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GAMETOPHYTIC SELF-COMPATIBILITY AND ITS INFLUENCE ON THE PHYSICAL CHARACTERISTICS OF PLUM FRUIT

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ABSTRACT - Gametophytic self-incompatibility can be one of the obstacles to achieving high yields in plum orchards. The objective of this work was to verify in the field the occurrence of gametophytic self-compatibility, and its influence on the physical characteristics of fruits in Japanese plum accessions. The work was installed at the Research Pole of IDR-Paraná in Ponta Grossa-PR, in 2021, selecting six accesses belonging to the active Plum-GAB site. The experimental design was completely randomized (DIC) with a split plot (6 x 2), with the plot having six accessions and the subplot having two methods of pollination, induced self-pollination and free pollination, with five replications. For the variables related to the physical quality of the fruits, the DIC was delimited, with the six accessions as treatment and four replicates of five fruits. The variables observed for self-compatibility were the 67ercentagem of apparent and effective fruiting. For the physical quality of fruits, the total mass, seed and 67erc, vertical and horizontal diameter, volume, 67erc yield and seed/fruit mass ratio were evaluated. Free pollination was higher in the 67ercentagem of apparent and effective fruiting, and the genotypes PR-1013 and PR-1095 were the materials that presented the highest percentages for these variables, as well as for the physical quality of the fruits. The Japanese plum accessions evaluated were classified as self-incompatible, with free pollination as the best pollination method for the production and quality of fruits of this species.
Key words: *Prunus salicina* Lindl, fruit quality, self-incompatibility.

AUTOCOMPATIBILIDADE GAMETOFÍTICA E SUA INFLUÊNCIA NAS CARACTERÍSTICAS FÍSICAS DE FRUTOS DE AMEIXEIRA

RESUMO - A autoincompatibilidade gametofítica pode ser um dos empecilhos para alcançar altas produtividades em pomares de ameixeira. O objetivo deste trabalho foi verificar a campo a ocorrência da autocompatibilidade gametofítica, e suas influências nas características físicas de frutos em acessos de ameixeira japonesa. O trabalho foi instalado no Polo de Pesquisa do IDR-Paraná em Ponta Grossa-PR, no ano 2021, selecionando seis acessos pertencentes ao BAG-Ameixa do local. O delineamento experimental foi inteiramente casualizado (DIC) com parcela subdividida (6 x 2), sendo a parcela os seis acessos e a subparcela dois métodos de polinização, a autopolinização induzida e polinização livre, com cinco repetições. Para as variáveis relacionadas a qualidade física dos frutos foi delimitado o DIC, com os seis acessos como tratamento e quatro repetições de cinco frutos. As variáveis observadas quanto a autocompatibilidade foram a porcentagem de frutificação aparente e efetiva. Para a qualidade física de frutos foram avaliadas a massa total, de caroço e polpa, diâmetro vertical e horizontal, volume, rendimento de polpa e relação caroço/massa de frutos. A polinização livre foi superior na porcentagem de frutificação aparente e efetiva, sendo os genótipos PR-1013 e PR-1095 os materiais que apresentaram as maiores porcentagens para estas variáveis, assim como para a qualidade física dos frutos. Os acessos de ameixeira japonesa avaliados foram classificados como autoincompatíveis, tendo a polinização livre como o melhor método de polinização para a produção e qualidade de frutos desta espécie.
Palavras-chave: *Prunus salicina* Lindl, qualidade de fruto, autoincompatibilidade.

INTRODUCTION

According to FAO (2018), Japanese plum production ranks second on a global scale among cultivated seed fruits, second only to peach trees, with a world production of approximately 12.608.678 tons each year. In Brazil, there was an advance in the export of plum fruits between the years 2018 and 2019, with an increase in the order of 39,4%, which represents 1.537 kg more fruit exported from one to the other, showing a warming of the market for this crop (ANUÁRIO, 2020).

In Paraná, production in 2018 was around eight thousand tons, which yielded a gross production value of 23 million reais, representing 1% of total fruit production in the state (PARANÁ, 2018). The Ponta Grossa Regional Center has significant representation in plum production, totaling 47% of Paraná production, mainly in the municipality of Arapoti (PARANÁ, 2020).

