



Overcoming dormancy in citrus rootstock seeds

Francine Madruga, Andreia da Silva Almeida, Cristina Rossetti, Carem Rosane Coutinho Saraiva, Josiane Cantuária Figueiredo, Guilherme Oliveira Pagel, Emanuele Klug, Keliane Corrêa Boeira

Universidade Federal de Pelotas – UFPEL, RS. E-mail: francinebonemann@hotmail.com

Abstract

Citrus is a fruit of great importance in Brazil, mainly in the socio-economic issue as it generates employment and income due to the production and export of concentrated orange juice, as well as other products. In view of the above, this work aimed to evaluate the dormancy overcoming of citrus seeds of the cultivars: Flying Dragon, San Diego, Índio, Cravo Santa Cruz, Riverside, BRS Sunki Tropical, Volkamerian, as well as the species *Poncirus trifoliata*. The seeds from Embrapa (Brazilian Agricultural Research Corporation) Clima Temperado, located in Pelotas-RS, as well as from Embrapa Cassava e Fruticultura, located in Cruz das Almas-BA, were submitted to the germination test as written in the Seed Analysis Rules and a germination test in which a small cut was made in the seed coat on the side of the embryo, around 0.1 mm with the help of a stylet, which was used 200 seeds (4 samples of 50 seeds per repetition) on germitest® paper, moistened with distilled water in an amount equivalent to 2.5 times its weight and kept inside a germinator at a constant temperature of 25 °C, photoperiod of 12 hours. The evaluations were carried out at the end of the 21 days after the installation of the test, providing data that were expressed in percentage of normal and abnormal seedlings and non-germinated seeds (hard, dormant or dead). of citrus seedlings at the end of the tests and the data obtained were submitted to analysis of variance and the means were compared by the Tukey test at a 5% significance level ($p \leq 0.05$), with the aid of the R- bio. It is concluded that, when evaluating germination and shoot and root length of seedlings cultivars Flying Dragon, Índio, Cravo Santa Cruz, BRS Sunki Tropical, Riverside and Volkameriano, San Diego and the species *P. trifoliata*, were satisfactory when submitted in the dormancy breaking process, but further studies are needed.

Keywords: agriculture; production; commercialization.

Superação de dormência em sementes de porta-enxertos de citros

Resumo

Os citros é uma frutífera de grande importância no Brasil, principalmente na questão sócio econômico pois gera emprego e renda por conta da produção e exportação de suco concentrado de laranja, bem como outros produtos. Diante do exposto este trabalho teve como objetivo avaliar a superação de dormência de sementes de citros das cultivares: Flying Dragon, San Diego, Índio, Cravo Santa Cruz, Riverside, BRS Sunki Tropical, Volkameriano, bem como a espécie *Poncirus trifoliata*. As sementes oriundas Embrapa (Empresa Brasileira de Pesquisa Agropecuária) Clima Temperado, localizada em Pelotas-RS, bem como da Embrapa Mandioca e Fruticultura, localizada em Cruz das Almas-BA, foram submetidas ao teste de germinação como escrito nas Regras de Análise de Sementes e a um teste de germinação na qual foi feito um pequeno corte no tegumento da semente do lado do embrião, em torno de 0,1 mm com ajuda de um estilete, a qual foi utilizada 200 sementes (4 amostras de 50 sementes por repetição) em papel germitest®, umedecido com água destilada em quantidade equivalente a 2,5 vezes o seu peso e mantidos no interior de um germinador à temperatura constante de 25 °C, fotoperíodo de 12 horas. As avaliações, foram realizada ao final dos 21 dias após a instalação do teste, fornecendo dados que foram expressos em porcentagem de plântulas normais, anormais e sementes não germinadas (duras, dormentes ou mortas). Além de ser realizado o comprimento da parte aérea e raiz das plântulas de citros no final dos testes e os dados obtidos foram submetidos à análise de variância e as médias, comparadas pelo teste Tukey ao nível de 5% de significância ($p \leq 0,05$), com o auxílio do pacote estatístico R-bio. Conclui-se, que ao avaliar a germinação e o

comprimento da parte aérea e raiz das plântulas cultivares Flying Dragon, Índio, Cravo Santa Cruz, BRS Sunki Tropical, Riverside e Volkameriano, San Diego e a espécie *P. trifoliata*, foram satisfatórios, quando submetidos no processo de quebra de dormência, porém se faz necessário mais estudos a respeito.

