agriculture

MDPI

Article

Parents' Selection Affects Embryo Rescue, Seed Regeneration and the Heredity of Seedless Trait in Table Grape Breeding Programs

Damiano Puglisi [1], Giuseppina Las Casas [2,*], Filippo Ferlito [2,3,*], Elisabetta Nicolosi [1,3], Mario Di Guardo [1,3], Francesco Scollo [3], Giuseppe Saitta [1], Stefano La Malfa [1,3], Alessandra Gentile [1,3] and Gaetano Distefano [1,3]

[1] Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy; damiano.puglisi@phd.unict.it (D.P.); elisabetta.nicolosi@unict.it (E.N.); mario.diguardo@unict.it (M.D.G.); saitta94@hotmail.it (G.S.); stefano.lamalfa@unict.it (S.L.M.); alessandra.gentile@unict.it (A.G.); gaetano.distefano@unict.it (G.D.)
[2] CREA, Research Centre for Olive, Fruit and Citrus Crops, Corso Savoia 190, 95024 Acireale, Italy
[3] AgriUniTech, University of Catania, Via Valdisavoia 5, 95123 Catania, Italy; info@agriunitech.com
* Correspondence: giuseppina.lascasas@crea.gov.it (G.L.C.); filippo.ferlito@crea.gov.it (F.F.); Tel.: +39-095-7653111 (G.L.C.)

Abstract: The development of new seedless cultivar represents one of the most important goals in table grape breeding programmes worldwide. The most common technique to obtain new seedless cultivars is embryo rescue, an approach that allows the isolation of immature embryos and their cultivation in vitro. In this study, a total of 23 crosses (developed employing one seeded and one seedless parent) were performed during two seasons (2017 and 2018) for a total of 1140 seedlings. For each cross, the principal parameters related to the efficiency of the pollination were measured (harvested bunches, collected berries, recovered embryos/seeds and plants obtained). Based on these traits, statistical analyses were performed to calculate the female and male parental efficiency and to compare the two techniques of propagation employed: embryo rescue (7.8% of plants obtained) and gamic propagation (8.4%). Finally, the segregation of the SSR marker p3_VvAGL11 was evaluated on the progeny of four crosses in which the same cultivar was used alternately as female or male parent (SugxIta/ItaxSug and CrixIta/ItaxCri). The parameters measured showed a positive correlation between berries, seeds and plants obtained, exclusively in combination with a seeded female parent. The crossing combinations investigated indicate that some genotypes outperformed others when used as female parent in terms of embryos/seeds produced. Therefore, the efficiency in terms of seedlings obtained for the seedless female parents is actually balanced with those obtained for the seeded ones. The proposed research aims to provide useful information to guide the choice of genotypes used in the genetic improvement programs of seedless grapes, to increase their efficiency.

Keywords: seedlessness; parental efficiency; cross combination; stenospermocarpy; p3_VvAGL11

Citation: Puglisi, D.; Las Casas, G.; Ferlito, F.; Nicolosi, E.; Di Guardo, M.; Scollo, F.; Saitta, G.; La Malfa, S.; Gentile, A.; Distefano, G. Parents' Selection Affects Embryo Rescue, Seed Regeneration and the Heredity of Seedless Trait in Table Grape Breeding Programs. *Agriculture* **2022**, *12*, 1096. https://doi.org/10.3390/agriculture12081096

Academic Editor: Michelle Wirthensohn

Received: 29 June 2022
Accepted: 20 July 2022
Published: 26 July 2022

1. Introduction

In recent years, the production sector of table grapes has experienced radical changes due to innovations both on the agronomical practices and on the availability of novel varieties that are contributing to the production of fruits with improved characteristics (fruit quality, yield, resistance to biotic/abiotic stress) [1–4]. Breeders all over the world are particularly interested in the constitution of seedless varieties bearing berries with increased size [1]; this trend is significantly driven by consumers seeking grapes that are more suitable for consumption by children. Despite the cultivation of the seedless cultivars often being limited by agronomical constraints, several breeding programs are aimed at developing novel varieties coupling seedlessness with optimal fruit quality traits (e.g., large berry size) and resistance to biotic/abiotic stress and [1,5,6].

Two types of seedlessness are known in *Vitis vinifera* L.: parthenocarpy, in which pollination and fertilization are not required, and stenospermocarpy, in which fertilization occurs, but embryo or endosperm development abort during the early steps of seed development [7]. The physiological stage in which the seed's development is interrupted determines the occurrence of woody or herbaceous rudiments of the seed [8]. Traditional breeding programs aimed at the development of seedless varieties are hampered by several issues regarding: (i) the impossibility to obtain seed from the stenospermocarpic female parents, (ii) the poor germination rate, especially in in the early ripening seeded grapes [9–11], (iii) the bud's fertility [12].

The main source of seedlessness in table grapes was the group of Kishmish grapes, ancient oriental varieties, among which is the white-berried 'Thompson seedless' or 'Sultanina' [13,14]. Many programs of genetic improvement used a 'Sultanina'-derived cultivar characterized by soft seeds, generally without seed coat lignification and so imperceptible chewing of the berry. Starting with this genetic pool, most of the cultivated seedless grapes were developed in California [15], Israel [16], South Africa, Chile, Australia and other countries [1]. In Italy, genetic improvement programs for seedless table grapes only began in recent years, mainly by private companies and research institutions in the south.

The first use of embryo rescue in grapes was reported in 1982 by Emershad and Ramming [17]; then, technical advancement made the use of zygotic embryos obtained from a seedless female parent an efficient and extensively utilized practice. This biotechnological approach consists of the cultivation and the development of isolated immature embryos in vitro [17,18] and nowadays is routinely employed in breeding programs for seedless varieties [19].

