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Short note

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Contrasting aspects of the physical and physiological dormancy by seeds from four peach rootstocks

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Abstract: Considering that cultivars of peach rootstocks selected to be propagated by seeds show variation in the degree of physiological dormancy and peculiarities regarding the limitation imposed by the endocarp, it is essential to define the cold stratification period and the ideal temperature to be used during the pre-germination treatment. Isolated for each cultivar, knowledge of these variables which will provide viability in the production of peach rootstocks via seeds, in the presence of the endocarp, at a larger scale. Two germination tests were carried out, in a completely randomized experimental design and 4 x 3 factorial scheme, with four replications, each one consisting of 25 endocarps. The first experiment was performed with four cultivars (Aldrighi, Capdebosq, Okinawa Roxo and Tsukuba 1) and three stratification temperatures (1°C, 4°C and 7°C) for a period of 90 days. For the second experiment, the same cultivars were used, two temperatures (7°C for 'Aldrighi' and 'Capdebosq' and 4°C for 'Okinawa Roxo' and 'Tsukuba') and three stratification periods (40, 80 and 120 days), followed by sowing in substrate under greenhouse conditions for a period of 45 days, with subsequent endocarp breaking and re-sowing. The pre-germination treatment at 7°C for 90 days is sufficient to obtain high germination percentage of 'Aldrighi' and 'Capdeboscq' seeds in the presence of the endocarp. Under the stratification conditions tested, the seeds of 'Okinawa Roxo' and 'Tsukuba 1' require rupture of the endocarp to reactivate the germinative embryonic process.

1. Introduction

The production of peach rootstocks [*Prunus persica* (L.) Batsch] is traditionally done by seed, being the simplest and the most inexpensive method when compared to clonal propagation (Martins *et al.,* 2014; Fischer *et al.,* 2016). Despite the fact that stone fruit production is very important in Brazil, the country still presents relatively low orchard average productivity, mainly in Rio Grande do Sul state (Fischer *et al.,* 2016). Factors associated with this low productivity include pest incidence, diseases, climate and seedling quality, mainly in the form of rootstock production; in general, they are obtained from seeds discarded by the canning industry and, therefore, with no genetic identity (Souza *et al.,* 2018).

Currently, at Federal University of Pelotas and Embrapa Temperate Climate, a wide range of rootstock cultivars for peach seedling production are available to growers (Souza *et al.,* 2018; Menegatti *et al.,* 2019 a; Souza *et al.,* 2019 a; Menegatti *et al.,* 2020). However, peach seeds of different genotypes exhibit considerable divergence in germination rate as a result of the variability in the degree of physical and physiological dormancy of the seeds, constituting a limitation for commercial seedling production of the specie (Souza *et al.,* 2017; Menegatti *et al.,* 2019 b). Souza *et al.* (2017) and Souza *et al.* (2019 b) suggest that the combination of physical and physiological dormancy presented by seeds of temperate climate species, such as peach, may be one of the main factors responsible for the variation in the germination percentage of rootstocks seeds. This variation requires the exposure of seeds to different combinations of time and low temperatures to overcome dormancy and, consequently, to the maximizing and homogeneity of germination. According to Souza *et al.* (2016) the overcoming of this dormancy can be achieved through techniques that promote the rupture of the endocarp, popularly known as the stone. Endocarp removal, suggested as a method for obtaining high germination percentages in peach seeds (Souza *et al.,* 2017), is an operation that results in an additional cost for the nurseryman, mainly due to the high cost of the procedure, which must be performed manually and individually in order not to compromise the physical integrity of seed structures (Wagner Júnior *et al.,* 2013).

Considering that cultivars of peach rootstocks selected to be propagated by seeds show variations in the degree of physiological dormancy and peculiarities regarding the limitation imposed by the endocarp, it is essential to define the cold stratification period and the ideal temperature to be used during the pre-germination treatment, isolated for each cultivar (cv.), which will provide viability in the production of peach rootstocks via seeds, in the presence of the endocarp, at a large scale. Therefore, this study aimed to evaluate contrasting aspects of physical and physiological dormancy shown by seeds of different peach rootstock cultivars.