The recommendation for the implementation of a plum orchard mainly takes into account the simultaneity between the flowering time of the producing cultivar and

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the pollinator, currently it is recommended to use more than one pollinator cultivar in the same orchard, so that the fertilization process and fruit formation have more chances to succeed (CASTRO et. al, 2008; DALBÓ; FELDBERG, 2009; SAPIR et al., 2004). The focus of genetic improvement for the Japanese plum tree in recent years has been on the selection of genetic resources that exploit characteristics such as early maturation, high level of physicochemical quality of fruits and in the post-harvest period, in addition to resistance/tolerance to diseases (RUIZ et al., 2016). It was noticed that verifying only these agronomic characteristics for the selection of genotypes and recommendation of cultivars for a commercial orchard was resulting in lower yields than expected by the plants and one of the reasons could be the genetic incompatibility between the plants of the cultivar or between cultivars many diferente (BANDEIRA et al., 2011; CONTI et al., 2013).

The Japanese plum tree shows the occurrence of the so-called gametophytic self-incompatibility (AGA), characterized as a pre-zygotic reproductive obstacle, as it impedes the growth of the pollen tube when entering the flower style, making self-fertilization impossible. This type of self-incompatibility between the pollen and the pistil of the flower of the same cultivar is commanded by a multi-allelic locus called the S-allele, that is, when the pollen grain has the S-allele similar to one of the two S-alleles present in the pistil, fertilization is interrupted, which can lead to large production losses (GUERRA et al., 2020).

According to Gordillo-Romero et al. (2020), understanding the AIG process is extremely important for the planning of orchards that aim to optimize their fruiting and production. In this sense, Herrera et al. (2018) mention that the AIG phenomenon should be taken into account in genetic improvement programs for the selection of self-compatible materials, thus minimizing the effects of impeding the free flow of pollen and ensuring fruiting in commercial orchards. It should be noted that AIG can also hinder the selection of compatible materials and seed multiplication, if the different materials have the same S-alleles in the flower's pollen and pistil (CACHI et al., 2017).

Therefore, the aim of this study was to verify in the field, the presence of gametophytic self-compatibility and its implications for fruit formation in different accessions of Japanese plum trees, belonging to the active germplasm bank (Plum-GAB) of the Rural Development Institute of Paraná IAPAR-EMATER (IDR-Paraná).

MATERIAL AND METHODS

The present work was conducted in the field at the Research and Innovation Pole of IDR-Paraná, located along the BR-376, towards Curitiba, at the geographic coordinates 25°08'57.2"S and 50°09'09.9 "W, with an altitude of approximately 850 m. The Japanese plum trees selected for this work are accessions belonging to the Plum-GAB of this research institution. The aforementioned Plum-GAB was implemented in 2005, and the crowns of the different plum accessions were grafted onto rootstocks of peach cv. Okinawa.

Along with the genotypes originating from the crossings carried out by the IDR-Paraná, this Plum-GAB also has commercial cultivars used by producers for many years and international introductions, totaling 326 accessions. The accessions evaluated were the accessions PR-1005, PR-1013, PR-1095, PR-1162, PR-1178 and BY-68-1119, being selected based on criteria such as resistance/tolerance to leaf scalding, physical quality-chemistry of fruits and phenological cycle, determined in evaluations of previous years in the genetic improvement program of IDR-Paraná.

The determination of the phenological cycle of the selected accessions was based on the phenological sequence proposed by Lopes et al. (2018). The evaluations of the phenological cycle were carried out once a week, between the months of July and November 2020 and are included in Table 1. In each evaluation, the phenological stage in which the accession plants were, beyond the evaluation date, was observed. with the objective of using this data for classification of materials based on their cycle. The classification of the phenological cycle was based on the methodology employed by Anzanello; Menin (2018), who separated Japanese plum cultivars into three groups, early group when fruit ripening and harvesting took place in November, intermediate cycle with maturation and harvesting were carried out in December, and when fruit maturation and harvesting took place happened only in January, the cultivars were classified as late.