If the embryo rescue enables the use of seedless cultivars also as the female parent, the efficiency in terms of seedlings generated varies strongly according to the cross combinations. Therefore, knowledge of the regeneration rates of the parents chosen in a mating scheme (alone and in combination) is a fundamental prerequisite to increase the odds of obtaining seedless grapes with positive repercussions on the time and costs of the whole breeding process [20]. So, the successful application of embryo rescue is strongly influenced by the choice of the parents and their cross-compatibility. These aspects play an important role in influencing the embryo recovery rate and the seedling development [21,22]. Zhu et al. [23], through the analysis of the seedlings of five segregating populations with the same male parent, showed that the success ratio of crosses is greatly influenced by the choice of the parents. Furthermore, the successful development of embryos is greatly influenced by their stage of maturity at the time of extraction and by the culture medium used [24]. Preliminary studies have also shown that reciprocal crosses determine a different efficiency both by crossing two seedless varieties [25], and by crossing a diploid variety with a tetraploid variety [26].

Table grapes, as well as all the tree crops, undergo a long juvenility period (up to 4–5 years) in which fruits cannot be evaluated. Nevertheless, the use of molecular markers can greatly help to speed up the selection process through marker-assisted selection (MAS). Microsatellite markers or Single Sequence Repeats (SSRs) are ideal tools to genetically characterize a germplasm and to infer phylogenetic relationships [27–29]. Several studies have compared SSRs with different molecular markers, including Single Nucleotide Polymorphisms (SNPs), which are considered to be very efficient for discriminating genetic diversity in a grapevine, concluding that SSRs are the most efficient markers for determining parent–progeny relationships [30–32].

Recently, a SSR marker named p3_VvAGL11 was detected. This marker is mapped within the promoter region of the AGAMOUS-like 11 gene of *V. vinifera* (VvAGL11), a major functional gene involved in seedlessness [33,34]. Successive works [22,28] have tested the efficiency of p3_VvAGL11 in discriminating between woody seeds and varieties characterized by either seedlessness or the occurrence of a rudimental-herbaceous seed. The combination of in vitro embryo culture and MAS can be very effective in detecting and excluding seedlings carrying negative alleles in early stages, and reducing the time and

costs needed for the development of a novel variety [8,27]. To this extent, the use of embryo culture followed by the screening of the seedlings with the VvAGL11 marker can greatly help breeding programs worldwide to reduce time and costs needed for the development of seedless table grape varieties [1,35].

The aim of this research is to evaluate the influence of the genetic background of selected parents in plantlet regeneration from aborted embryos and seeds. Furthermore, the effect of the genotype employed alternately as male or female parent in transmitting the seedless traits, using the SSR marker p3_VvAGL11, was evaluated. To achieve these goals, 23 cross combinations were carried out using male and female parents from both seedless and seeded cultivars, monitoring the differences in seed germination and embryo regeneration.

2. Materials and Methods

2.1. Site, Plant Material and Experimental Design

The research was carried out in a commercial table grape farm located in south Italy (Sicily: lat. 37°04′56″ N, long 14°33′32″ E; 270 m elevation), in an area characterized by extensive cultivation of table grape.

Vines of five- to six-year-old vines, grafted onto 140 Ruggieri rootstock and planted at a density of 1500 vines per hectare in order to obtain uniform behavior of the grapevine root system [36], were used. Vines were trained using the overhead system called 'Tendone' in Italian [37]. The structure was covered by a thin white net. Above the net, and also in the laterals, a 0.16 mm-thick white polyethylene was applied a week after the first leaf unfolded and spread away from the shoot (BBCH-11) [38]. Seeded vines' architecture consists of a trunk 1.40 m in height, with four main branches, each with 4 fruiting canes pruned to 8–10 nodes; thus, there are 36–40 buds per vine. The seedless cultivar retained several fruiting canes for a total of 100–120 buds per vine. The branches were maintained by wires at 45 degrees. A drip irrigation system was used, and water was supplied every 3–4 days to maintain the soil near the drippers above 75% of the field capacity. All agronomic practices were applied uniformly across treatments and following the standard commercial practice of the area. Fertigation was commonly applied. Five seedless female parents—'Sublima' (Sub), 'Sugraone' (Sug), 'Crimson' (Cri), 'Summer Royal' (SuR), 'Luisa' (Lui)—and three seeded female parents: 'Italia' (Ita), 'Italia2' (Ita2), 'Muscat of Hamburg' (MoH) were used as maternal lines. Except for 'Summer Royal' and 'Muscat of Hamburg', all of the mentioned cultivars were also used as male parent. In addition, the seedless 'Pink Muscat' (PMu) and the seeded 'Black Magic' (BlM) and 'Victoria' (Vic) were used as male parent (Table 1).

Table 1. Cultivars used and cross combination acronyms: 24 cross combination were performed using seedless and seeded parent.

	Female Parent	Male Parent	Cross Acronym
seedless female parent	Sublima	Sugraone	SubxSug
	Sublima	Italia2	SubxIta2 (2017) SubxIta2 (2018)
	Sugraone	Italia2	SugxIta2
	Sugraone	Italia	SugxIta
	Sugraone	Muscat of Hamburg	SugxMoH
	Crimson	Muscat of Hamburg	CrixMoH
	Crimson	Italia	CrixIta
	Summer royal	Black magic	SuRxBlM
	Summer royal	Victoria	SuRxVit
	Summer royal	Italia2	SuRxIta2
	Luisa	Sublima	LuixSub
	Luisa	Italia2	LuixIta2
	Luisa	Italia	LuixIta

Table 1. *Cont.*

	Female Parent	Male Parent	Cross Acronym
seeded female parent	Italia	Sublima	ItaxSub
	Italia	Luisa	ItaxLui
	Italia	Sugraone	ItaxSug
	Italia	Crimson	ItaxCri
	Italia	Pink muscat	ItaxPMu
	Italia2	Sublima	Ita2xSub
	Italia2	Sugraone	Ita2xSug
	Italia2	Pink Muscat	Ita2xPMu
	Muscat of Hamburg	Sugraone	MoHxSug
	Muscat of Hamburg	Pink muscat	MoHxPMu