2. Materials and Methods

The present study was carried out with plant material obtained from ripe fruits harvested from clonal matrix plants of peach cvs. Aldrighi, Capdeboscq, Okinawa Roxo and Tsukuba 1, from Federal University of Pelotas Peach rootstock Germplasm Collection. Two different experiments were performed, both referring to germination tests. In the first one, the endocarps were packed in tulle bags and placed in plastic boxes (40 x 27 x 10 cm) and covered with fine vermiculite, previously and periodically moistened with distilled water and fungicide solution (Benlate 500 - 2 g L^{-1}). Then the plastic boxes were placed in Biochemical Oxygen Demand (BOD), in different temperatures, where they remained for 90 days in cold weather for stratification, with four cvs. (Aldrighi, Capdebosq, Okinawa Roxo and Tsukuba 1) and three stratification temperatures (1°C, 4°C and 7°C), with four repetitions, each one consisting of 25 endocarps.

At the end of the germination test (at 90 days), the percentage of seed germination was determined, with germinated seeds considered as those that presented radicle protrusion of at least two millimeters (MAPA, 2009). With these data, it was possible to determine the ideal temperature to be used during the pre-germinative treatment of the seeds of each peach rootstock cv. in the presence of the endocarp.

From these results, the second experiment was performed in which the endocarps were conditioned and kept under similar conditions to the previous experiment but were subjected to different stratification periods using the temperatures predetermined in experiment I, specifically 7°C for cvs. Aldrighi and Capdebosq and 4°C for cvs. Okinawa Roxo and Tsukuba 1, and three stratification periods (40, 80 and 120 days), with four repetitions, each one consisting of 25 endocarps. At 40, 80 and 120 days, the germination percentage was determined according to MAPA (2009), and the endocarps in which the seeds did not germinate during the cold stratification process were sown, at 1.0 cm depth, in polystyrene trays of 72 cells (114 cm^3 per cell) containing as substrate composed of 25% orchard soil + 25% vermiculite + 25% medium sand + 25% commercial. The trays were kept under controlled greenhouse conditions, with irrigation as needed, for a period of 45 days.

After this period, the percentage of seedlings emerged under greenhouse conditions was determined (MAPA, 2009), and the endocarps in which the seeds did not germinate were removed from the trays and broken to extract the seeds using a manual lathe. The seeds were then re-sown in the polystyrene trays, under the same conditions mentioned above, and kept under greenhouse conditions to evaluate seedling emergence.

The percentage of seedling emergence was daily monitored, and the experiment was finished at 15 days after the direct re-seeding during which, in three consecutive evaluations, no new seedlings emerged in any of the treatments.

All analyses was performed with the program SigmaPlot version 10.0 (Systat, 2006).

3. Results and Discussion

The seeds of cvs. Aldrighi and Capdeboscq presented superior results regarding to germination percentage in the presence of endocarp when stratified at 7°C for 90 days (Table 1), suggesting that such conditions are adequate to overcoming seed dormancy and appropriate for inducing germination. A temperature of 7°C employed in cold stratification, for a period of 90 days the cvs. Aldrighi and Capdeboscq provided average values of germination percentage of 82 and 80% respectively, results considered satisfactory according to MAPA (2009). Souza *et al.* (2017) evaluated the stratification of the seeds of these same cultivars, under identical conditions, but for a period of 60 days, registered lower values, which were: 56.5 and 21%, respectively, indicating that the period of 60 days is not sufficient to overcome the dormancy process and, consequently, to obtain satisfactory germination results.

These results also suggest that the 30-day increase, from the 60 days suggested by Souza *et al.* (2017), to 90 days proposed in the present study, for the pre-germinative treatment of seeds from these cultivars. in the presence of endocarp, might have promoted significant changes in the hormonal balance that controls the process of physiological dormancy, thereby making the seed metabolism more active and the embryo able to resume development. Additionally, it was observed that when the stratification temperature was reduced to 4°C and 1°C, the germination percentages decreased significantly for 'Capdeboscq' and 'Aldrighi', being about 54% and 15%, respectively (Table 1).