Therefore, of the six accessions that were evaluated regarding their phenological cycle in the 2020/2021 season, three were classified as early cycle, being them PR-1005, PR-1095 and PR-1162. The others were classified as intermediate cycle, framing accessions PR-1013 (fruits ripe from 12/08/2020), PR-1178 (there was no fruiting) and BY-68-1119 (fruits ripe from 01/01/21). Guided by the phenological sequence proposed by Lopes et al. (2018), the AIG evaluations began when the flowers of the plants of the selected accessions were in the white bud stage, which corresponds to stage D.

Adjusting the methodology of Silveira et al. (2011), four random branches in five plants of each studied access, together with their inflorescences were covered, aiming to favor the induced self-pollination between the flowers. As a control, four other branches were also marked on five plants of each assessed access, allowing free pollination between the flowers of the different accesses present in the Plum-GAB. The floral buds present in each branch were computed before covering with white non-textured fabric (TNT), so that the calculation of apparent and effective fruiting of possible fertilized flowers could be quantified. Each covered and uncovered branch had an average of 15 flowers, totaling 60 flowers per plant and 240 flowers per genotype, for each treatment. Then, the flower buds were discovered and counted again 30 days after the covering, when the accesses were between the F (fall of petals) and G (setting) stages, popularly known as chumbinho.

The resulting data in relation to AIG were statistically analyzed in a completely randomized design (DIC), in a split plot, with six plum accessions selected and the subplot two pollination methods (induced self-pollination and free pollination), with five replications for each pollination, each repetition being composed of four covered and uncovered branches in five plants of each assessed access. The variables analyzed were the percentage of apparent fecundation, characterized as the percentage of flowers that had fallen petals and did not result in senescence, supposedly forming a fruit and percentage of effective fruiting, counted from the count of fruits that had normal development until the phenological stage G.

As there was no fruit formation that reached the maturity stage in any induced self-pollination treatment, the fruits collected from the free pollination treatment were evaluated for their physical characteristics in a DIC, with six accessions as treatment and four replicates of five fruits, totaling 20 fruits per genotype. Thus, the accessions were deferred through the variables of ripe fruit, seed and pulp, measured with the aid of an analytical balance with precision of four decimal places after the decimal point, expressing the values in grams (g). In addition, the fruits were also analyzed for their horizontal and vertical diameter, using a manual caliper, counting the values in centimeters (cm).

TABLE 1 - Monitoring of the phenological cycle of accesses submitted to AIG evaluation at the Plum-BAG of the IDR-Paraná Research Pole in Ponta Grossa-PR, in 2020.

Genotypes	Year 2020																				
	July				August				September				October					November			
	Days																				
	06	15	22	31	07	14	21	27	03	10	18	23	01	08	16	22	30	06	13	24	30
PR-1005	B	B	C	D	E	F	F	G	G	G	H	H	H	H	H	H	I	I	I	I	X
PR-1013	A	A	A	A	B	C	D	E	F	G	G	G	H	H	H	H	H	H	H	H	H
PR-1095	C	C	D	E	E	E	E	F	F	G	H	H	H	H	H	H	H	H	I	I	I
PR-1162	C	D	E	E	F	F	G	G	G	H	H	H	H	H	H	H	H	H	H	I	I
PR-1178	A	A	A	A	B	B	C	C	D	D	E	E	F	G	G	X	X	X	X	X	X
BY-68-1119	A	A	A	A	A	A	A	B	B	C	D	E	F	G	H	H	H	H	H	H	H

A = dormant yolk, B = yolk swelling, C = budding, D = white bottons, E = full bloom, F = petal drop, G = effective fruiting = H = green fruit development, I = ripe fruit, X = only leaves.

Also in relation to the physical characteristics of the fruit, the volume was verified from a 1.000 ml beaker previously filled with water at an established value, noting the displacement value of the water after immersion of the fruit in the beaker (GONÇALVES et al., 2013). The pulp yield was calculated by subtracting the value of the seed mass from the fruit mass, dividing this result by the fruit mass, multiplying the final result by 100, denoting the values in percentage (%) (ARAÚJO et al., 2015). Finally, the seed mass/fruit mass ratio was determined by multiplying by 100 the quotient between seed mass and fruit mass, being a dimensionless value (BAUCHROWITZ et al., 2019).