2.2. Crossing

A total of 23 crosses were performed in two years, 2017 and 2018 (Table 1). The combination SubxIta2 was repeated in both years to verify any eventual environmental effect. The crossing periods depend on the flowering time of the cultivars used and this spanned from 14 March 2017 to 26 April 2017 and from 12 April 2018 to 14 May 2018. The pollen was sampled when the plants were from stage BBCH-65 and BBCH-68, when the pollen was out from the anthers, while the female parent was used at the stage BBCH-60 ("First flowerhoods detached from the receptacle" stage). The inflorescences were emasculated by hand or tweezers. The open flowers were eliminated before the emasculation. Artificial pollination was carried out using inflorescences of the male parent; pollinated inflorescences were immediately bagged and marked to record the cross and the pollination date. A total of 212 bunches were pollinated between 2017 and 2018. After the initial fruit development, (10 days) the bags were removed to allow the regular growth of the bunches. The name of the cross is given by the female parent followed by the male parent (♀× ♂).

2.3. Embryo Rescue and Plant Development

Immature berries were collected 2 months after pollination, about 30–40 days before the harvesting period. The berry surface was treated with a 1% solution of Plant Preservative Mixture (PPM, Plant Cell Technology—Washington, DC, USA), to preserve the skin until sterilization. Later, the berries were sterilized in 70% ethanol solution for 30 s and in 2% sodium hypochlorite added to Tween20 (0.1%) solution for 20 min, followed by 3 washes in sterile water. The berries were sectioned longitudinally, and the ovules were excised and placed on a culture medium consisting of 2.5 g/L McCown Woody Plant Medium (WPM), including vitamins (Duchefa Biochemie), supplemented with 30 g/L sucrose, 10 mg/L indole-3-acetic acid (IAA), 15 mg/L gibberellic acid (GA_3), 4 g/L gelrite (Duchefa Biochemie); 2.5 g/L of activated charcoal (AC) was added to the medium, to avoid the embryo itself producing toxic substances [19,39].

The plates were stored in a climatic chamber at a temperature of 25 ± 1 °C and with a photoperiod of 16 h of light, with a light intensity of 100 μM s^{-1} m^{-2}.

Every six weeks, until the beginning of germination, the embryos were transferred in a new substrate, and when the plants showed 3–4 leaflets and a root system, they were transferred to jiffy® (Kanagawa, Japan). After 2–3 weeks, the plants were transplanted and acclimatized.

2.4. Plant Developed from Seeded Female Parent

Seeds from mature berries obtained from crosses with seeded female parents were extracted and dried for 20 days at room temperature, then layered on sand for 90 days at 4 °C [40]. Seeds were then treated with 1 g/L of gibberellic acid (GA_3) for 24 h at room temperature and washed in sterile water, dried for a week and sown in a vase [41]. After germination and initial growth, the plantlets were transplanted in jiffy® and acclimatized.

2.5. DNA Extraction and Marker Assisted Selection (MAS)

DNA was extracted from 1 mg of young leaves using the Cetyl-trimethylammonium bromide (CTAB) method [42], after tissue homogenization by Tissuelyser (QIAGEN, Hilden, Germany). DNA extraction was performed on the progeny of 4 crosses in which the same cultivar was used alternately as the female or male parent (SugxIta/ItaxSug and CrixIta/ItaxCri) and on the three genotypes used as parents. SSR analysis was performed as reported in Bennici et al. [23]. Image acquisition and fragment size estimation were performed using GelDOCTM XR+ system, equipped with Image LabTM 6.1 software (BIO-RAD—Hercules, CA, USA).

2.6. Efficiency of the Cross Pollination and Statistical Analysis

For each cross, the number of pollinated inflorescences, harvested bunches, collected berries, and recovered embryos/seeds and plants obtained were registered. The percentage of embryos or seeds was calculated as a function of the number of opened berries. Moreover, the female and the male parental efficiency was calculated as number of embryos/seeds that developed a plant out of the total of embryos/seeds collected (conversion ratio).

The Student *t*-test was carried out for reciprocal crossings (i.e., in which the same parents are used once as male/female and once as female/male) considering the ratios of bunches collected on pollinated inflorescences, number of embryos or seeds on number of berries, and finally the plant obtained on number of embryos or seeds; the analysis was achieved using R 4.0.3 statistical software (R Foundation for Statistical Computing: Vienna, Austria) [43].

Pearson correlation analysis, among all the parameters collected, was computed using the psych package [44] implemented in R 4.0.3 statistical software [43]. To evaluate the segregation of the SSR marker p3_VvAGL11 on the progeny of the SugxIta, ItaxSug, CrixIta and ItaxCri, the χ^2 test of independence was computed with R 4.0.3 statistical software [43].

3. Results and Discussion

Seedlessness in table grapes represents one of the main objectives for the development of novel varieties. In light of this, 23 cross combinations were made, adding up to almost 6000 embryos and more than 8000 seeds obtained in total. All the raw data concerning the number of berries, embryos or seeds and the plants obtained are summarized in the Supplementary Materials (Table S1).

3.1. Fertilization and Seed/Fruit Set Evaluation

Among the seedless × seeded crosses, 69% of pollinated inflorescences underwent the complete ripening process (the remaining showed the rachis wilting and/or did not set berries), while 87% of bunches correctly developed in the seeded × seedless crosses (Table S1). The lower efficiency registered in the seedless × seeded crosses is probably due to the high rate of flower and fruit fall proper of seedless cultivars. However, when the pollination was successfully performed, the average berry number collected from each bunch did not differ significantly in crosses with the seedless (42 bunches) or seeded (44) maternal line, suggesting that the development of the berries is independent from the development of the seed (Table S1).