Table 1 - Mean germination values (%) of four peach rootstocks, in accordance of the temperature used in the pre-germination cold stratification treatment for 90 days

Temperature (°C)	Germination (%)			
	Aldrighi	Capdeboscq	Okinawa	Tsukuba 1
1°C	15	12.5	2	4
4°C	54	52.5	10	11
7°C	82	80	2	0

The seeds of cvs. Okinawa Roxo and Tsukuba 1 showed low average germination percentages at the end of the stratification period, regardless of the temperature tested. The best results were obtained in the treatment in which the endocarps remained in stratification at 4°C for 90 days, which were: 10% and 11%, respectively (Table 1). These results are superior to those obtained by Souza *et al.* (2017), who reported zero germination rate for these two cvs., when the seeds in the presence of the endocarp were stratified at 7°C, for a period of 60 days.

Although these results suggest that the prolongation of seed stratification period in the presence of the endocarp and the reduction in temperature from 7°C to 4°C, allow expressive increases in the germination percentage of seeds of these cultivars, these values are still considered unsatisfactory according to MAPA (2009). Based on the best results obtained in the first experiment, seeds from the four rootstocks were subjected to stratification temperatures that were considered most appropriate, with reductions and extensions of the stratification period. Thus, 'Aldrighi' and 'Capdeboscq' endocarps were placed to germinate at 7°C and 'Tsukuba 1' and 'Okinawa Roxo' were stratified at 4°C.

In figure 1 A and B, the effects of the prolongation of the cold stratification period (in days) on the seed germination percentage in the presence of the endocarp are shown under the ideal temperatures of 7°C for 'Aldrighi' and 'Capdeboscq', and 4°C for 'Okinawa Roxo' and 'Tsukuba 1', that were previously described as ideal for pre-germinative treatment. The prolongation of the cold stratification period induced an increase in the germination percentage, with the maximum obtained at 120 days independent of cv. evaluated, however, the most significant increase in germination rate was registered for 'Aldrighi' (Fig. 1). These results corroborate with the research done by Wagner Júnior *et al.* (2013), in which they observed superiority in the effect of cold stratification on seeds of some *Prunus* cvs. according to the number of hours of accumulated cold increases.

It is important to highlight that at 120 days of stratification at 7°C, 'Aldrighi' and 'Capdeboscq' presented an average germination percentage of 82% (Fig. 1A), similar to that obtained before 90 days of cold stratification at this same temperature (Table 1). These results indicate, with greater reliability, the use of pre-germinative stratification treatment at 7°C for 90 days to overcome seed dormancy of these cultivars, in the presence of the endocarp, and may have greater impact on the large-scale production of rootstocks.

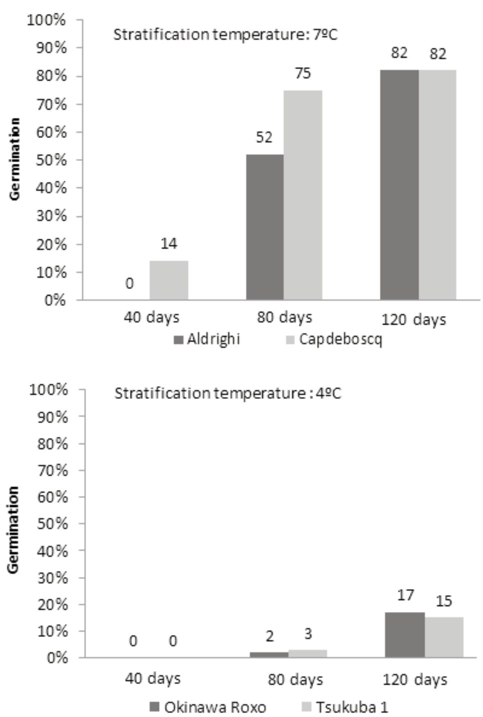

Fig. 1 - Mean values of seed germination (%) in the presence of the endocarp, of the peach rootstocks cultivars, in Biochemical Oxygen Demand (BOD), in accordance of the period used in the pre-germination treatment.