Statistical calculations were performed using the R software, on the Rstudio platform (R STUDIO, 2019), in which the data that resulted in a level of significance between each other in the analysis of variance were submitted to Tukey's test at the level of 5% probability, with the purpose of to indicate the best pollination method, in addition to accessions with better pollination results and physical quality of fruits, enabling the recommendation or not of the need for a pollinator cultivar in the establishment of a commercial orchard.

RESULTS AND DISCUSSION

For the percentage of apparent fruiting of Japanese plum accessions, interactions between genotypes and pollination methods were observed, with the PR-1013 genotype being superior to the other materials evaluated in free pollination, reaching 66.34% in induced self-pollination. genotypes PR-1013 and PR-1095 had the highest percentages, with 40.14 and 48.28% respectively (Table 2). The variation between different Japanese plum genotypes is reported by Conti et al. (2013) for pollen germination in the stigma, where the crosses between the cultivars América x Reubennel and América x Pluma 7, obtained the highest germination percentages when compared to the others.

Analyzing the simple effect of the pollination methods, there was a statistically significant difference for the PR-1005, PR-1013, PR-1162 and BY-68-1119 genotypes, with free pollination being the one that resulted in the highest percentages of apparent fruiting (Table 2). Similar results are cited by Radovic et al. (2020) in quince genotypes, where the fruiting percentage both initial (three weeks after full flowering) and final (harvest time) was higher in free pollination compared to manual self-pollination. This is also observed in the passion fruit crop, where open pollination resulted in higher fruit production compared to manual self-pollination, for the species *Passiflora cincinata* Mast. and *Passiflora quadrangularis* Linn. (BRITTO et al., 2018).

Regarding the effective fruiting of Japanese plum accessions evaluated, the interaction between the form of pollination and Japanese plum accessions was also verified, where the materials PR-1013 and PR-1095 stood out from the others for free pollination, reaching a average of 37.40 and 30.0% of effective fruiting. On the other hand, no significant difference was found between accessions for induced self-pollination (Table 3). This interaction was also observed by Carvalho et al. (2020), where the PR-1013 genotype together with the PR-1246 and PR-1156 genotypes presented the highest percentage of effective fruiting when free-pollinated, compared to the other materials studied.

Checking the simple effect of the pollination method, it is observed that the results were statistically significant, characterizing free pollination as the main method for fruit development, classifying accessions as self-incompatible (Table 3). Krahl et al. (2015) also infers that cross-pollination for plants of *Camaridium ochroleucum* Lindl. results in greater fruit formation, and that the bee species that pollinate these plants, although not efficient in removing pollen, are able to cross between the plants when they perform their body cleaning after visiting the flowers.

Although the PR-1095 and PR-1162 genotypes showed effective fruiting of 3.98 and 2.98%, in that order, for induced self-pollination, it was not possible to characterize them as self-compatible or pseudo-compatible. This happens because very low effective fruiting values are considered negligible to attest to such a condition of gametophytic compatibility (BULLOCK, 1985; LLOYD; SCHOEN, 1992). However, the assertion of reproductive systems considered self-compatible or self-incompatible is delicate, since individuals of the same species when subjected to different cultivation environments, may present different degrees of incompatibility in response to environmental changes (MATIAS et al., 2016).

TABLE 2 - Apparent fruiting of Japanese plum accessions present in the IDR-Paraná Plum-GAB, in relation to different pollination methods.

Japanese plum accessions	Free pollination	Induced self-pollination
PR-1005	37.42 BCa*	7.50 BCb
PR-1013	66.34 Aa	40.14 Ab
PR-1095	41.23 Ba	48.28 Aa
PR-1162	39.33 Ba	17.60 Bb
PR-1178	0.00 Da	0.00 Ca
BY-68-1119	21.78 Ca	7.48 BCb

*Means followed by different lowercase letters in the lines and capital letters in the columns differ statistically from each other by Tukey test, at the 5% level.