Among the seedless × seeded crosses, the cultivar 'Luisa' successfully developed all the pollinated inflorescences (15/15); on the contrary, 'Sugraone' was characterized by the lowest fruit set efficiency with 'only' 9 bunches collected from 25 pollinated inflorescences (38%) (Figure 1). This difference is probably related to the low fertility rate of 'Sugraone', as reported by Ferrara and Mazzeo [12]. Moreover, the small size of the flower, and in particular of the calyptra, could also influence the success rate of the crossing operation; in fact, the flower of 'Sugraone' is particularly small and delicate, compared to Luisa's inflorescences.

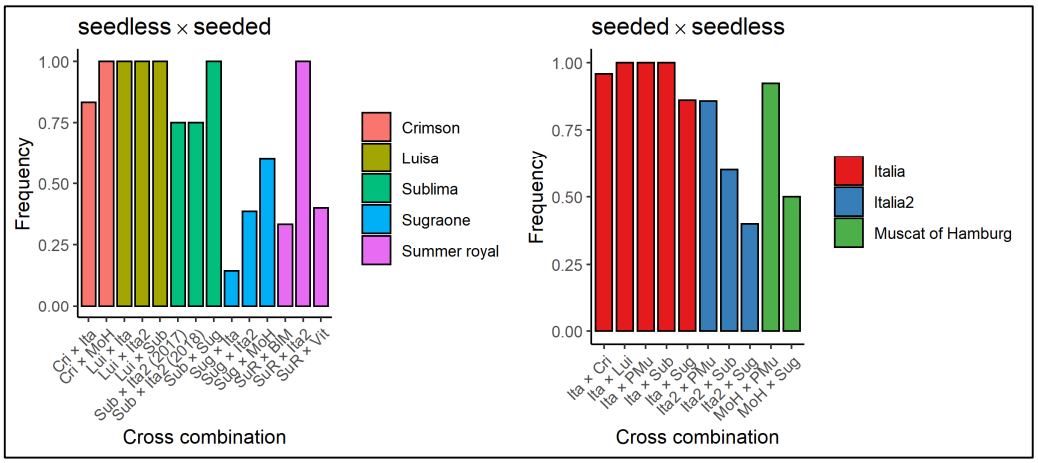

Figure 1. Ratio of the bunches collected and pollinated inflorescences: seedless × seeded; seeded × seedless.

As for the seeded × seedless crosses, the female parents 'Italia' and 'Italia2' were characterized, respectively, by the highest (96%) and the lowest (62%) number of mature, well developed, bunches (Figure 1). Moreover, for each of the three seeded cultivars 'Italia', 'Italia2' and 'Muscat of Hamburg', the worst result in terms of harvested bunches was always recorded in the combination with the cultivar 'Sugraone' used as male parent. Therefore, 'Sugraone' showed the lowest fertility both as pollen donor and as female recipient among all the parents tested.

The mean value of embryos and seeds obtained from the collected berries was 1.4 for the seedless × seeded crosses and 1.6 for the seeded × seedless ones. In total, 7.8% and 8.4% of plants were obtained from embryo rescue seed extraction, respectively (Table S1).

As for the average number of embryos recovered per berry in each cross (Figure 2), the best performance was observed in 'Luisa' (1.8) while both 'Sublima' and 'Sugraone' registered an average of 0.94 embryos per berry. The number of seeds collected per berry was highly variable (Figure 2), with values ranging from 0.13 for ItaxLu to 2.8 for MoHxSug.

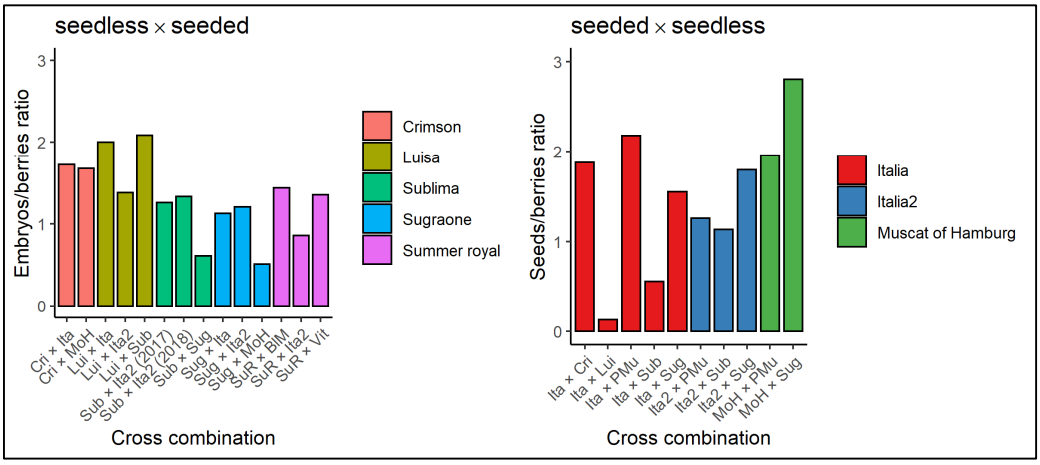

Figure 2. Ratio of the number of embryos or seeds and the number of berries: seedless × seeded; seeded × seedless.

3.2. Embryo and Seed Plant Regeneration Efficiency

The parental efficiency was evaluated by the conversion ratio; a parameter reflecting the number of embryos (seedless × seeded) or seeds (seeded × seedless) effectively developed into plants. This value showed an average of 12%, which is similar to what was reported by Z. Li et al. and Tian et al. [19,45]. On the other hand, a great variability was

found among the parentals (Figure 3). In our analysis, 'Sugraone' registered the lowest efficiency in terms of developed bunches and number of embryos, but those embryos showed the highest survival rate, with 36% effectively developed in adult plants. An opposite behavior was registered for 'Crimson' and 'Luisa', which were both characterized by the production of a high number of embryos and a low conversion rate in plants ('Crimson' 2%; 'Luisa' 4%) (Table S1). It is probable that the few berries developed in the crosses with 'Sugraone' all contain viable embryos, on the contrary 'Crimson' and 'Luisa' can also grow berries in which the development of the embryo does not proceed correctly.