Besides ensuring germination efficiency, the use of this pre-germination treatment to overcome seed dormancy of these cutlivars excludes mechanical procedures such as endocarps rupture, a method commonly indicated for many of the seeds of the *Prunus* genus of commercial interest in which the endocarp acts as a physical limitation to the initiation of the germination process (Souza *et al*., 2019 c).

The superior seed germination rate and the lower degree of physical dormancy has promoted the use of 'Capdeboscq' as a rootstock in the production of peach seedlings in southern Brazil (Souza *et al.,* 2018) for a long time. However, the use of this genotype is not currently recommended due to attributes such as the high susceptibility to different species of the genus *Meloidogyne* (Almeida *et al*., 2015, Souza *et al.,* 2019 c), a parasitic nematode, and the adherence of the fruit pulp in the endocarp. Removal of the pulp necessitates the cleaning of the endocarps, facilitating the potential development of pathogens that may compromise the phytosanitary quality of the postharvest seeds. These characteristics are also exhibited by 'Aldrighi', discouraging the use of these particular cultivars, and promoting of the use of cutlivars with opposite characteristics.

The increase in germination percentage with an extension of the stratification period was also observed in 'Okinawa Roxo' and 'Tsukuba 1' seeds kept at 4°C (Fig. 1B). However, at 120 days of stratification, satisfactory germination rates were not reached (MAPA, 2009), indicating that the increase in the number of cold hours beyond this period is not the main factor determining the low germination rate of these cultivars.

Thus, it is suggested that physical limitations imposed by the presence of the endocarp may contribute decisively to the low seed germination rate of these cvs., as already evidenced by Wagner Júnior *et al.* (2013) and Fischer *et al.* (2016), which suggests that the endocarp is a highly lignified structure, that confers high resistance to water absorption. This factor could compromise seed germination due to the difficulty of the embryo to overcome such a barrier during initial stages of the germination process when imbibition of water is critical.

For these same four cultivars, Souza *et al.* (2017) obtained germination rate of 100% when seeds were stratified without endocarp at 7°C for 25 days. On the other hand, when the seeds were stratified at 7°C for 60 days, without endocarp removal, there were 0 and 0,7% of germination rates for 'Tsukuba 1' and 'Okinawa Roxo', respectively, suggesting that in addition to the presence of the endocarp, the early maturation of the fruits of these cvs., in relation to the other genotypes, directly influence into the physiological quality of the seeds, especially in relation to the embryo maturation. According to Pérez-Jiménez *et al.* (2012) when an embryo is not fully developed, it requires maintenance under specific conditions for a period of time to induce full development in order to ensure the establishment of a new seedling.

The differences in germination percentage of seeds previously treated with cold stratification by different temperatures and periods, followed by sowing in the presence of the endocarp in trays containing commercial substrate, and kept for 45 days under greenhouse conditions are shown in figures 2 and 3. Seeds of ‘Aldrighi’ stratified previously at 7°C during the periods of 40 and 80 days (Fig. 2), and the seeds of ‘Capdeboscq’ stratified for 40 days (Fig. 2B), followed by sowing in the greenhouse did not demonstrate a continuity in seed germination. For these treatments, a higher percentage of germination was expected during maintenance in the greenhouse given that during the period they were kept in BOD the values were considered low. Different behaviour was exhibited by seeds submitted to stratification treatment at 7°C for 120 days, for these two cultivars (Fig. 2A and B), since they had already shown high germination percentage in BOD, not showing significant increases in the percentage of germination in the greenhouse.

