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Germination potential and initial growth of seedlings of peach rootstocks genotypes

Potencial germinativo e crescimento inicial de *seedlings* de genótipos de porta-enxertos de pessegueiro

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The selection and use of peach rootstock genotypes capable of producing seeds with high germination rates, vigor, and uniformity in the early growth stages are crucial for enhancing the production efficiency of nurseries and peach farmers. This study aimed to evaluate the germination potential and morphological traits associated with the initial vigor of seedlings from different peach rootstock genotypes. Seeds from the cultivar 'Capdeboscq' and four peach rootstock selections ("Okinawa Roxo", "NR0060408", "NR0160305" and "NR0170302") were used. The experiment followed a completely randomized design with five treatments (genotypes) and four replicates of 10 seeds per replicate. Germination potential was evaluated through germination percentage, average emergence time, and emergence speed index. The initial seedling growth was assessed 40 days after emergence, recording plant height, stem diameter, number of leaves, and leaf area. The rootstock selections "NR0060408", "NR0160305" and "NR0170302" demonstrated superior phenotypic performance compared to 'Capdeboscq', which serves as a standard comparison in Brazil.
Keywords: seedling production, *Prunus persica*, phenotypic evaluation.

A seleção e o uso de genótipos de porta-enxertos de pessegueiro capazes de produzir sementes com altas taxas de germinação, vigor e uniformidade nas fases iniciais de crescimento são cruciais para aumentar a eficiência produtiva de viveiros e produtores de pêssego. Este estudo teve como objetivo avaliar o potencial de germinação e as características morfológicas associadas ao vigor inicial de mudas de diferentes genótipos de porta-enxertos de pessegueiro. Foram utilizadas sementes da cultivar 'Capdeboscq' e de quatro seleções de porta-enxertos de pessegueiro ("Okinawa Roxo", "NR0060408", "NR0160305" e "NR0170302"). O experimento seguiu um delineamento inteiramente casualizado com cinco tratamentos (genótipos) e quatro repetições de 10 sementes por repetição. O potencial de germinação foi avaliado por meio da porcentagem de germinação, tempo médio de emergência e índice de velocidade de emergência. O crescimento inicial das mudas foi avaliado 40 dias após a emergência, registrando-se a altura da planta, diâmetro do caule, número de folhas e área foliar. As seleções de porta-enxertos "NR0060408", "NR0160305" e "NR0170302" demonstraram desempenho fenotípico superior em comparação com 'Capdeboscq', que serve como padrão de comparação no Brasil.
Palavras-chave: produção de mudas, *Prunus persica*, avaliação fenotípica.

1. INTRODUCTION

The production of peaches [*Prunus persica* (L.) Batsch] is concentrated in the South and Southeast regions of Brazil, with the states of Rio Grande do Sul and São Paulo being the largest producers (137.55 and 33.07 thousand tons, respectively) [1]. Over the last few years, the country has been the thirteenth largest producer of peaches and nectarines in the world [2].

Despite the significant socio-economic importance of stone fruit production in Brazil, national productivity is still relatively low when compared to other world producers, and especially in Rio Grande do Sul [3-5], where the average productivity of peach orchards is up to 50% lower than in the state of São Paulo [1]. This low productivity has often been attributed to the quality of the plant material used to produce the nursery plants, especially in terms of the type and method of obtaining rootstocks [6-8], which directly influence the productivity and longevity of the plants [9, 10].

Currently, a large part of peach tree rootstock production is carried out using seeds from the canning industry [11]. However, as this type of material is a varietal mixture of scion cultivars, the seeds have different levels of dormancy, which implies limitations in germination and seedling precocity, as well as high genetic variability during plant growth and in the degree of tolerance to biotic and abiotic stresses [5, 7, 12-15].