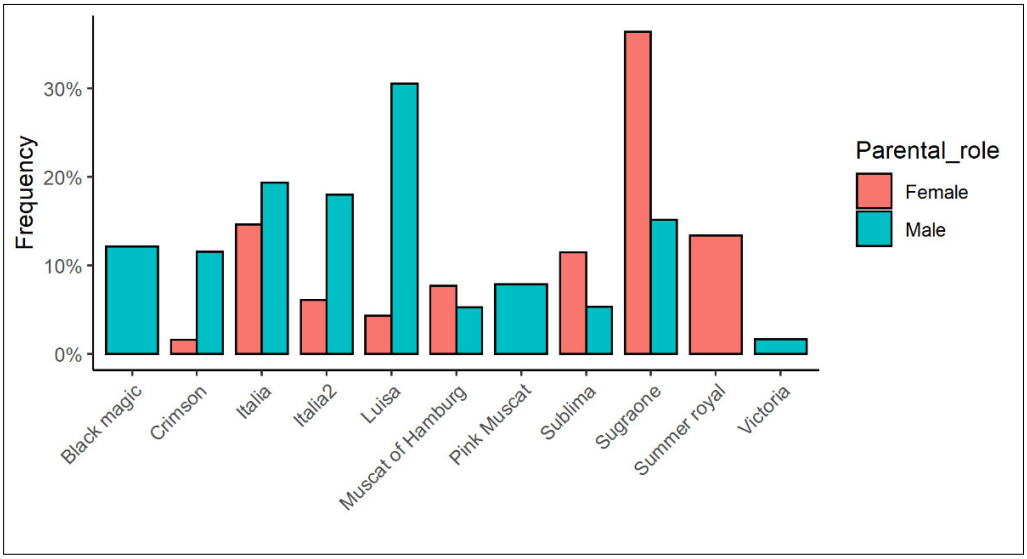

Figure 3. Parental efficiency of each cultivar measured as number of plantlets per embryos/seeds along with all cross combination.

Among the male parents, the best result was achieved for 'Luisa', which showed a parental efficiency of the 30%; on the contrary, 'Victoria' gave the worst performance with only 1.6% of germinated seeds (Table S1).

Some cultivars, as supposed, showed a high difference in terms of efficiency when used as male or female parent ('Crimson' ♀1.6%, ♂11%; 'Italia2' ♀6%, ♂18%; 'Luisa' ♀4%, ♂30%; 'Sugraone' ♀36%, ♂15%), demonstrating that the genetic contribution of the genotype changes according to its role (Figure 3).

The number of plantlets obtained varied strongly based on the different genetic combinations (Figure 4). The crosses, Ita2xSub and Ita2xSug, despite the number of seeds obtained (respectively 34 and 145) did not produce offspring (Figure 4). This is probably caused by the early developmental status of the embryos in the seeds rather than the cross combination itself, as it occurs for the germination of the excised ovule [46].

For seedless × seeded crosses, the average number of plants among the crosses was the 15%; 'Sugraone', although rit egistered the lower efficiency in terms of developed bunches and number of embryos, showed the highest survival rate with the 36% (50% for SugxIta2 and SugxIta; 10% SugxMoH) of embryos effectively developed in adult plants. In seeded × seedless crosses, only ItaxLui showed a higher frequency (30%) compared to the overall average (10%) (Table S1). In this case, the good result does not seem to be determined by the maternal parent 'Italia', but by the specific genetic combination with the 'Luisa' cultivar. In fact, in this combination, a very low number of seeds is obtained; however, these had a very high germination rate. It can be assumed that seeds that were unable to develop, were aborted prematurely and were subsequently discarded at the time of collection.

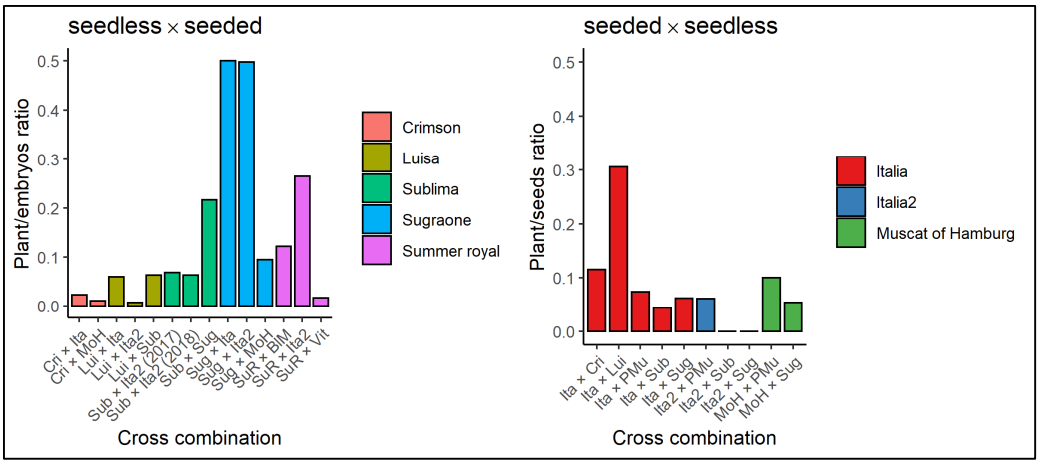

Figure 4. Ratio of the plant and the number of embryos or seeds: seedless × seeded; seeded × seedless.

