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Prediction Model for Breeding Hardy Geraniums

Mehrdad Akbarzadeh [1,2,3,*], Paul Quataert [1], Johan Van Huylenbroeck [1], Stefaan P. O. Werbrouck [2,*] and Emmy Dhooghe [1,2]

[1] Plant Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Caritasstraat 39, 9090 Melle, Belgium; paul.quataert@ilvo.vlaanderen.be (P.Q.); johan.vanhuylenbroeck@ilvo.vlaanderen.be (J.V.H.); emmy.dhooghe@ugent.be (E.D.)

[2] Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

[3] Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University, Ahvaz 613, Iran

* Correspondence: mehrdad.akbarzadeh@ugent.be (M.A.); stefaan.werbrouck@ugent.be (S.P.O.W.); Tel.: +32-493121249 (M.A.)

Abstract: Key message. The success of interspecific hybridization in hardy geranium (*Geranium* sp.) can be predicted by considering the genetic distance between parental genotypes and using the logistic regression model developed in this study. Hardy geranium is a popular ornamental plant known for its architecture, hardiness, prolonged flowering, and diverse colors in leaves and flowers. In ornamental breeding, the pursuit of novel trait combinations is never-ending. Even in *Geranium*, certain combinations of valuable traits have not yet been achieved. Interspecific hybridization can increase diversity; however, success remains low due to pre- and postzygotic barriers. Crossing success can be predicted by response criteria such as pollen tube growth (tube_length), seed development (seed_dev), and seed setting (seed_set). Within a collection of 42 *Geranium* genotypes and during two consecutive breeding seasons (years), we evaluated tube_length, seed_dev, and seed_set for 150, 1155, and 349 crosses, respectively. These crosses varied in four parental differences (variables): chromosome number (Chrom), DNA/chromosome (DNA), style length (Style), and genetic distance expressed as the Jaccard distance (cJaccard = 1 − Jaccard). Using logistic regression models has confirmed that most often, the success rate decreased with increasing parental distance. The most consistent association was seen in seed_dev in combination with cJaccard. The model was used to predict the number of crosses necessary to have 10 successful crossing products by taking into account the uncertainty in the model. These findings provide valuable guidance for future planning of interspecific breeding experiments in *Geranium*. By incorporating the genetic distance between parental genotypes, breeders can enhance the efficiency and success of hybridization efforts.

Keywords: Geraniaceae; incongruity; interspecific hybridization; Jaccard; pollen tube; pre- and postzygotic barriers

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1. Introduction

Geranium, the largest genus of the Geraniaceae, is divided into three subgenera: *Erodioidea*, *Robertium*, and *Geranium* [1]. Hardy geraniums are well-suited to temperate climates and thrive in well-watered soils. These plants can grow in a wide range of environments, as long as the climate is suitable [2]. Successful intersubgeneric crosses have been carried out between species of *Geranium* and *Erodioidea*, although the most common combinations occur within the subgenus *Geranium* [2]. Under natural conditions, crosses between members of *Robertium* and the other two subgenera are not successful, confirming the occurrence of incongruity and fertilization barriers in hardy geranium. Hardy geraniums with commercially appealing traits typically showcase characteristics such as large flowers, an extended flowering period, unique flower or leaf colors, distinctive morphologies, and unusual plant architecture (e.g., compactness).

Creating new plant combinations, especially in ornamentals, remains a crucial approach to increasing diversity within a plant assortment [3]. Hardy geraniums show an enormous variation, with a minority of cultivars combining particularly attractive traits. Interspecific hybridization is the most obvious way to create new cultivars, by introducing new and desirable traits into existing cultivars. This method has been used in other herbaceous ornamental breeding programs, including *Asclepias* sp. [4], *Pavonia* [5], ornamental pepper [6], and many others.

Successful hybridization requires parent plants with viable pollen and receptive stigmas as well as essential congruity. Crossing barriers have either a genetic or a morphological background [7]. These barriers can be classified as different flowering times of the species, varietal differences, self or class incompatibility, pollen failure, low rate of pollen tube growth, fertilization failure, developmental arrest after the initial cell divisions after fertilization, or the production of nonviable seeds [7].