On the other hand, the seeds of ‘Okinawa Roxo’ and ‘Tsukuba 1’ did not show high germination percentage either under BOD conditions or when submitted to different stratification periods at 4°C followed by a period under greenhouse conditions (Fig. 3). This suggests once more the necessity of rupturing of the endocarps for the continuity of the germinative process by the embryo. Souza *et al.* (2019 c) and Menegatti *et al.* (2019 a) point out that in some peach rootstock cvs. the physical barrier imposed by the endocarp may be more determinant in germination than the low temperature and the cold stratification period. The results show that physiological seed dormancy is always present and it needs to be overcome with cold stratification so that the germination occurs, but besides physiological dormancy, the presence of the endocarp may also limit or even prevent

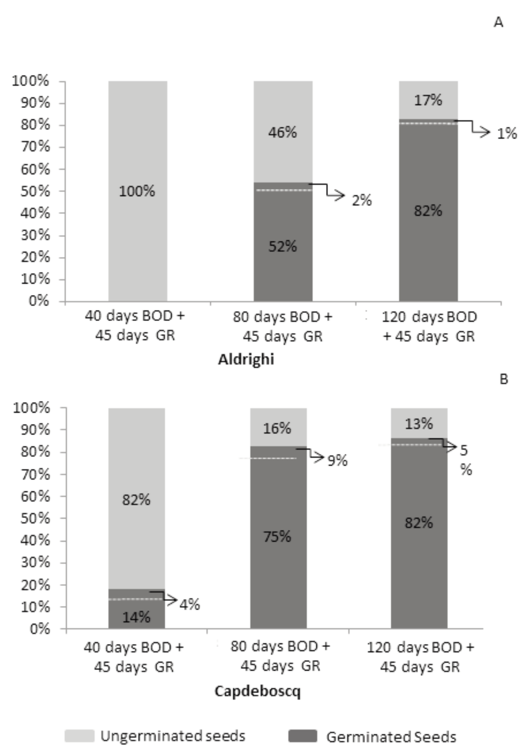

Fig. 2 - Mean values of increase in percentage (%) of germinated seeds after pre-germination stratification treatment in accordance with the period, (A) 'Aldrighi' and (B) 'Capdeboscq', at 7°C, followed by sowing of the seeds in the presence of the endocarp in trays containing commercial substrate and kept for 45 days in greenhouse (GR). Dotted lines on darker bars indicate the limit of the average germination percentage obtained during stratification in Biochemical Oxygen Demand (BOD), and the additional germination obtained after 45 days in greenhouse.

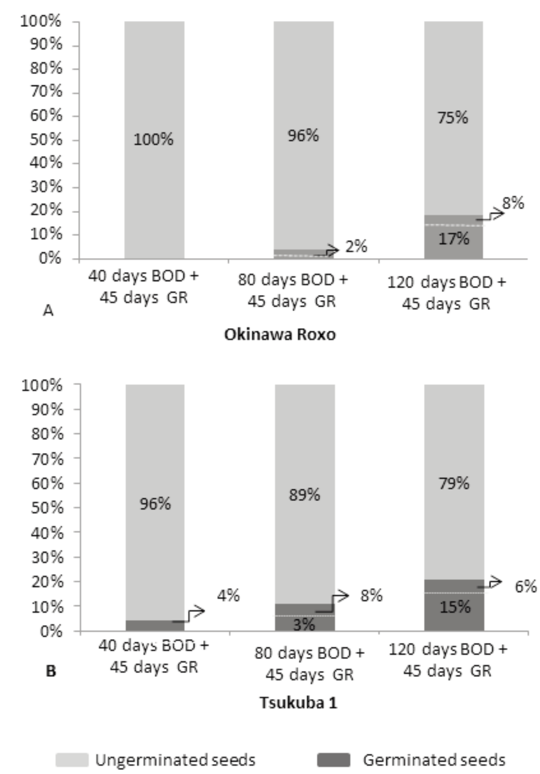

Fig. 3 - Mean values of percentage increase (%) of germinated seeds after pre-germination stratification treatment in accordance of the period, (A) 'Okinawa Roxo' and (B) 'Tsukuba 1', at 4°C, followed by seed sowing in the presence of the endocarp, in trays containing commercial substrate and kept for 45 days in greenhouse (GR). Dotted lines on darker bars indicate the limit of the average germination percentage obtained during stratification in Biochemical Oxygen Demand (BOD), and the additional germination obtained after 45 days in greenhouse.

seed germination of some cultivars resulting in non-uniform seedlings, as demonstrated by Souza *et al.* (2019 c), Souza *et al.* (2017), and the present study.