The use of seeds to produce rootstocks is a viable propagation method that is economically attractive and easy to carry out when compared to vegetative and in vitro propagation [4, 5, 8]. However, the production of high-quality seedlings requires the use of seeds from genotypes that have been properly selected for use as rootstocks [12]. Among the cultivars selected for use as rootstocks for peach trees in Brazil, only six are registered with the RNC/MAPA (National Register of Cultivars): 'A9', 'Flordaguard', 'Nanomais', 'Okinawa', 'Rigitano', and 'Sharpe' [16]. In addition to these, there are two other scion cultivars ('Aldrighi' and 'Capdeboscq') that have been widely used as rootstocks in the past [17], with 'Capdeboscq' still being used as a reference in current rootstock evaluation studies. Of the six registered rootstocks, two ('Rigitano' and 'Sharpe') have presented problems of graft incompatibility with peach trees [18-21]. Therefore, the options for peach rootstocks are quite limited in Brazil.

This situation is partly due to the fact that *Prunus* spp. breeding programs in Brazil have focused mainly on the development of new scion cultivars for peach, nectarine, and Japanese plum trees [22-24]. However, due to the importance and influence of rootstocks on the longevity and productivity of orchards, studies aimed at understanding the genetic variability and individual performance of new rootstocks are essential to support the development of new rootstock cultivars with superior characteristics to those that already exist [25]. In this sense, the rootstock cultivars 'Okinawa' and 'Flordaguard' and the selections "Okinawa Roxo", 'Tsukuba-1', 'Tsukuba-2' and 'Tsukuba-3' have been suggested as substitutes for rootstocks produced from the seeds of scion cultivars from the canning industry [4, 21, 25-28]. These materials differ mainly in their chilling requirement, vigor and resistance to soil phytonematoids [9, 26], as well as the nutritional demand required throughout their growth [29, 30].

The quality of a nursery can be defined by the genetic, health and morphophysiological characteristics of the seedlings and the grafted plants produced [31], and the time taken to form the plants ready for sale is decisive for the nurseryman's economic success. In this way, the use and selection of peach rootstock genotypes capable of producing seeds with high homogeneity and germination percentage, vigor, and homogeneity of seedlings in the initial growth phase is fundamental to improving the productive efficiency of nurseries.

The initial vigor of the seedlings can be used as an indicative parameter of the vigor that the rootstock will provide to the peach cultivar [7, 32]. Therefore, this study sought to test the hypothesis that new selections of peach rootstocks have superior phenotypic performance in relation to the material used as a standard for comparison in Brazil, which is 'Capdeboscq'. To this end, the germination potential and morphological characteristics related to the initial vigor of seedlings of five peach rootstock genotypes were evaluated.

2. MATERIAL AND METHODS

The experiment was conducted in a greenhouse between August and October of 2023. We used seeds from five genotypes of peach rootstock, including one cultivar ('Capdeboscq') and four rootstock selections of *Prunus persica* ("Okinawa Roxo", "NR0060408", "NR0160305" and "NR0170302"), the last three of which were obtained by controlled crossing at the Federal University of Pelotas (UFPel). The seeds used were obtained from mature fruit harvested from mother plants maintained in the germplasm collection of *Prunus* spp. rootstocks located at the Centro Agropecuário da Palma (31º48'09"S; 52º30'25"W) at UFPel. The period corresponding to the fruit harvest of the genotypes used is shown in Table 1.

Table 1. Period of harvest and maturation interval of fruit harvested from mother plants of rootstock peach tree (<u>Prunus persica</u>) maintained in the collection of germplasm of rootstock <u>Prunus</u> spp. at the Federal University of Pelotas in 2023.

Genotype	Reference and/or Crossing	Fruit harvest period	Fruit maturation interval
‘Capdeboscq’	Finardi (1998) [33]	01/10 – 02/04/2023	25 days
“Okinawa Roxo”	Obtained by open pollination of the ‘Okinawa’	01/10 – 12/14/2023	35 days
“NR0060408”	‘Capdeboscq’ × ‘Nemaguard’	01/30 – 02/14/2023	15 days
“NR0160305”	‘Nemaguard’ × ‘Capdeboscq’	01/30 – 02/14/2023	15 days
“NR0170302”	‘Nemaguard’ × ‘Tsukuba-1’	01/30 – 02/05/2023	06 days

The fruit pulp was removed by hand, and the endocarps were washed in running tap water and dried in the shade. Subsequently, the endocarps were stored in a refrigerator at a temperature of 2±6ºC. In May 2023, the endocarps were broken, and the seeds were distributed in Petri dishes containing two sheets of Germitest paper moistened with 2 mL of commercial fungicide solution (1.5 g L^{-1} of Sialex® 500 + 2.5 g L^{-1} of Orthocide® 500). The seeds were then returned to the refrigerator for stratification, at a temperature of 2±6ºC without light for a period of 80 days. The humidity of the Germitest paper sheets was monitored every other day. When the Germitest paper showed low humidity level, an additional 2 mL of the above commercial fungicide solution was added per Petri dish.