The Student *t*-test carried out for reciprocal crossings on reproductive performances (bunches collected on pollinated inflorescences, number of embryos or seeds on number of berries, and plant obtained on number of embryos or seeds) always showed statistical significance, with p-value < 0.05. So, the genetic background, which affects the plantlets' regeneration and the obtaining of a high number of seedlings, seems to be linked to the specific cross combinations and to the maternal or paternal role of selected genotypes.

The parameters measured on seedless × seeded and on seeded × seedless crosses showed a significant correlation for several traits in analysis. In Figure 5 (seedless female parents), the highest correlation was detected for the correlation of bunches and embryos (r = 0.919 ***) and of berries and embryos (r = 0.896 ***), while the lowest was detected for embryos and plants (r = 0.112). In Figure 5 (seeded female parents), the correlation between the investigated parameters is generally significant, (r between 0.993 *** and 0.827 **), achieved for the correlation between all investigated parameters with the plants obtained, were significant.

The different correlation estimated between berries and pollinated inflorescences in cross combinations with seedless (r = 0.624 *) and seeded (r = 0.961 ***) parents was probably linked to the different physiological development of the bunches determined by the presence of the seed. On the other hand, the number of berries was not affected by the presence of the seeds, as the number of berries showed a similar significant correlation both with the number of embryos (r = 0.896 ***) and the number of seeds (r = 0.979 ***) collected. Finally, in seedless × seeded crosses, berries and embryos collected showed a low correlation with the number of plants obtained, (r = 0.267 and r = 0.112, respectively); however, in seeded × seedless crosses, a strong correlation was detected between plants obtained and both berries and seeds collected (r = 0.875 *** and r = 0.934 ***, respectively) (Figure 5).

The choice of the cultivars for genetic improvement programs should consider that, more than the number of embryos (or seeds) per berries, the effect of cross combination, in developing plants from the embryos or the seeds, plays a significant role. This is reflected by the results of the different crosses: CrixIta was characterized by a high ratio of embryos per berry but a low conversion level, while, on the contrary, SugxIta2, was characterized by a lower number of embryos but a much higher conversion rate, thus making the latter a more effective cross for breeding purposes compared to CrixIta (Figure S1).

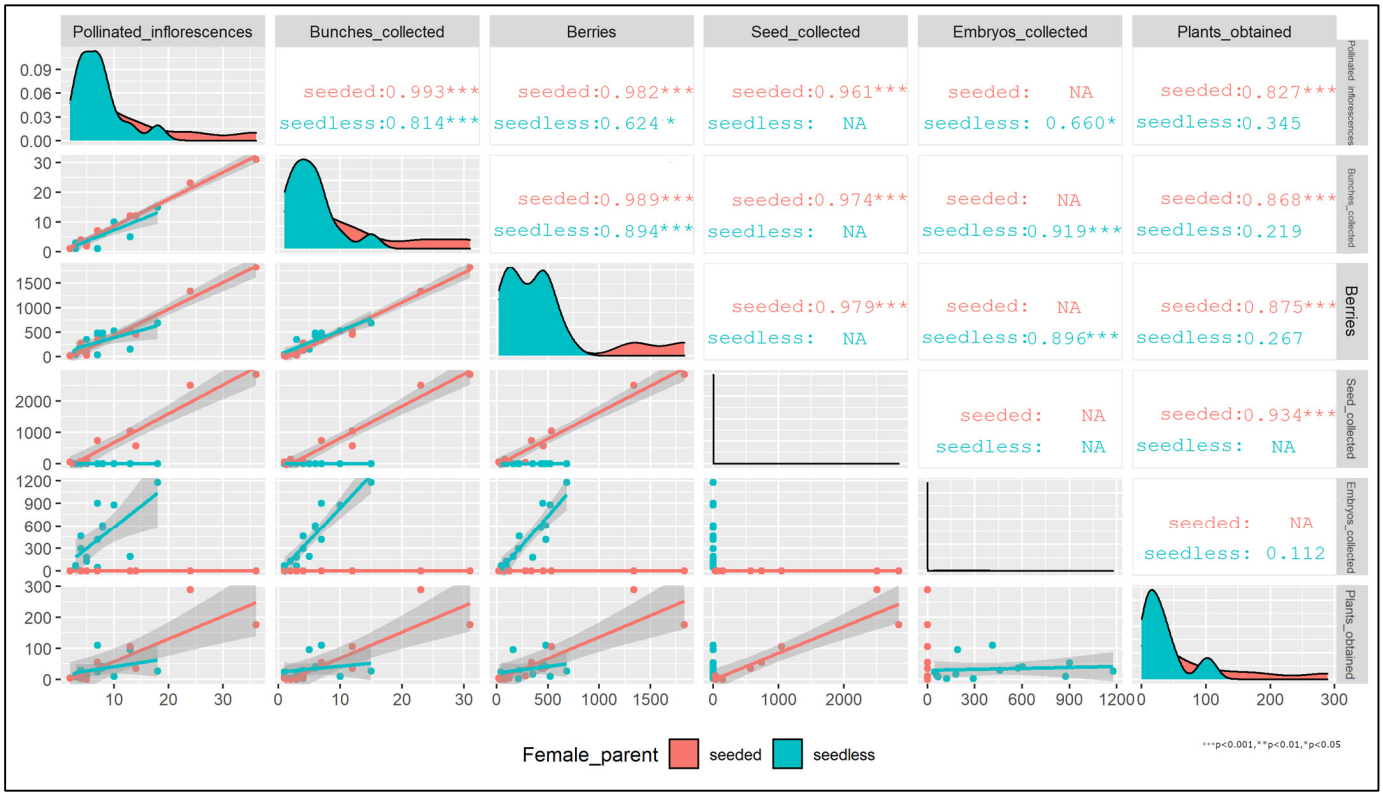

Figure 5. Pairwise correlations of crossing efficiency parameters: on the upper triangle, the pairwise Pearson correlations computed among all the parameters collected is shown; on the lower triangle the pairwise correlation between the parameters collected is shown; the diagonal line shows the histogram of each parameter in the two groups (seeded, seedless).

