is a major obstacle for cultivation because of the dormancy and the low germination rates, becoming necessary to study the physical requirements for germination of *Punica granatum* seeds (Rawat *et al*., 2010) Seed germination is affected by environmental factors, such as temperature and substrate, which can be manipulated to optimize the percentage, speed and uniformity of germination, resulting in more vigorous seedlings and reducing the production cost (Nassif *et al*., 2004). The substrate is an important component to grow *Punica granatum* seedlings, and it shall be in accordance with the physical and chemical properties for the specie. The ideal substrate should be available for easy

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acquiring and transport, absent from pathogens and weeds, rich in essential nutrients, retain sufficient amounts of water, oxygen and nutrients, lodge proper pH, present good texture, structure and offer support to grow seedlings (Silva *et al*., 2001). Because of the many functional and nutraceutical properties, the fruit has received global importance (Sumner *et al*., 2005). However, this specie still has narrow importance in Brazil, resulting in few researches related to the production, particularly on the propagation. Therefore, studies on seeds germination and emergence are essential for plant breeding purposes, as well as for the own propagation. Thus, the aim of this study was to evaluate different substrates for seed germination and emergence of *Punica granatum*.

MATERIALS AND METHODS

The experiment was carried out at the Plant Production sector of the Northern Parana State University, Luiz Meneghel *campus*, Bandeirantes, Brazil, in laboratory and seedling nursery. There were used 12 physiological mature pomegranate fruits harvested from different plants. The seeds were manually extracted from the fruits, and submitted to fermentation in distilled water and sugar solution (10:1, v/v), during 72 hours to remove the sarcotesta. Thereafter, the seeds were washed on sieve under running water with manual friction between pomegranate seeds and steel mesh, during three minutes. Then, the seeds were laid to dry on absorbent sheets at ambient temperature for three days. Previously, the seeds were immersed for three minutes in fungicide suspension of metalaxyl-m + fluodioxonil (Maxim XL®) 0.2%. The germination and emergence tests were performed with the following substrates: washed and autoclaved sand (WAS), fine texture vermiculite, carbonized rice husk (CRH), commercial substrate (Tropstrato HT®) and the mixtures - washed and autoclaved sand + carbonized rice husk and Tropstrato HT® + carbonized rice husk, both mixtures at the 1:1(v/v) proportion.

The germination test was performed in clear plastic boxes (11.0 x 11.0 x 3.5 cm), containing one-centimeter thickness of the substrates and the mixtures. Twenty-five seeds were arranged inside the boxes and moistened each two days. After sowing, the clear boxes were transferred to germination chambers (Biochemical Oxygen Demand - B.O.D.), regulated at alternated temperatures of 20-30°C (8h - 16h) in the absence of light. The characteristics evaluated were the percentage of germinated seeds (radicle length equal or greater than 2 mm), normal seedlings, abnormal seedlings and hard seeds (Brasil, 2009). The evaluations were performed at 7, 14, 21, 28, 35 and ended at 42 days after sowing, when occurred the germination stabilization. The germination speed index was calculated by Maguire's formula (1962). The emergence test was carried out at the seedling nursery with polyethylene cover and black shade net 50%. The sowing was conducted in styrofoam seed trays of 128 plugs, filled with the substrates and mixtures. Each plot consisted of 32 plugs, and inside each one was sown one seed at one centimeter deep. The data counts started 11 days after sowing, with the first seedlings emergence and were realized each three days up to the stabilization, which occurred at 35 days after sowing. It was considered emerged seedling, the ones that presented expanded cotyledon leaves. The determination of emergence percentage was realized considering the total number of emerged seedlings, and the emergence speed index by the formula proposed by Maguire (1962). In both tests, the experimental design was completely randomized with six treatments and four replications of 25 seeds to the germination test and 32 seeds to the emergence test. The data were analyzed by Assistat program and the means were compared by Tukey's test at 5% probability. The data, when necessary, were transformed to arcsin (x/100)^1/2).