The majority of pollen grains capable of long-distance transport exhibit tolerance to desiccation and/or have the ability to form long pollen tubes [8]. In the absence of incongruence, the pollen on the stigma undergoes five successive events: adhesion, hydration, germination, penetration of the pollen tube, and growth through the transmitting tissue [9]. Prezygotic barriers occur from the start; in some crosses, pollen is even shed at the stigma surface. This is most common in species with dry stigmas [10,11]. Studies in *Arabidopsis* have confirmed that as the genetic distance between parent species increases, the attachment of pollen decreases [12]. Incongruity can also be observed as an atypical behavior of the pollen tube, such as growth in the wrong direction, callose plugging, or growth that stops before entering the ovules. Longer or more intense callose plugs in apples and tobacco, respectively, are indications of incongruity [13]. *Geranium* has a dry stigma [10], with five carpels and ten ovules producing five seeds under optimal conditions [14]. In addition, a study of pollen tube growth in *Geranium caespitosum* showed that after 2 h, fewer self- than cross-pollinated tubes entered the ovules, but after 24 h the difference between the number of tubes from self- and cross-pollination entering the ovules had decreased [15]. In *Geranium maculatum*, 30 min was sufficient for pollen tubes to reach the ovules with a growth rate of 0.133 mm/min [14]. However, achieving optimal conditions is not always possible. In such cases, embryo rescue has been well-documented to increase breeding success in interspecific hybridizations [16–21].

A highly significant positive correlation was observed between transgression frequency and genetic distance in eudicot plants [22], making it important for breeders to find the effect of parental differences on hybrid production rate. Some studies highlight that knowledge of genetic distance is a good predictor of success in breeding programs [23]. However, in *Sarcococca*, genetic distance, ploidy level, and genome size did not represent a true hybridization barrier [24], while in *Helleborus*, a general relationship between genetic distance and hybrid offspring was established [25]. Shortening the breeding cycle is a desirable goal in breeding programs and the use of breeding programs and modeling can greatly help breeders in parental selection, predicting crossing performance, and selecting strategies. The objective of this study was to develop a model that can predict crossbreeding success in hardy geraniums through interspecific hybridization. This novel model is based on analyzing various parental differences including chromosome numbers (Chrom), DNA/chromosome (DNA, pg/chromosome), style length (Style), and genetic distances (cJaccard). By observing pre- and postzygotic barriers during two crossing seasons of *Geranium*, we constructed a logistic regression model. To the best of our knowledge, there is no existing research that directly compares our unique combination of factors. This study offers a fresh and comprehensive approach to predicting cross-success in hardy geraniums, providing valuable insights for breeding programs aiming to enhance efficiency and achieve successful hybridization outcomes.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

A *Geranium* collection comprising 42 genotypes was used for this study. The collection represented the three subgenera of *Geranium* (*Geranium*, *Erodioidea*, and *Robertium*) and included 18 species, 18 interspecific hybrids, and 6 genotypes with unknown backgrounds (Table 1).

Table 1. Different genotypes of *Geranium* sp. used in this study.

Codes	Genotypes	Subgenera	Parents
G01	*G.* 'Anne Thomson'	*Geranium*	Parent **1:** *G. procurrens*, Parent **2:** *G. psilostemon*
G02	*G.* 'Azure rush'	*Geranium*	Parent **1:** *G. wallichianum*, Parent **2:** *G.* 'Rozanne'
G03	*G.* 'Bob's Blunder'	*Geranium*	unknown
G04	*G.* 'Brookside'	*Geranium*	Parent **1:** *G. pretense*, Parent **2:** *G. clarkei*
G05	*G.* 'Catherine Deneuve'	*Geranium*	Parent **1:** *G. psilostemon*, Parent **2:** *G.* × *oxonianum* or *G.procurrens*
G06	*G.* 'Chantilly'	*Geranium*	Parent **1:** *G. gracile*, Parent **2:** *G. renardii*
G07	*G.* 'Dragon Heart'	*Geranium*	Parent **1:** *G. psilostemon*, Parent **2:** *G. procurrens*
G09	*G. pratense* 'Galactic'	*Geranium*	*G. pratense*
G10	*G. cinereum* 'Jolly Jewel Red'	*Erodioidea*	*G. cinereum*
G13	*G.* × *riversleaianum* 'Mavis Simpson'	*Geranium*	Parent **1:** *G. endressii*, Parent **2:** *G. traversii*
G14	*G.* 'Orion'	*Geranium*	Parent **1:** *G.* 'Brookside', Parent **2:** *G. himalayense*
G16	*G.* 'Rozanne'	*Geranium*	Parent **1:** *G. wallichianum*, Parent **2:** *G. himalayense*
G17	*G.* 'Salome'	*Geranium*	Parent **1:** *G. lambertii* × *G. procurrens*, Parent **2:** *G. sanguineum*
G18	*G.* 'Sanne'	*Geranium*	Parent **1:** *G. sessiliflorum*, Parent **2:** *G.* × *oxonianum*
G19	*G. nodosum* 'Silverwood'	*Geranium*	*G. nodosum*
G21	*G. wallichianum* 'Sylvia's Surprise'	*Geranium*	*G. wallichianum*
G22	*G.* 'Tanya Rendall'	*Geranium*	*G.* × *antipodeum*
G24	*G.* 'Tiny Monster'	*Geranium*	Parent **1:** *G. sanguineum*, Parent **2:** *G. psilostemon*
G27	*G.* × *cantabrigiense* 'Biokovo'	*Robertium*	Parent **1:** *G. maccrorhizum*, Parent **2:** *G. dalmaticum*
G30	*G. cinereum* 'Laurence Flatman'	*Erodioidea*	*G. cinereum*
G35	*G. endressii*	*Geranium*	*G. endressii*
G37	*G. endressii* 'Trevor Bath'	*Geranium*	*G. endressii*
G38	*G. himalayense* 'Baby Blue'	*Geranium*	*G. himalayense*
G39	*G. himalayense* 'Derrick Cook'	*Geranium*	*G. himalayense*
G42	*G. macrorrhizum* 'Czakor'	*Robertium*	*G. maccrorhizum*
G44	*G. macrorrhizum* 'White Ness'	*Robertium*	*G. maccrorhizum*
G45	*G. maculatum* 'Album'	*Geranium*	*G. maculatum*
G46	*G. maculatum* 'Elizabeth Ann'	*Geranium*	*G. maculatum*
G49	*G.* × *oxonianum* 'Katherine Adele'	*Geranium*	Parent **1:** *G. versicolor*, Parent **2:** *G. endressi*
G50	*G.* × *oxonianum* 'Southcombe Double'	*Geranium*	Parent **1:** *G. versicolor*, Parent **2:** *G. endressi*
G54	*G. phaeum* 'Angelina'	*Erodioidea*	*G. phaeum*
G57	*G. pratense* 'Algera Double'	*Geranium*	*G. pratense*
G61	*G. pratense* 'Purple Ghost'	*Geranium*	*G. pratense*
G62	*G. psilostemon*	*Geranium*	*G. psilostemon*