3.3. Validation of the Molecular Marker for Seedlessness according to the Different Parental Lines and the Direction of the Cross

Through the segregation of the p3_VvAGL11 marker, we evaluated the influence of the parents in conferring the seedlessness trait, also taking into consideration their employment as female or male parents. Molecular marker analysis was conducted on crosses obtained from the seeded 'Italia' crossed with the seedless cultivars 'Crimson' or 'Sugraone'; each of the three parental lines was employed both as maternal and paternal lines, generating four crosses (ItaxCri, CrixIta, ItaxSug and SugxIta). The two seedless parents ('Sugraone' and 'Crimson') have a heterozygous allelic configuration for the p3_VvAGL11 marker, presenting amplicons with sizes equal to 184 and 194 bp (Figure S2). This genetic configuration characterize samples carrying either herbaceous or aborted seeds [22,28]. The seeded parent 'Italia' is instead characterized by the 184 bp allele in homozygous status. This implies that the offspring of both crosses can show two genotypes: 184/184 (woody seed) and 184/194 (seedless or herbaceous seed).

The expected segregation in all crosses tested is 1:1, while Figure 6 shows that, in three cases, the segregation of the character was in favor of the genotype with woody seed (with similar relative frequencies: 60.4% in CrixIta; 60.7% in ItaxCri; 60.8% in ItaxSug). SugxIta was instead characterized by an opposite behavior with 71.4% of offspring showing a 184/194 genetic allelic configuration. Marker p3_VvAGL11 shows differential efficiency due to the genetic background; for germplasm originated from 'Crimson' and 'Sugraone' it shows good prediction power, with values of false negative equal to 9% and 7%, respectively, and false positive values of 0% and 12% [24].

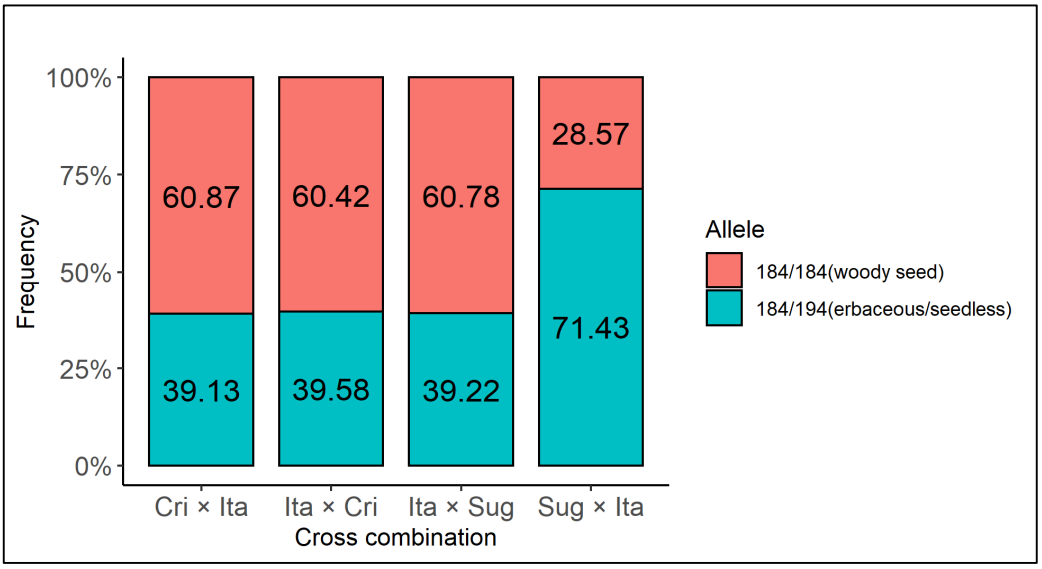

Figure 6. Distribution of allele p3_VvAGL11 in hybrids of 4 cross combinations in which the parental lines are used both as maternal and paternal line.

The χ^2 test, based on the SSR scoring of p3_VvAGL11, confirmed that the observed and expected frequencies of the two genotypes (woody on one side and herbaceous or aborted on the other) did not show significant differences in the reciprocal crossings having as parents 'Crimson' and 'Italia' ($\chi^2 = 0$; p-value = 1). As for the two crosses deriving from 'Sugraone' and 'Italia', the observed and expected frequencies of the two genotypes differ significantly ($\chi^2 = 4.95$; p-value = 0.025). This suggests that there is an effect of the genotype employed alternately as male or female parent in transmitting the seedless traits.

4. Conclusions

This research, carried out through 23 cross combinations, provides useful elements for the planning and implementation of breeding programs for table grapes. The choice of the female parent affects the determination of the number of embryos or seeds obtained, as observed on 'Sugraone', which produced a low number of embryos compared to 'Italia', which produced many seeds, reflecting the different degree of fertility reported by Ferrara and Mazzeo [12]. The genetic background and the specific combination of maternal and paternal lines affect fertilization and regeneration as supported by statistical analysis, to obtain a high number of seedlings, which is the aim of a genetic improvement program. Molecular marker analysis has shown that the role of a genotype as male or female parent also affects the probability of obtaining seedless progeny. Regarding the use of seeded or seedless female parents, the efficiency is the same as long as we observed that number of berries collected, embryos or seeds extracted and seedlings obtained were in substantial balance. Finally, the correlation identifies significant values between berries and plants obtained, exclusively in combination with the seeded female parent.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12081096/s1, Figure S1: Ratio of the plant and the berries; Figure S2: Amplification results for molecular SSR marker p3_VvAGL11; Table S1: Performed crossing combination.