After this period of cool, humid stratification, the seeds were sown in polystyrene trays with 72 cells (114 dm^3 per cell) containing commercial Carolina Soil® substrate supplemented with 4 g L^{-1} of Osmocote® 14-14-14 slow-release fertilizer (N-P-K), and 4 g L^{-1} of Filler-type dolomitic agricultural limestone (CaO = 26.50%; MgO = 15.00%; PRNT = 76.16%).

The experimental design was a completely randomized, consisting of five treatments (genotypes) and four repetitions, with 10 seeds per repetition. After sowing, seedling emergence was monitored every other day until stabilization. The following parameters were then estimated:

Emergence velocity index – EVI [34]: $EVI = \frac{E1}{N1} + \frac{E2}{N2} + \ldots \frac{En}{Nn}$

Where: E1, E2, ..., En = Number of seedlings emerged in the first count, in the second count, and so on until the nth count; N1, N2, ..., Nn = Number of days from the first count, from the second count, and so on until the n^{th} count.

Average emergency time – AET [35]: $AET = \frac{\sum NiTi}{\sum Ni}$

Where: Ni = Number of seedlings emerged per day; Ti = Time to emergence in days.

Germination percentage – G%: $G\% = \frac{G}{N} \times 100$

Where: G = Number of seeds germinated; N = Number of seeds placed to germinate.

Subsequently, 40 days after emergence (DAE), the plant height (PH, in cm), the stem diameter 10 cm above the collar of the plant (SD, in mm) and the number of leaves (NL, in leaves $plant^{-1}$) were measured.

The leaf area (LA) of the plants was also estimated using the non-destructive method proposed by Sachet et al. (2015) [36]:

$LA = 6{,}852 + 0{,}823W \times L - 0{,}691W^2 - 1{,}614L/W$

Where: W = Width of leaf limb; L = Length of leaf limb.

For each variable, the variance components were estimated using the mathematical model of the completely randomized design, given by: $Y_{ij} = \mu + G_i + \varepsilon_{ij}$

Where, Y_{ij} is the mean value observed for the response variable in plot ij, μ is the overall mean, G_i is the fixed effect of the genotype (‘Capdeboscq’, “Okinawa Roxo”, “NR0060408”, “NR0160305” and “NR0170302”), ε_{ij} is the effect of the experimental error [37].

Once the significance of the factor under study had been verified, the means were grouped using the Scott-Knott test at a 5% probability of error. All the analyses were carried out using Microsoft Office Excel and the software R [38].

3. RESULTS AND DISCUSSION

According to Souza et al. (2016) [4], the endocarp of peach seed acts as a physical barrier to the germination process and as a barrier to the transfer of cold necessary to overcome physiological dormancy during stratification. Therefore, breaking the stones for subsequent stratification of the seeds enhances the expression of maximum vigor in *Prunus persica* seeds [5]. As a result, low coefficients of experimental variation have been observed in germination tests of peach tree genotypes with seeds without endocarps [4, 5, 39], allowing the obtainment of reliable germination percentage results even when using a reduced number of seeds per repetition.

The genotypes differed significantly in terms of germination percentage (G%), which varied between 77.5% (Cultivar ‘Capdeboscq’) and 100% (Selection “NR0060408”). ‘Capdeboscq’ had the lowest G% compared to the other genotypes, which did not differ significantly from each other. As for EVI and AET, there was no statistical difference between the treatments, although numerically, selections “NR0170302” and “NR0160305” had the highest EVI (1.63) and lowest AET (13.55 days), respectively (Table 2).