Table 1. *Cont.*

Codes	Genotypes	Subgenera	Parents
G64	*G. renardii*	*Geranium*	*G. renardii*
G69	*G. sanguineum* 'Album'	*Geranium*	*G. sanguineum*
G71	*G. sylvaticum* 'Album'	*Geranium*	*G. sylvaticum*
G73	*G. versicolor*	*Geranium*	*G. versicolor*
G75	*G.* 'Bloomtime'	*Geranium*	Parent **1:** *G. wallichianum*, Parent **2:** *G. himalayense*
G76	*G. wallichianum* 'Havana Blue'	*Geranium*	*G. wallichianum*
G77	*G. wlassovianum*	*Geranium*	*G. wlassovianum*
G80	*G.* 'Blushing Turtle'	*Geranium*	*G. sanguineum*

All plants were planted in duplicate in an open field at ILVO, Melle, Belgium (50°59′31.6″ N, 3°47′07.3″ E); in addition, at least two plants of each genotype were kept in the greenhouse. Plants in the greenhouse were grown in 2 L pots (peat substrate Saniflor NPK 12:14:24, EC 45 mS·m^{-1}), except *G. cinereum*, which was potted in a stone mixture (Kift). Plants were grown under natural conditions (greenhouse ventilation set point was 10 °C).

2.2. Parental Difference

To test pre- and postzygotic barriers, 1654 crosses were carried out, of which 41, 96, and 236 cross combinations were performed for pollen tube length (tube_length, 2020 and 2021, Table S2), seed setting (seed_set, 2020, Table S3), and seed development (seed_dev, 2021, Table S4), respectively. Four different measures were used to characterize the parental difference between two parents (P1 and P2) in each cross: Chrom, DNA, Style, and cJaccard (cJaccard = 1 − Jaccard, which is the complement of Jaccard similarity and represents Jaccard distance) (Tables S2–S4). Chrom expresses the parental difference between the chromosome number in a cross [26]. DNA is the difference in genome size/chromosome number (pg/chromosome) [26]. Style is the difference in style length (mm), which was measured using a ruler when the style was fully developed. The Jaccard similarity coefficient is calculated using statistical software based on AFLP markers (0 and 1), and the formula [27] is:

$$J = \frac{a}{a + b + c}$$

where J is the Jaccard similarity coefficient, a represents the total number of attributes where parent P1 and P2 both have a value of 1, b represents the total number of attributes where the attribute of P1 is 0 and the attribute of P2 is 1, and c represents the total number of attributes where the attribute of P1 is 1 and the attribute of P2 is 0.

By using cJaccard, all four indices represent a distance, thereby ensuring that the regression coefficients of the logistic regression have the same interpretation: a negative slope implies a lower success rate for an increasing parental distance.