Palavras-Chave: agricultura; produção; comercialização.

Introduction

Citrus is a culture originating from the Asian continent, belonging to the Rutaceae family and to the Citrus, Fortunella, Poncirus genera and other genera of the Auratioideae subfamily, these citrus genera were introduced in Brazil through the propagation of seeds, during the colonization of the Portuguese in the 20th century. XVI (CONCEIÇÃO *et al.*, 2017).

The world production of this crop in the 2020/2021 crop reached approximately 2.4 billion boxes of 40.8 kg, which is equivalent to a 4% increase in productivity compared to previous years, according to a report by the Department of Agriculture of the United States (USDA, 2018). In Brazil According to the Brazilian Institute of Geography and Statistics (IBGE, 2018), in the 2020 harvest, the country produced 17 tons of orange fruits in an area of 592,814 hectares, with an average yield of around 29,561 kg ha, being the largest producer world of fruits, with more than one million hectares of citrus plants in its territory.

This production is both for *in natura* or industrial commercialization and for the production of essential oils, jellies, candies, liqueurs, concentrated orange juice and in ornamentation as a source of extra income for producers of this culture (NEVES; TROMBIN, 2017).

But for this, it is important to use seeds to obtain rootstocks, aiming at obtaining vigorous seedlings that will perform well in the field (SARMIENTO *et al.*, 2016). In addition, is important take into account the attributes that are related to the quality of a seed, such as: physical, genetic, sanitary and physiological, which are essential for the good performance of cultures, considering that they carry all the genetic potential of the cultivar (GUIMARÃES *et al.*, 2006).

The propagation of citrus can be carried out in two ways: sexual and asexual, but the way most used today by citrus growers is the asexual way by the use of rootstock, among which they present some advantages, among which one can mention the uniformity of the seedlings, since the

rootstocks used are polyembryonic (PASSOS *et al.*, 2006).

The sexual propagation of citrus is a method that aims to reproduce, perpetuate the species and obtain citrus rootstocks (SEAPI, 2018). In citrus, the vigor of a rootstock is directly related to the polyembryony of seeds, so that the smaller amount of embryos per seed favors the larger size of the embryo and the germination of the zygotic embryo (SOARES *et al.*, 2000).

But to carry out the propagation of citrus seeds sexually and quickly, it is important to overcome the dormancy that, according to (OLIVEIRA *et al.*, 2006) and (OLIVEIRA; SCIVITTARO, 2007), some citrus rootstock varieties have shown germination uniformity problems, possibly as a consequence of some type of dormancy, due to the integuments that surround the seeds, acting as a physical barrier to water imbibition and gas diffusion or even by the presence in the integument, of some inhibitor of embryo development.

Given the above, this study aimed to evaluate the dormancy overcoming of citrus seeds of the cultivars: Flying Dragon, San Diego, Índio, Cravo Santa Cruz, Riverside, BRS Sunki Tropical, Volkamerian, as well as the species *Poncirus trifoliata*.

Material and Methods

The present study was carried out from June 2022 to August 2022, at the "Flavio Rocha" Seed Analysis Didactic Laboratory, of the Department of Plant Science, Faculty of Agronomy Eliseu Maciel, at the Federal University of Pelotas, located in the Municipality of Capão do Leão- RS.

The experimental design was completely randomized, in an 8x2 factorial scheme (eight rootstocks and two different periods), totaling 64 treatments and four replications with fifty seeds each.