Although characteristics such as fruit and seed size are genetically determined, they can be influenced by the conditions of the growing environment and the management adopted [5]. The same is true of seed longevity post-harvest, which is genetically determined and varies from genotype to genotype, but its viability is easily affected during storage by factors such as moisture content, temperature, and phytosanitary status [40]. For peach trees, proper post-harvest handling of the endocarps is decisive in ensuring the viability and physiological quality of the seeds. It is therefore recommended that the endocarps be cleaned by removing all the pulp, followed by drying in the shade, phytosanitary treatment of the endocarps with a fungicide recommended for the crop, and storage in a ventilated, dry place [5].

According to Bruna (2007) [41] and Silva et al. (2013) [42], the germination potential of peach seeds is associated with the percentage of dry matter accumulated in the seeds, which is generally higher in cultivars with a longer cycle. In this scenario, the adoption of management practices, such as post-harvest fertilization, phytosanitary treatments, and green pruning, can help to increase the plant's reserves during the winter dormancy period and, consequently, promote the accumulation of dry matter in the seeds during fruit growth, especially in shorter-cycle cultivars [41].

The cultivar ‘Capdeboscq’ has been widely used as a rootstock for grafting peach trees in southern Brazil due to the ease with which it can be obtained from canning factories [11, 33] and its high germination potential [24], even without pre-germination procedures such as stratification of the stones in a controlled environment [5]. Although the processing of the endocarps used in this study was identical, the germination percentage observed for the ‘Capdeboscq’ was lower than that of the other genotypes, which were developed exclusively for use as rootstocks (Table 2). In general, the results obtained for the germination percentage were satisfactory, especially when compared to the germination rate obtained in the traditional method of producing rootstocks in the field, where the percentages close to or below 30% are generally obtained [43].

The genotypes differed significantly in terms of the height and stem diameter of the seedlings at 40 DAE, with the best results being seen in selection “NR0170302” (PH = 33.44 cm; SD = 2.11 mm), followed by selections “NR0160305” (PH = 28.77 cm; SD = 2.01 mm) and

"NR0060408" (PH = 27.68 cm; SD = 1.92 mm), which were superior to "Okinawa Roxo" and 'Capdeboscq' (Table 2). The use of seeds to produce peach rootstocks is a viable, economically attractive, and simple method of propagation compared to vegetative propagation by cuttings and in vitro [4, 5, 8], as well as making it possible to obtain seedlings free from some diseases [44]. However, it is necessary to use seeds from genotypes that have been properly selected for the role of rootstock, capable of producing seeds with a high germination rate, homogeneous seedlings, and, preferably, containing some level of resistance/tolerance to biotic and abiotic stresses [12, 13]. In addition, it is essential to use seeds that are in a good state of health and to provide suitable stratification conditions [5], in order to optimize the overcoming of dormancy, obtaining a high germination rate and higher EVI, accompanied by low AET values, to produce batches of plants that are homogeneous in terms of their growth.

Table 2. Summary of the analysis of variance and mean comparison test for variables evaluated in seeds and seedlings of genotypes of rootstocks of peach trees (Prunus persica) grown in a greenhouse during the year 2023.

Sources of variation	Mean Square(1)		
	G%	EVI	AET
Genotype	292,50*	0,181ns	41,788ns
Residue	61,667	0,096	93,450
CV_{exp} (%)	8,68	23,90	15,32
Overall mean	90,50	1,29	16,29
Genotype	Mean		
Cultivar 'Capdeboscq'	77,50 b	1,11	16,20
Selection "Okinawa Roxo"	92,50 a	1,38	17,65
Selection "NR0060408"	100,00 a	1,21	17,00
Selection "NR0160305"	87,50 a	1,15	13,55
Selection "NR0170302"	95,00 a	1,63	17,05

Sources of variation	Mean Square			
	PH	SD	NL	LA
Genotype	470,074*	1,844*	67,289*	54953,923*
Residue	18,131	0,068	7,483	3506,755
CV_{exp} (%)	15,15	14,00	14,76	28,10
Overall mean	27,92	1,86	18,47	208,63
Genotype	Mean			
Cultivar 'Capdeboscq'	23,44 c	1,69 c	16,53 c	172,10 c
Selection "Okinawa Roxo"	26,28 b	1,57 c	18,41 b	169,72 c
Selection "NR0060408"	27,68 b	1,92 b	17,85 b	231,56 b
Selection "NR0160305"	28,77 b	2,01 b	19,40 a	208,94 b
Selection "NR0170302"	33,44 a	2,11 a	20,18 a	260,82 a