2.3. Crossing Success Criteria

The success rate of these crosses was assessed based on the following crossing success response criteria:

(1) Pollen tube growth (tube_length) using aniline blue staining tests (see further);
(2) Seed development (seed_dev) is defined as the percentage of crossed flowers with at least one swollen ovule with white, yellow, or green testa or mature seed with brown testa, 7–35 days after pollination (for 2021);
(3) Seed set (seed_set) as the percentage of crossed flowers with at least one mature seed with brown testa, 25–35 days after pollination (for 2020).

The difference between seed_set and seed_dev is in the harvesting stage, in which seed_set means all seeds remained on the plant until full maturation, while for seed_dev, seeds were harvested immature or mature and rescued in tissue culture.

For the aniline blue staining 48 h after pollination, the pollinated pistils were harvested and placed in FAA (formaldehyde:acetic acid:ethanol (70%) 1:1:18) for 24 h. After a washing step with water, the pistils were transferred to NaOH (6 M) for 16 h. Then, the pistils were transferred into 0.033 M K_3PO_4 + 0.1% aniline blue (Acros Organics, Geel, Belgium) and kept in this solution for 3 h (in the dark), after which they were squashed and the pollen tubes were examined using fluorescence microscopy (Leica DMIRB, Wetzlar, Germany).

2.4. Statistical Analysis

To predict the success of hybridization in hardy geraniums, the following univariate logistic regression model was fitted:

$$log\frac{\pi}{1-\pi} = \beta_0 + \beta_1 pd$$

In the formula, π represents crossing success, *pd* represents the parental difference, and β_0 and β_1 represent the slopes of the regression line in the logit scale. For each response criterion, four parental difference measures (variables) were examined for their quality as predictors: Chrom, DNA, Style, and cJaccard.

A quasi-binomial error distribution was considered [28], i.e., an extension of the binomial distribution so that proportions (such as tube_length) can be modeled. Additionally, the quasi-model can cope with overdispersion, ensuring that standard errors (and therefore confidence limits) are correctly estimated [29].

If the probability of success is π, the average number of crosses required to achieve 10 successes is $10/\pi$. To take into account the uncertainty of the model, we also calculated this number for the lower and upper limits of the confidence intervals to predict the value of π. Statistical analysis was conducted using R [30] in conjunction with RStudio (version 2022.07.2). The regression models were fitted with the glm function from R-base followed by the emmeans function from the emmeans package [31] to calculate the predicted values and 95% confidence intervals. For drawing the regression models along with confidence bands, the function geom_smooth from the ggplot2 package [32] was used, with method = "glm" and family = "quasibinomial".

3. Results

3.1. Pre-Zygotic and Post-Zygotic Barriers

Prezygotic barriers were evaluated using aniline blue staining for some cross combinations (Table S2). This resulted in different scenarios. Sometimes, germinated pollen stopped early on the stigma, as depicted for the cross between *G. himalayense* 'Baby Blue' (G38) x *G. sanguineum* 'Album' (G69) (Figure 1a). The cross *G. nodosum* 'Silverwood' (G19) x *G. phaeum* 'Angelina' (G54) also clearly showed a high degree of prezygotic incongruity due to curled pollen tubes, disoriented pollen tube growth, and short pollen tubes (Figure 1b). Figure 1c shows the cross *G.* 'Blushing Turtle' (G80) x *G.* 'Brookside' (G04) in which the pollen tubes stopped before entering the ovules. Often, the pollen tubes contained many callose plugs, but they could grow throughout the style (Figure 1d, *G.* 'Brookside' (G04) x *G. sanguineum* 'Album' (G69)). Based on pollen tube growth, the parental difference between cJaccard was the variable that best expressed the success or failure of pollen tube growth (Tables S2–S4).

During two consecutive crossing seasons, more than 1504 crosses (crosses for tube_length excluded) were performed for harvesting seeds. In some combinations, seed development was observed by swelling of the carpels (ovules) and embryo formation, but not all combinations could produce mature seeds. This indicates the existence of postzygotic barriers. Their impact was assessed by comparing the seed production in the first crossing season (2020), where seeds were harvested at maturity, with the second season's success (2021), where immature seeds and embryos were saved (Table 2). In 2020, it was found that

5.2% of all combinations were successful in producing mature seeds, and 2.08% of them were able to obtain a healthy seedling, while in 2021, when in vitro rescue of seeds and embryos was included, successful combinations improved to 15.25%, and the probability of a successful combination with healthy seedlings increased to 6.35%. Although different crosses were performed in 2020 and 2021, the overall mean parental genetic distance (expressed as cJaccard) was similar for all crosses (Table 2). These data confirmed that some postzygotic barriers can be overcome by embryo rescue treatments. Our results showed that waiting for maturation reduced seed production from 3.03% to 0.85%, and healthy seedling development from 0.57% to 0.17%, suggesting that efficiency would increase with the rescue of immature seeds and embryos (Table 2).