The following citrus cultivars were evaluated: Flying Dragon, San Diego, Índio, Cravo Santa Cruz, Riverside, BRS Sunki Tropical, Volkamerian, as well as the species *Poncirus trifoliata*, from Embrapa (Brazilian Agricultural

Research Corporation) Clima Temperado, located in Pelotas. -RS, as well as Embrapa Mandioca e Fruticultura, located in Cruz das Almas-BA.

The germination evaluations were carried out in two ways, the first being performed as described in the Rules for Seed Analysis, in which 200 seeds (4 samples of 50 seeds per repetition) were used on germitest® paper, moistened with distilled water in an equivalent amount to 2.5 times its weight and kept inside a germinator at a constant temperature of 25 °C, photoperiod of 12 hours. The evaluations were performed at the end of 45 days after installation of the test, providing data that were expressed as percentage of normal and abnormal seedlings and non-germinated seeds (hard, dormant or dead) (BRASIL, 2009).

The second form of the germination test was performed through a small cut in the seed coat on the side of the embryo, around 0.1 mm with the help of a stylet, which was used 200 seeds (4 samples of 50 seeds per repetition) on germitest® paper, moistened with distilled water in an amount equivalent to 2.5 times its weight and kept inside a germinator at a constant temperature of 25 °C, photoperiod of 12 hours. The evaluations were carried out at the end of the 21 days after the test installation, providing

data that were expressed in percentage of normal and abnormal seedlings and non-germinated seeds (hard, dormant or dead).

In addition to the germination test, the length of the seedlings was measured, after obtaining a constant number of seedlings, 20 seedlings were removed from each sample and the distance (cm) from the apex of the seedling was measured using a graduated ruler. root to the region of insertion of the cotyledonary leaves of the citrus seedlings, both at 45 days after the end of the test and at 21 days.

The data obtained were submitted to analysis of variance and the means were compared by the Tukey test at a 5% significance level ($p \leq 0.05$), with the aid of the R-bio statistical package (BHERING, 2017).

Results and Discussion

According to the results, it can be observed that there was a statistical difference in germination and for the length of the shoot and root, for Flying Dragon, San Diego, Índio, Cravo Santa Cruz, Riverside, BRS Sunki Tropical, Volkamerian, as well as the species *P. trifoliata*, evaluated during 21 and 45 days (Table 1).

Table 1. Represents the germination (%) at 21 and 45 days of analysis and the length (cm) of the shoot and root at 21 and 45 days of citrus seeds of different cultivars. Lion's Capon, 2022.

Citros	Germinação 21 dias	Germinação 45 dias	Comprimento (P.A), 45 dias	Comprimento (Raiz), 45 dias	Comprimento (P.A), 21 dias	Comprimento (Raiz), 21 dias
Flying Dragon	28 c	28 c	4.2 b	4.9 c	4.9 b	4.2 d
Riverside	46 b	44 a	5.7 a	7.9 b	6.4 a	7.5 a
Cravo	45 b	44 a	5.7 a	10.3 a	5.8 a	6.4 b
Sunki Tropical	45 b	46 a	6.1 a	7.1 b	4.4 b	5.5 c
Volkameriano	49 a	49 a	5.6 a	7.5 b	4.6 b	6.4 b
Índio	42 b	44 a	4.7 b	6.4 b	5.7 a	3.6 d
San Diego	45 b	39 b	5 a	3.2 d	4.4 b	3.6 d
<i>P. Trifoliata</i>	4 d	3 d	5.9 a	6.7 b	5.8 a	7.4 a
CV %	5,16	7,76	14,71	10,47	9,24	8,47

*Means followed by the same letter in the columns do not differ significantly from each other by Tukey's test at 5% significance.

During the period of 21 days, it can be observed that the cultivar that had the highest germination was Volkamerian with 49%, followed by Riverside with 46%, Cravo, BRS Sunki Tropical, San Diego with 45%, Flying Dragon with 28% and the species *P. trifoliata* with 4%. The germination

results at the end of the 45 days period were similar to the results at the end of 21 days for both cultivars and species studied, where the cultivar Volkamerian remained 49% germination both at 21 days and at 45 days, the same occurred with the Flying Dragon that remained at

21 and 45 days with 28% germination, the cultivar Índio increased from 42% at 21 days to 44% at 45 days, Cravo went from 45% at 21 days to 44% at 45 days, Riverside, which went from 46% at 21 days to 44% at 45 days, San Diego from 45% germination at 21 days to 39% at the end of 45 days and the species *P. trifoliata*, went from 4% at the end of 21 days of germination at 3% at the end of 45 days of germination.