(1) G% – Germination percentage (%); EVI – Emergence velocity index; AET – Average emergence time (days); PH – Plant height 40 days after emergence (cm); SD – Stem diameter of plants 40 days after emergence (mm); NL – Number of leaves 40 days after emergence (leaves $plant^{-1}$); LA – Leaf area of plants at 40 days after emergence (cm^2). $CV_{exp.}$ – Coefficient of variation experimental (%). * Indicates significant effect by F test at 5% probability of error. ns Indicates non-significant effect by F test at 5% probability of error. Means of variables followed by the same lowercase letter in the column do not differ by the Scott-Knott test at a 5% probability of error.

In experimental conditions similar to our study, Picolotto et al. (2007) [45] obtained seedlings of the cultivars 'Aldrighi', 'Capdeboscq', and 'Okinawa' with an average height of 19.15 cm and a stem diameter of 1.46 mm, 40 days after germination. When assessing the average height of seedlings from 'Okinawa' and 'Capdeboscq' rootstocks at 58 days after sowing, Schmitz et al. (2014) [9] obtained average heights of 12.72 and 15.30 cm, respectively. On the other hand, studying eight peach rootstocks ('Flordaguard', 'Aldrighi', 'Capdeboscq', 'Okinawa', "Okinawa Roxo", 'Tsukuba-1', 'Tsukuba-2' and 'Tsukuba-3'), Souza et al. (2017) [5] obtained average plant heights ranging from 13.90 to 23.07 cm and average stem diameters ranging from 1.88 to 2.99 mm, at 22 days after sowing. According to Fischer et al. (2016) [13], the production of rootstocks from seeds can result in seedling populations with satisfactory levels of homogeneity in relation to seed germination, height, and stem diameter, as long as seeds from genotypes selected for this purpose are used.

The number of leaves and leaf area of the seedlings differed significantly, with selections “NR0170302” and “NR0160305” having the highest numbers of leaves (20.18 and 19.40 leaves plant^{-1}, respectively) and selection “NR0170302” having the largest leaf area (260.82 cm^2), followed by selections “NR0060408” (231.56 cm^2) and “NR0160305” (208.94 cm^2) (Table 2). One of the main limitations of the current peach tree seedling production system in southern Brazil is the time needed for the rootstock to reach the grafting stage or for the seedlings to reach the minimum standards for commercialization [25]. Therefore, there is a preference among nurseries and producers for rootstock cultivars with superior characteristics, such as resistance to pests and soil pathogens, precocity in the production of seedlings that will form the new orchard, and the ability to induce high production efficiency and fruit quality [25, 26].

In this scenario, the development of superior rootstock genotypes, such as the selections “NR0170302”, “NR0060408” and “NR0160305”, has become increasingly necessary to optimize the stone fruit production sector, especially with regard to the seedling production system. More detailed evaluations of the potential use of these new selections are necessary, as even genotypes obtained from reciprocal crosses, such as the selections “NR0060408” and “NR0160305”, have shown some important different characteristics, such as the germination potential of the seeds (Table 2). The new peach rootstock genotypes developed at UFPel have sought to add important characteristics such as adaptation to the subtropical climate, present in ‘Capdeboscq’ and ‘Tsukuba-1’, with tolerance to gall nematodes (*Meloidogyne* spp.) and vigor, present in ‘Nemaguard’. In addition, it is necessary to validate these genotypes in the field in the future, assessing their potential for use when grafted with different scion cultivars, to expand the range of rootstock options that are useful in the face of the great variability of edaphoclimatic conditions in which peach, nectarine and Japanese plum trees are grown in Brazil.

4. CONCLUSION

The peach rootstock selections “NR0060408”, “NR0160305” and “NR0170302” presented superior phenotypic performance to the cultivar ‘Capdeboscq’, used as a comparison standard in Brazil.

The evaluated genotypes have potential for use in more efficient seedling production systems. In the context of climate change, selecting genotypes with greater vigor, uniformity, and adaptability may contribute to the establishment of orchards that are more resilient to abiotic stresses. These findings highlight the importance of rootstock selection as a strategy to enhance peach tree adaptation under future climatic conditions.

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