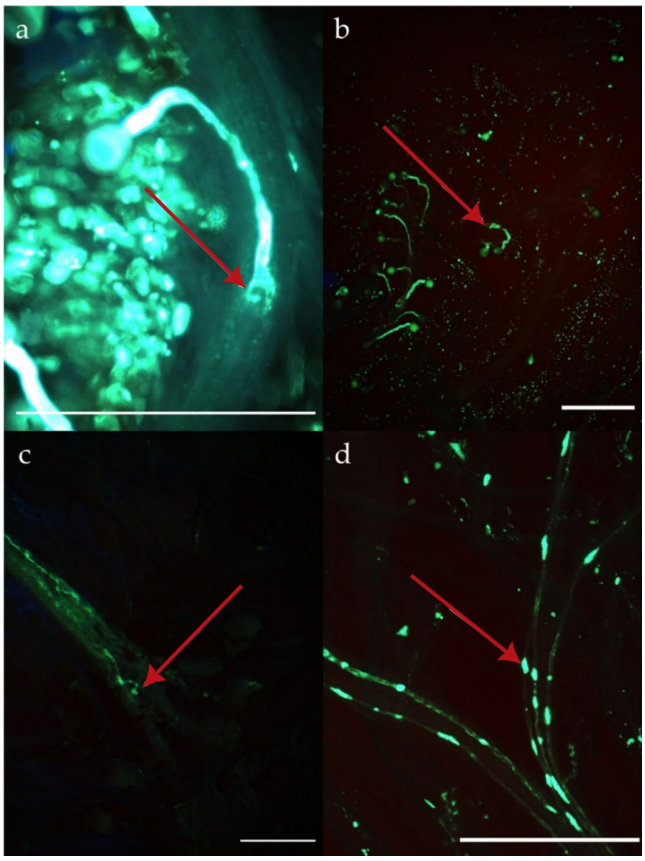

Figure 1. Details of pollen tube growth visualized by aniline blue staining after 48 h in different crossing combinations: (**a**) *G. himalayense* 'Baby Blue' (G38) x *G. sanguineum* 'Album' (G69) (arrow shows that the pollen tube stopped), (**b**) *G. nodosum* 'Silverwood' (G19) x *G. phaeum* 'Angelina' (G54) (arrow shows that the pollen tube grew in the wrong direction), (**c**) *G.* 'Blushing Turtle' (G80) x *G.* 'Brookside' (G04) (arrow shows that the pollen tube stopped before it entered the oocyte), and (**d**) *G.* 'Brookside' (G04) x *G. sanguineum* 'Album' (G69) (arrow shows the pollen tube with callose plug) (bar scale = 0.5 mm).

Table 2. Success rate of different crosses and combinations of the first and second breeding seasons.

Growing Season	2020	2021
Number of all crosses	349	1155
Number of combinations	96	236
Number of harvested seeds	15	175
Number of combinations that produced seeds	5	36
Number of combinations that produced healthy seedlings	2	15
Number of healthy seedlings	3	33
Average of cJaccard of crosses	0.81	0.78

3.2. Prediction of Crossing Success

To describe and predict the crossing success for future breeding programs of hardy geraniums, univariate logistic regression models were examined using tube_length, seed_dev, and seed_set as the response criteria (Figures 2 and 3). The explanatory variables expressing parental difference were Chrom, DNA, Style, and cJaccard. Figure 2 displays the regression models, while Figure 3 shows the estimated slopes along with their 95% confidence intervals. Parameter estimates (intercept and slope), 95% confidence intervals, t-values, and corresponding *p*-values for all fitted models can be found in Tables S5–S7. For most combinations, the slope of the parental difference is negative (decreasing regression lines). With increasing parental difference, the success rate, as assessed by tube_length, seed_set, and seed_dev, decreases. In addition, some of the 95% confidence limits for the slope estimates do not cover zero, implying that we can reject the null hypothesis of no slope at a significance level of 5%. More specifically, for cJaccard, the slope is consistently negative, while for Style, Chrom, and DNA, the slopes were not. Conversely, when considering the response variables, seed_dev consistently exhibited a negative relationship with all explanatory variables, making it the most suitable predictor of breeding success in *Geranium*.