RESULTS AND DISCUSSION

A. Laboratory:

The results of the germination test are presented in the tables 1 and 2. It is observed at 42 days after sowing (Table 1), when occurred the germination stabilization, the substrates: washed and autoclaved sand (WAS); Tropstrato HT®; vermiculite and the mixture Tropstrato HT® + carbonized rice husk (CRH) provided higher percentage of germinated seeds (50-55%) and twice higher than CRH and the mixture (WAS + CRH). These results are consistent with those observed by Pacheco *et al*., (2008), who observed good performance on *Tabebuia aurea* germination using vermiculite (87%), Tropstrato HT® (87%) and sand (84%).

Table 1. Percentage of germinated seeds (GS) and germination speed index (GSI) of *Punica granatum* using different substrates at 7,14, 21, 35 and 42 days (d) after sowing

Substrates	**GS (%)						GSI
	7d	14d	21 d	28 d	35 d	42 d	
WAS	*0.0 b	*3.0 b	*11.0 bc	*30.0 a	*39.0 abc	*55.0 a	*1.91 bc
CRH	0.0 b	0.0 b	1.0 d	10.0 b	17.0 c	25.0 b	0.76 e
Vermiculite	0.0 b	0.0 b	3.0 cd	27.0 a	41.0 ab	51.0 a	1.63 cd
Tropstrato HT®	4.0 b	25.0 a	35.0 a	40.0 a	45.0 a	55.0 a	3.11 ab
WAS + CRH	0.0 b	0.0 b	2.0 cd	8.0 b	19.0 bc	26.0 b	0.79 de
Tropstrato®+CRH	10.0 a	26.0 a	31.0 ab	41.0 a	43.0 ab	50.0 a	3.39 a
SMD	5.60	14.74	16.77	19.80	25.62	23.47	1.28
CV(%)	93.94	53.70	41.44	19.29	19.95	15.15	13.35

WAS-washed and autoclaved sand, CRH- carbonized rice husk. *Equal letters do not differ by Tukey's test at 0.05 probability level.
**Data transformed to "arcsin ((x/100)^1/2)".

Similar effect for normal seedlings was observed, highlighting the four substrates mentioned above (Table 2). However, just the Tropstrato HT® and the mixture (Tropstrato HT®+ CRH) provided faster and more uniform germination, as can be seen by the values obtained from GSI or during the period evaluated (Table 1).

Some substrates, such as Tropstrato HT® (a mix between pine bark + peat + vermiculite), presents higher water holding capacity due to its composition (Pereira *et al*., 2012) and this characteristic can influence positively the seed germination, like the results observed by these authors on *Cucumis melo* seeds germination.

Table 2. Percentage of normal seedlings (NS), abnormal seedlings (AS) and hard seeds (HS) of *Punica granatum* using different substrates

Substrates	**NS(%)	**$AS_{(ns)}$	HS
WAS	*45.0 a	*10.0	*45.0 b
CRH	19.0 b	6.0	75.0 a
Vermiculite	39.0 ab	12.0	49.0 b
Tropstrato HT®	48.0 a	7.0	45.0 b
WAS + CRH	20.0 b	6.0	74.0 a
Tropstrato®+CRH	46.0 a	4.0	50.0 b
SMD	25.86	10.32	23.47
CV(%)	19.39	49.21	18.56

ns- not significant.
WAS-washed and autoclaved sand, CRH- carbonized rice husk.
*Equal letters do not differ by Tukey's test at 0.05 probability level.
**Data transformed to "arcsin((x/100)^1/2)".

Table 3. Emergence percentage (EP) and emergence speed index (ESI) of *Punica granatum* seedlings, using different substrates

Substrates	**$EP_{(ns)}$	**ESI
WAS	*12.0	*0.56 bc
CRH	11.0	0.34 c
Vermiculite	28.0	1.12 abc
Tropstrato HT®	25.0	1.72 a
WAS + CRH	31.0	1.31ab
Tropstrato®+CRH	29.0	1.85 a
SMD	21.27	0.88
CV(%)	32.30	28.12

ns- notsignificant.
WAS-washed and autoclaved sand, CRH- carbonized rice husk.
*Equal letters do not differ by Tukey's test at 0.05 probability level.
**Data transformed to "arcsin((x/100)^1/2)".