TABLE 3 - Fruiting of Japanese plum accessions present in the IDR-Paraná Plum-GAB, in relation to different pollination methods.

Japanese plum accessions	Free pollination	Induced self-pollination
PR-1005	21.20 BCa*	0.00 Ab
PR-1013	37.40 Aa	0.00 Ab
PR-1095	30.00 ABa	3.98 Ab
PR-1162	20.00 Ca	2.98 Ab
PR-1178	0.00 Da	0.00 Aa
BY-68-1119	12.20 Ca	0.00 Ab

*Means followed by different lowercase letters in the lines and capital letters in the columns differ statistically from each other by Tukey test, at the 5% level.

As for the physical characteristics of the fruits from free pollination, there were significant differences in all variables described in Table 4, with the PR-1013 and PR-1095 genotypes being more prominent than the others. These genotypes were the only ones to surpass or approach the average of 68 g per fruit for the cultivar Reubennel mentioned by Danner et al. (2010), showing the commercial potential of the fruits of these genotypes in relation to their mass.

Mean fruit mass values ranged between 69.18 and 35.57 g for the PR-1013 and PR-1005 genotypes, respectively, excluding the material PR-1178 that did not show fruiting in this season (Table 4). These values are on the same level as the results found for 10 Japanese plum cultivars cultivated in Caldas-MG, which ranged from 26 to 53 g, for cultivars Kelsey 31 and Gema de Ouro, in that order (SILVA et al., 2008).

The lack of fruiting in the PR-1178 access may have occurred due to different factors, as mentioned by Conti et al. (2013), where external factors such as water stress, pollen handling, nutritional status of the plant or periods of intense rain during the flowering season can hinder pollination and fruit development, in addition to internal factors, as reported by Castro et al. (2008), being the receptivity of the stigma, male-sterility and self-incompatibility limiting factors to fruiting.

For the mean horizontal and vertical fruit diameter, discrepant results were found between the materials evaluated, the genotype PR-1013 with the largest diameters both horizontal and vertical (5.33 and 4.71 cm, respectively) and the genotype PR-1005 with the lowest values (3,74 and 4,01, in that order). Oliveira et al. (2019) describe that the Gulfblaze and Reubennel cultivars alternated between the horizontal diameters of 39.6 to 47.5 mm in the 2014 and 2015 seasons under the effect of different rootstocks, showing the similarity and even superiority of the PR-1162 genotypes, PR-1095 and PR-1013 for such commercial cultivars.

In the variable fruit volume, there was statistical significance between the materials studied, with the PR-1013 and PR-1095 genotypes reaching greater volume in relation to the others, followed by the materials PR-1162, BY-68-1119 and PR-1005 that did not differentiated from each other, and the PR-1178 genotype that did not produce fruit (Table 4). Fruit volume is an important parameter to be evaluated as it is closely related to the increase or decrease in fruit mass, length and diameter (MARTINS et al., 2003).

TABLE 4 - Biomass, vertical diameter, horizontal diameter and fruit volume of Japanese plum accessions, present in the IDR-Paraná Plum-GAB, resulting from effective fruiting in free pollination.

Japanese plum accessions	Biomass (g)	Vertical diameter (cm)	Horizontal diameter (cm)	Fruit volume (mL)
PR-1005	35.57 B*	3.74 B	4.01 C	32.50 B
PR-1013	69.18 A	5.33 A	4.71 A	65.55 A
PR-1095	64.20 A	5.04 A	4.62 AB	57.50 A
PR-1162	40.14 B	3.92 B	4.32 ABC	36.25 B
PR-1178	0.00 C	0.00 C	0.00 D	0.00 C
BY-68-1119	37.01 B	3.96 B	4.06 BC	34.50 B
CV(%)	10.45	3.36	6.34	12.54

*Means followed by different capital letters in the columns differ statistically from each Other, by Tukey test, at the 5% level.