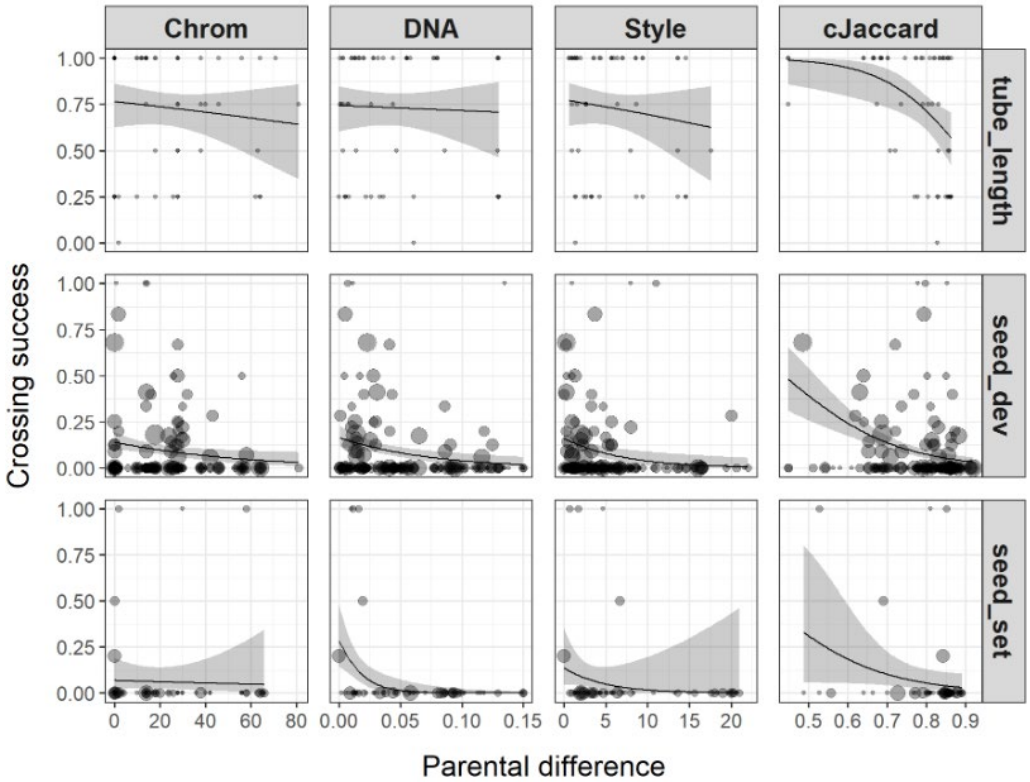

Figure 2. Univariate logistic regression model with response criteria tube_length, seed_dev, and seed_set with Chrom, DNA, Style, and cJaccard as explanatory variables. The size of the points is proportional to the number of observations with the same coordinates. The interval represents the 95% confidence limit. The graphs were created using the function geom_smooth from the R package ggplot with the options method = "glm" and family = "quasibinomial" [30].