The positive results for Tropstrato HT® were also observed by Franco *et al*. (2007), whom assessing different substrates under initial development of six citrus rootstocks, observed the highest germination percentage for the Tropstrato HT® (93.89%) and Nunes *et al*. (2014) who found germination percentage greater than 80% in *Erythrina cristagalli* seeds. Other authors for woody species also observed the beneficial effect of the substrates sand and vermiculite on the seed vigor. Nogueira *et al*. (2003) analyzing different substrates in *Hancornia speciosa* seeds reported the highest germination percentage on sand, attributing this to the fact of the sand gather beneficial features, such as porosity and sterility. These results are also according to Lopes *et al.* (2002), who in studies with *Mutingia calabura* seeds, observed that the sand fa cilitated the seed germination. In the vermiculite case, Andrade *et al.* (2000) and Miranda *et al.* (2012) testing different substrates for *Genipa americana* and *Anadenanthera peregrine* germination, respectively, noted that this substrate promoted the highest germination percentage values (89.7% and 96%). The percentage of abnormal seedlings was low, presenting no differences between the studied substrates. On the other hand, higher percentages of hard seeds (HS) and four times lower germination speed occurred on the CRH and WAS + CRH substrates. According to Guerrini and Trigueiro (2004) carbonized rice husk mixed to another substrate can promote reduction in the micropores proportion of the substrate, reducing the water retention capacity, which likely contributed to slow down the germination and increase the non-germinated seeds percentage in these treatments, knowing that the sand also is a fast drainage substrate. Steffen *et al.* (2010) found out that pure CRH did not promote the seedlings growth in *Antirrhinum majus* production.

B. Seedling Nursery:

The results for the emergence test are presented in the Table 3. There was no significant difference to seedling emergence by Tukey's test, for the analyzed treatments. The means ranged from 11% for the CRH substrate to 31% for the WAS + CRH mixture. However, an increasing around 20% on emergence percentage can be significant to the seedlings producers. The Tropstrato HT® and vermiculite substrates, as well as the mixtures (Tropstrato HT® + CRH and SWA + CRH) promoted faster seedling emergence, as can be seen at the higher ESI obtained. In these substrates, the emergence speed index ranged from 1.12 to 1.85, significantly different from the sand and CRH substrates. This means that the seedlings on sand and CRH substrates may become more vulnerable to the adverse environmental conditions, because it emerged more slowly and spent more time in the initial growth stage (Martins *et al*., 1999). This probably occurred due to the lower water holding capacity of these substrates (Danner *et al*., 2007; Vieira and Pauletto 2009). Other authors working on different species found similar results. Danner *et al*. (2007) analyzing different substrates in *Plinia sp.* propagation, observed the highest ESI utilizing commercial substrate (1.31), and the lowest value for the mixture of native forest soil + medium granulation sand + vermicompost (0.77), attributing this result to the sand presence in the mixture. Lima *et al*. (2010) studying *Sicana odoriferous* also obtained higher ESI using commercial substrate (1.4340 a) and underperformed with sand substrate (1.1080 b). Pacheco *et al.* (2011) working on *Dimorphandra mollis* seeds obtained elevate ESI for Tropstrato HT® (1.40) and vermiculite (1.30) substrates, as well as Souza *et al.,* (2014) who observed high ESI for *Cucurbita spp*. seeds for the same commercial substrate. In summary, in this study was evident the importance of environmental conditions control, such as temperature and water availability, on germination and seedlings emergence. When the tests were performed in laboratory, under controlled conditions, the results were superior in comparison to those obtained on seedling nursery.

Conclusion

The substrate (Tropstrato HT®) and the mixture Tropstrato HT® + carbonized rice husk (CRH), provided higher, faster and more uniform germination of *Punica granatum* seeds. The Tropstrato HT® and vermiculite substrates, as well as the mixtures (Tropstrato HT® + CRH and washed and autoclaved sand + CRH), promoted faster *Punica granatum* seedlings emergence.

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