Regarding the length of both the shoot and the root, there was a statistical difference both at 21 days and at the end of 45 days of evaluation. In the Flying Dragon cultivar, the shoot length increased from 4.2 cm at the end of 45 days to 4.9 cm at 21 days, root length was from 4.9 cm at 45 days to 4.2 cm at 21 days, to Riverside the shoot length was from 5.7 cm at 45 days to 6.4 cm at 21 days and the roots from 7.9 cm to 7.5 cm at the end of 21 days, Cravo passed the length of the aerial part from 5.7 cm to 5.8 cm at the end of 21 days, as for the root length it was from 10.3 cm at 45 days to 6.4 cm at the end of 21 days.

For the cultivar BRS Sunki Tropical, the length of the seedlings increased from 6.1 cm at the end of 45 days to 4.4 cm at the end of 21 days, whereas the length of the roots was from 7.1 cm to 5.5 cm. cm at the end of 21 days, Volkamerian the length of the shoot was from 5.6 cm to 4.6 cm at the end of 21 days, whereas the length of the roots was from 7.5 cm to 6.4 cm at the end of 21 days. days, on the other hand, the length of the shoot was from 4.7 cm to 5.7 cm at the end of 21 days, the length of the roots from 6.4 cm to 3.6 cm at the end of 21 days, San Diego the length of the shoot seedlings was from 5 cm at the end of 45 days to 4.4 cm at the end of 21 days, root length was from 3.2 cm to 3.6 cm at 21 days, for the species *P. trifoliata*, the shoot length was from 5.9 cm to 5.8 cm at the end of 21 days, root length from 6.7 cm to 7.4 cm over 21 days.

According to (MORELLI, 2021), when evaluating the germination of Swingle citrumelo seeds, he observed a variation in the results when subjected to different moisture content, the same (CONCEIÇÃO, 2015), when evaluating seed germination during storage periods different from species *P. trifoliata* and the cultivar limoiero Cravo, observed. As for the evaluation of the length of the shoot and root of citrus seedlings of the lime tree Rangpur as well as the species *P. trifoliata*, there was also a statistical difference, according to (SANTOS, 2020), in its evaluation.

These results can be attributed to a characteristic of each variety, as the seeds of *P. trifoliata* and its hybrids are more sensitive than those of other rootstocks used in the production of citrus seedlings (CARVALHO; SILVA, 2013).

In addition to the characteristics of each species, another important aspect is the physiological quality of the seeds which, according to (FRANÇA-NETO *et al.*, 2011), is important for the success of a culture, as they generate vigorous plants that will perform well in the field. For the determination of the physiological quality of a seed is done through a seed vigor test, as it is an increasingly routine instrument used by the seed industry, aiming to guarantee the physiological quality of the seeds (MARCOS FILHO, 2005).

The evaluation of shoot and root length is very important because it has the purpose of providing complementary information to those obtained in the germination test and that make it possible to estimate the potential for seedling emergence in the field (FRANCO *et al.*, 2007). The determination of the average length of normal seedlings or their parts is performed, considering that the samples that express the highest values are more vigorous (NAKAGAWA, 2000).

Conclusion

According to the results presented, it can be concluded that when evaluating germination and shoot and root length of seedlings cultivars Flying Dragon, Índio, Cravo Santa Cruz, BRS Sunki Tropical, Riverside and Volkamerian, San Diego and the species *P. trifoliata*, were satisfactory when submitted to the dormancy breaking process, but further studies are needed.