In addition to presenting the highest fruit mass, the PR-1013 genotype also denotes the significantly higher seed mass among the studied accessions, with an average of 2.03 g per seed, followed by the PR-1095 genotype, then the accessions PR-1005, BY-68-1119 and PR-1162, which had no statistical differences between them (Table 5). Bauchrowitz et al. (2019) evaluated 10 genotypes belonging to the Plum-GAB of the IDR-Paraná, observing a variation in seed mass from 2.13 to 5.10 g among the materials, and no accession in the current study reached the minimum value for this variable, indicating that there is great variability within the Plum-GAB in relation to seed mass.

As for the pulp mass variable, following the other results of the physical attributes of the fruits, the PR-1013 and PR-1095 genotypes stood out statistically over the others, reaching 67.15 and 62.62 g per fruit, in this order, followed by the others accesses. According to Bauchrowitz et al. (2018), some accessions of the Plum-GAB IDR-Paraná, such as the PR-1238, can reach up to 90 g of pulp mass per fruit when the plants are managed with manual thinning. None of the material in the present study reached the value mentioned by the aforementioned author, this may have occurred because the fruit thinning was not performed.

TABLE 5 - Seed biomass, pulp biomass, pulp yield and seed/fruit biomass ratio of Japanese plum accessions, present in the IDR-Paraná Plum-GAB, resulting from effective fruiting in free pollination.

Japanese plum accessions	Seed biomass (g)	Pulp biomass (g)	Pulp yeld (%)	Seed/ fruit biomass ratio
PR-1005	1.18 C*	34.40 B	96.70 C	3.30 A
PR-1013	2.03 A	67.15 A	97.05 BC	2.95AB
PR-1095	1.58 B	62.62 A	97.54 AB	2.46 BC
PR-1162	0.90 C	39.25 B	97.77 A	2.22 C
PR-1178	0.00 D	0.00 C	0.00 D	0.00 D
BY-68-1119	1.08 C	35.93 B	97.09 BC	2.90 AB
CV(%)	12.28	10.52	0.28	9.86

*Means followed by different capital letters in the columns differ statistically from each other by the Tukey test, at the 5% level.

The pulp yield showed a different result in relation to the physical attributes of the fruits, denoting the PR-1162 and PR-1095 genotypes as the best materials for this variable, with an average of 97.77% and 97.54% yield, respectively. Second Chitarra; Chitarra (1990), pulp yield is an interesting quality attribute in terms of industrial raw material, as there is a proportional relationship between yield and the amount of raw material produced, which tends to generate greater profit for the industry processing, for example. For the Seed/fruit mass ratio, it was observed that the accessions PR-1005, PR-1013 and BY-68-1119 were significantly superior to the other accessions, reaching a ratio of up to 3.30 for the PR-1005 genotype, with the highest value, but this is not interesting as it reduces the potential of the fruits for the processing industry. Based on Coelho et al. (2010), for the improvement of the cashew tree species, it is necessary to identify materials with higher fruit weight and lower seed/fruit mass ratio, which can make the species more attractive for the establishment of orchards with an agro-industrial focus.

According to Alves et al. (2013), the seed/pulp ratio, which results in the pulp yield of the fruits, is an important parameter to be taken into account in the genetic improvement of gabiroba, aiming to select fruits with a higher ratio seed/pulp, which can improve their quality in relation to commercialization and use in the industry.

The accessions evaluated during this study were considered self-incompatible in field tests, as they did not show fruit formation that completed the maturation process and subsequent harvest in induced self-pollination, requiring pollinating cultivars in the establishment of an orchard. Free pollination is the best pollination method for fruit production in the studied Japanese plum genotypes. The PR-1013 and PR-1095 genotypes were presented as an option for launching as Japanese plum cultivars, as they presented physical characteristics of fruit quality superior to materials that are cultivated in Brazil. Regarding flowering times, the PR-1095 genotype could be the pollinator of the PR-1013, PR-1005 and PR-1162 genotypes, as well as the PR-1178 could pollinate the BY-68-1119 accession, as they present the stage in full bloom at the same time.

CONCLUSION

The Japanese plum accessions evaluated were classified as self-incompatible, with free pollination as the best pollination method for the production and quality of fruits of this species.

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