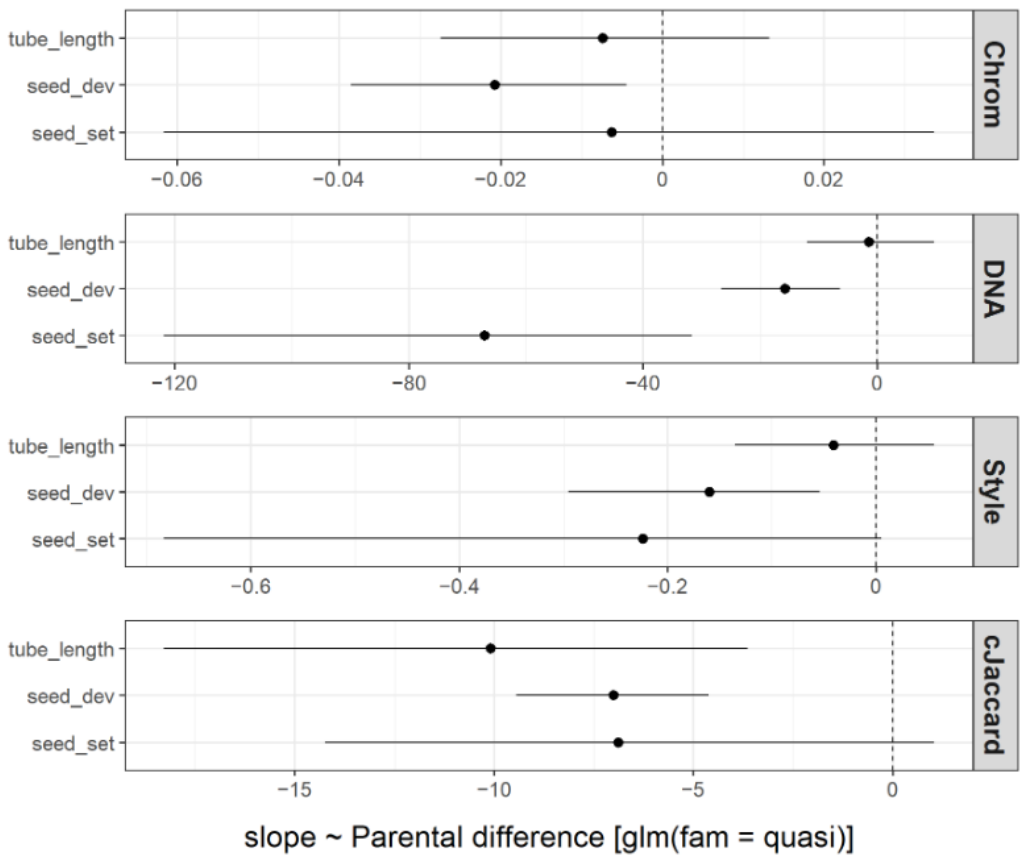

Figure 3. The slope parameters and their 95% confidence interval of the logistic regression model. Negative values whose confidence interval does not pass 0 on the X-axis are significantly negatively correlated with the variables Chrom, DNA, Style, and cJaccard. The confidence intervals were calculated with the emmeans function of the R package emmeans [30].

3.3. Prediction of Required Cross Number to Have 10 Crossing Products

The combination of Jaccard distance (cJaccard) and seed development (seed_dev) as the response variable yielded the best model. According to Table S7, the model for the probability π_{sd} of achieving at least one success for seed development (seed_dev) is as follows:

$$log\frac{\pi_{sd}}{1-\pi_{sd}} = 3.07 - 7.00\ cJaccard$$

We utilized the predicted probabilities to evaluate the number of crosses necessary to achieve 10 successful crossing products on average, which is represented by the equation $10/\pi_{sd}$. Figure 4 provides a graphical representation of this equation for both the fitted value and the lower and upper limits of the predictions. Although the confidence limits for π_{sd} decrease for decreasing Jaccard distance, the uncertainty around the predicted number of crosses increases due to the reduced probability of success at large parental distances. The table displayed within the graph presents the results for some specific values of the Jaccard distance. When the cJaccard of the parents is 0.5, the success rate of crossing is 39%. This implies that harvesting 10 developed seeds requires 25 (18–38) crosses. On the other hand, if the cJaccard value increases to 0.8, the success rate will drop to 7%, and to achieve 10 successful crosses, 136 (99–197) crosses are necessary (Figure 4).

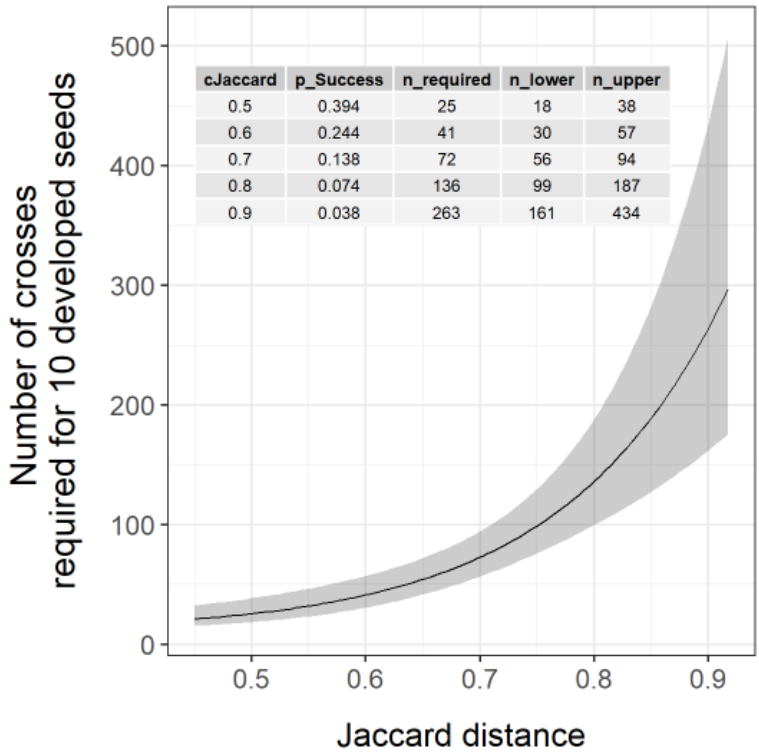

Figure 4. Number of crosses required to obtain 10 flowers with at least one developed seed as predicted by the model based on seed_dev and the Jaccard distance (cJaccard) as a prediction parameter. The estimated coefficients of the model are according to Table S7.