References

- BRAZIL. Ministry of Agriculture, Livestock and Supply. Secretary of Agricultural Defense. **Rules for seed analysis**. Brasília: MAPA/ACS, 2009.
- BHERING, L.L. Rbio: A tool for biometric and statistical analysis using the R platform. **Crop improvement and applied biotechnology**, v.17, p.187-190, 2017. <https://doi.org/10.1590/1984-70332017v17n2s29>
- CARVALHO, S.A.; SILVA, L.F.C. Monitoring the viability of citrus rootstock seeds stored under refrigeration. **Brazilian Journal of Fructiculture**, v.35, n.1, p.338-345, 2013.

<https://doi.org/10.1590/S0100-29452013000100027>

CONCEIÇÃO, P.M.; AZEVEDO, F.A.; SOUZA, A.J.B. PRÓSPERO, A.G.; MORELLI, M.; FORTI, V.A. Ideal point for harvesting 'Limeira-IAC382' trifoliolate orange fruits for seed extraction. **Brazilian Journal of Fruticulture**, v.42, n.1, p.1-9, 2017. <https://doi.org/10.1590/0100-29452020535>

CONCEIÇÃO, P. M.; AZEVEDO, F. A.; HENDRIKX, W.; MARTINELLI, R.; PACHECO, C. DE A.; CARVALHO, S. A. Qualidade de sementes de porta-enxertos de citros do Jardim Clonal do Centro APTA Citros Sylvio Moreira/IAC. **Citrus Research and Technology**, v.36, n.1, p.9-14, 2015. <https://doi.org/10.5935/2236-3122.20150002>

FRANÇA-NETO, J.B.; KRZYZANOWSKI, F.C.; HENNING, A.A. Soybean seed quality and its importance in productivity. **MT Foundation Soy Research Bulletin**, v.15, p.424-428, 2011.

GUIMARÃES, R.M.; OLIVEIRAI, J.A.; VIEIRA, A.R. Physiological aspects of seeds. **Agricultural Report**, v.27, n.232, p. 40, 2006.

IBGE. Systematic Survey of Agricultural Production: Monthly survey of forecast and monitoring of agricultural crops in the calendar year - **LSPA**. 2018. Available at: <http://www.ibge.gov.br>. Accessed on: 5 Aug. 2022.

MARCOS FILHO, J. **Physiology of seeds of cultivated plants**. Piracicaba: Fealq, 2005. 495p.

NAKAGAWA, M.; OAK, N. **Seeds: science, technology and production**. 4. ed. Jaboticabal: FUNEP, 2000. 588p.

NEVES, M.F.; TROMBIN, V.G. Citriculture Yearbook. São Paulo: Citrus BR, 2017. 57 p.

OLIVEIRA, R.P.; SCIVITTARO, W.B.; RADMANN, E.B. Chemical scarification of the seed to favor the emergence and growth of the Trifoliata rootstock. **Brazilian Agricultural Research**, v.41, n.9, p.1429-1433, 2006. <https://doi.org/10.1590/S0100-204X2006000900012>

OLIVEIRA, R.P.; SCIVITTARO, W.B. Formation of Trifoliata rootstock: sowing time and seed coat in

seedling emergence. **Rural Science**, v.37, n.1, p.281-283, 2007. <https://doi.org/10.1590/S0103-84782007000100047>

PASSOS, O.S.; PEIXOUTO, L.S.; SANTOS, L.C.; CALDAS, R.C.; SOARES FILHO, W.S. Characterization of hybrids of *Poncirus trifoliata* and other citrus rootstocks in the State of Bahia. **Brazilian Journal of Fruticulture**, Jaboticabal, v.28, n.3, p.410-413, 2006. <https://doi.org/10.1590/S0100-29452006000300016>

SARMIENTO, A.I.P; GIULIANA, J.C.; SOUZA, P.V.D. Morphology of fruits and seeds of citrus rootstocks cultivated in a protected environment. **Revista U.D.C.A Actualidad & Divulgación Científica**, v.19, p.17-24, 2016.

USDA. Foreign Agricultural Service. **Citrus: world markets and trade**. 2018. Available at: <https://www.usda.gov/>. Accessed on: 06 Aug. 2022.