Among non-germinated seeds of ‘Aldrighi’ and ‘Capdeboscq’ after pre-germination treatment, followed by sowing and maintenance in trays for 45 days in the greenhouse, when the endocarp was ruptured, a high percentage of nonviable seeds was found (mostly rotten), This percentage was highest with the cold stratification treatment for 40 days (Table 2). Moreover, the results showed that the increase of the cold stratification period induced a reduction in the number of dead seeds of these same cultivars. These results show that the presence of the endocarp, involving the seeds of these cultivars., doesn’t totally restrict the entry of water and cold to overcome dormancy, but probably the accumulation of 40 and 80 days of cold was not enough to promote effective changes in hormonal balance.

This response was not observed in the great majority of ‘Okinawa Roxo’ and ‘Tsukuba 1’ seeds (Table 2), which when extracted from the endocarp were intact, with no turgidity, i.e., no evidence of water entering, which allowed the re-seeding and maintenance in the greenhouse again, for a period of 15 days. After this period, it was possible to observe a significant increase in the germination rate directly proportional with the extension of the stratification period employed, reaching a maximum value of 60 and 58%, respectively, after the stratification period of 120 days.

The results obtained in this study reinforce the results published by Souza *et al.* (2017), which compared the seed germination rate of ‘Tsukuba 1’ after cold stratification in the presence and absence of the endocarp and found that the germination rate was zero in the presence of the endocarp. In contrast, the authors obtained approximately 95% germination when the physical barrier (endocarp) was eliminated. Similar response was also obtained by Souza *et al*. (2017), having found that seeds without endocarp of ‘Okinawa Roxo’ and ‘Tsukuba 1’ achieved germination percentages greater than 90%.

In ‘Okinawa Roxo’ and ‘Tsukuba 1’, the presence of the endocarp, besides preventing the entry and absorption of water by the embryo, evidenced by the absence of turgidity in the seeds present inside the endocarp even after stratification for 120 days, may also have prevented the leaching of the inhibitor substances present in the seeds. This factor would make the germination process in the presence of the endocarp more demanding regarding the stratification period. This behavior occurs especially in cultivars with greater resistance to rupture of the endocarp, as is the case with these two previously mentioned cultivars (Souza *et al.,* 2017) and ‘Okinawa Roxo’ according to work by Fischer *et al.* (2016).

4. Conclusions

Pre-germinative treatment at 7°C for 90 days is efficient to obtain high germination percentages of seeds of ‘Aldrighi’ and ‘Capdeboscq’ in the presence of the endocarp. Total removal of the endocarp from the seeds of cultivars ‘Okinawa Roxo’ and ‘Tsukuba 1’ enables a more efficient overcoming of physical and physiological dormancy and allows for a greater optimization germination rate these cultivars that could benefit large-scale peach rootstock production.

Pre-germination treatment at 4°C for 80 days is not efficient to obtain high germination percentages of seeds of ‘Okinawa Roxo’ and ‘Tsukuba 1’ in the

Table 2 - Mean values of the percentage of non-germinated seeds after pre-germination stratification treatment, 'Aldrighi' and 'Capdeboscq', at 7°C (B) 'Okinawa Roxo' and 'Tsukuba 1' at 4°C, for different periods, and kept for 45 days in greenhouse, percentage of dead seeds after this period, percentage of seeds germinated after the whole process followed by endocarp rupture and re-sowing and percentage of lost seeds

	days in BOD at 7°C + 45 days in GR						days in BOD at 4°C + 45 days in GR					
	Aldrighi			Capdeboscq			Okinawa Roxo			Tsukuba 1		
	40	80	120	40	80	120	40	80	120	40	80	120
% S Non-germinated	100	46	17	82	16	13	100	96	75	96	89	79
% S dead	94	12	11	78	12	8	0	2	1	0	3	2
% S germinated after rupture-endocarp	0	17	2	0	2	3	32	44	60	20	40	58
% S lost	6	17	4	4	2	2	68	5	14	76	46	19

%S= percentage of seeds; GR= greenhouse; BOD= Biochemical oxygen demand.

presence of the endocarp.

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