4. Discussion

Prezygotic barriers such as incorrect pollen growth directions, twisted and/or short pollen tubes, and high density of callose plugs observed in *Geranium* are often observed in interspecific or intergeneric crosses in ornamentals [33,34]. When pollen germinates, the accumulation of callose can indicate the incompatibility of pollen grains and tubes, which may also appear in the papillae of the stigma after rejection [35]. The number of callose plugs in *Geranium* is not a real impediment to fertilization since many callose plugs are present in the compatible parent combinations with a low cJaccard, but in plants with a larger size of plugs, the pollen tube may stop earlier. Callose also plays a crucial role in protecting the elongating pollen tube from tensile and compressive stresses and prevents reflux of pollen tube contents, thus maintaining turgor pressure and tube integrity [36,37]. Qin et al. confirmed that in *Arabidopsis thaliana*, pollen tubes without callose plugs were shorter than those with plugs [38]. Further, in *Hibiscus moscheutos*, the number of callose plugs can also be used as an indicator of pollen tube growth rate [39].

Our observations confirm that crossing barriers in *Geranium* primarily resulted from pollen failure, aberrant pollen tube growth, failed fertilization, and arrested growth before the formation of viable seeds. In some combinations, pollen tubes fail to adhere to the stigma, which is common in plants with dry stigmas. In other crosses, swollen pistils are observed without embryo formation, indicating successful pollen tube growth through the style tissue but unsuccessful fertilization. Eventually, in some cases, embryos were formed but never reached the mature stage. Techniques such as reciprocal crosses, mixed or mentor pollination, style manipulation, or stigma treatment can help overcome prezygotic barriers [34,40,41]. Postzygotic barriers often result from genetic discrepancy and chromosome degeneration during cell division of the zygote [42]. Lack of endosperm development, abortion of the embryo, albinism, or lack of vigor are the main observations in postzygotic abortion [3]. In the interspecific hybridization of *Lilium*, embryonic development is influenced by endosperm development, and the lack of embryo development in

the endosperm can be resolved by early embryo rescue [42]. *Geranium* seeds typically have little endosperm [43], and during the mature stage, both the nucellus and endosperm have disappeared [44]. Embryo rescue has been successfully applied to *Pelargonium* sp., a close relative of *Geranium* [45,46].

Our findings strongly support the concept that crossing success diminishes as parental distance increases. In our study, the best measure of parental distance was the Jaccard distance, the complement of the Jaccard similarity. With all variables for parental differences of crossing success, the slope with cJaccard is negative. Notably, the strongest relationship was found with seed_dev, making it the most reliable predictor of crossing success in *Geranium*. This is probably due in part to the high number of replicates and good data coverage. Previous research also supports the importance of cJaccard in determining cross compatibility [26], and based on hybrids described in the literature, we hypothesized that good cross compatibility was correlated with a cJaccard of 0.5 and that the maximum value of cJaccard for hybridization is 0.87. According to our logistic regression model utilizing our hybridization data, cJaccard values of 0.5 and 0.87 correspond to a seed development crossing success of 39% or 4.6%, respectively. These rates are considered indicative of good and poor success, respectively. To our knowledge, this is the first instance where ornamental breeders can quantitatively estimate the number of crosses required to attain a desired success rate based on specific variables of parental differences. This newfound knowledge has the potential to significantly enhance the efficiency of hybrid production in hardy geraniums. By accurately predicting crossing success rates, breeders can strategically plan their breeding programs, optimize resource allocation, and ultimately achieve higher success rates in geranium hybridization.

5. Conclusions

In conclusion, our study demonstrates that the success of interspecific hybridization in *Geranium* can be predicted by considering the genetic distance between parental genotypes and utilizing the logistic regression models developed in this study. We have also discovered that harvesting immature seeds and saving embryos can increase the success rate of hybridization. The logistic regression model we have developed provides breeders with the ability to estimate the number of required crosses to achieve a specific success rate based on parental differences, enabling more realistic and efficient breeding programs in *Geranium*. The increasing success rate of interspecific hybridization through the use of predictive models and advanced breeding techniques can have significant implications for the development of new cultivars.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9060617/s1, Table S1: Different genotypes of *Geranium* sp. used in this study; Table S2: Cross combinations that were carried out for pollen tube growth evaluation with their respective parental difference variables; Table S3: Cross combinations that were carried out for seed_set evaluation with their respective parental difference variables; Table S4: Cross combinations that were carried out for seed_dev evaluation with their respective parental difference variables; Table S5: Intercept and slope of the logistic regression model in the logit scale for pollen tube growth; Table S6: Intercept and slope of the logistic regression model in the logit scale for seed_set; Table S7: Intercept and slope of the logistic regression model in the logit scale for seed_dev.

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