Author Contributions: Conceptualization: F.F., E.N. and G.D.; methodology: D.P. and G.L.C.; validation, D.P. and G.L.C.; investigation: D.P., G.L.C., F.F., E.N., F.S., G.S. and G.D.; data curation: D.P. and M.D.G.; writing—original draft preparation: D.P. and G.L.C.; writing—review and editing: F.F., E.N., M.D.G., S.L.M., A.G. and G.D.; supervision: G.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Akkurt, M.; Tahmaz, H.; Veziroğlu, S. Recent Developments in Seedless Grapevine Breeding. *S. Afr. J. Enol. Vitic.* **2019**, *40*, 2. [CrossRef]
2. Karaagac, E.; Vargas, A.M.; de Andrés, M.T.; Carreño, I.; Ibáñez, J.; Carreño, J.; Martínez-Zapater, J.M.; Cabezas, J.A. Marker Assisted Selection for Seedlessness in Table Grape Breeding. *Tree Genet. Genomes* **2012**, *8*, 1003–1015. [CrossRef]
3. Nicolosi, E.; Ferlito, F.; Amenta, M.; Rapisarda, P. Shelf-Life of Minimally Processed Table Grapes Packed in Snack-Size Containers. *Acta Hortic.* **2018**, *1209*, 417–424. [CrossRef]
4. Nicolosi, E.; Ferlito, F.; Amenta, M.; Russo, T.; Rapisarda, P. Changes in the Quality and Antioxidant Components of Minimally Processed Table Grapes during Storage. *Sci. Hortic.* **2018**, *232*, 175–183. [CrossRef]
5. Ponce, M.T.; Agüero, C.B.; Gregori, M.T.; Tizio, R. Factors Affecting the Development of Stenospermic Grape (*Vitis vinifera*) Embryos Cultured in Vitro. *Acta Hortic.* **2000**, *528*, 667–671. [CrossRef]
6. Nookaraju, A.; Barreto, M.S.; Karibasappa, G.S.; Agrawal, D.C. Synergistic Effect of CPPU and Benzyladenine on Embryo Rescue in Six Stenospermocarpic Cultivars of Grapevine. *Vitis-J. Grapevine Res.* **2007**, *46*, 188–191. [CrossRef]
7. Stout, A. *Seedlessness in Grapes*; Technical Bulletin 238; New York State Agricultural Experiment Station: Geneva, NY, USA, 1936.
8. Mejía, N.; Hinrichsen, P. A New, Highly Assertive Scar Marker Potentially Useful to Assist Selection for Seedlessness in Table Grape Breeding. *Acta Hortic.* **2003**, *603*, 559–564. [CrossRef]
9. Ramming, D.W.; Emershad, R.L.; Spiegel-Roy, P.; Sahar, N.; Baron, I. Embryo Culture of Early Ripening Seeded Grape (*Vitis vinifera*) Genotypes. *HortScience* **1990**, *25*, 339–342. [CrossRef]
10. Garcia, E.; Martinez, A.; Garcia De La Calera, E.; Perez, L.J.; Cenis, J.L.; Carrefio, J. In Vitro Culture of Ovules and Embryos of Grape for the Obtention of New Seedless Table Grape Cultivars. *Acta Hortic.* **2000**, *528*, 663–666. [CrossRef]
11. Perl, A.; Sahar, N.; Spiegel-Roy, P.; Gavish, S.; Elyasi, R.; Orr, E.; Bazak, H. Conventional and Biotechnological Approaches in Breeding Seedless Table Grapes. *Acta Hortic.* **2000**, *528*, 613–618. [CrossRef]
12. Ferrara, G.; Mazzeo, A. Potential and Actual Bud Fruitfulness: A Tool for Predicting and Managing the Yield of Table Grape Varieties. *Agronomy* **2021**, *11*, 841. [CrossRef]
13. Dangi, G.S.; Mendum, M.L.; Prins, B.H.; Walker, M.A.; Meredith, C.P.; Simon, C.J. Simple se-quence repeat analysis of a clonally propagated species: A tool for managing a grape germplasm collection. *Genome* **2001**, *44*, 432–438. [CrossRef] [PubMed]
14. Ibáñez, J.; Vargas, A.M.; Palancar, M.; Borrego, J.; De Andrés, M.T. Genetic relationships among table-grape varieties. *Am. J. Enol. Vitic.* **2009**, *60*, 35–42.
15. Ramming, D.W.; Tarailo, R.E.; Sayed, A.B. 'Crimson Seedless': A New Late-maturing, Red Seedless Grape. *Hortscience* **1995**, *30*, 1473–1474. [CrossRef]
16. Perl, A.; Sahar, N.; Eliassi, R.; Baron, P.; Spiegel-Roy, P.; Bazak, H. Breeding of new seedless table grapes in Israel conventional and biotechnological approach. *Acta Hortic.* **2003**, *623*, 185–187. [CrossRef]
17. Emershad, R.L.; Ramming, D.W. In-ovulo embryo culture of *Vitis vinifera* L. cv. 'Thompson Seedless'. *HortScience* **1982**, *17*, 576.
18. Cain, D.W.; Emershad, R.L.; Tarailo, R.E. In Ovulo Embryo Culture and Seedling Development of Seeded and Seedless Grapes (*Vitis vinifera* L.). *Vitis* **1983**, *22*, 9–14.
19. Tian, L.; Wang, Y.; Niu, L.; Tang, D. Breeding of Disease-Resistant Seedless Grapes Using Chinese Wild Vitis Spp. I. In Vitro Embryo Rescue and Plant Development. *Sci. Hortic.* **2008**, *117*, 136–141. [CrossRef]
20. Li, J.; Wang, X.; Wang, X.; Wang, Y. Embryo Rescue Technique and Its Applications for Seedless Breeding in Grape. *Plant Cell Tissue Organ Cult.* **2015**, *120*, 861–880. [CrossRef]
21. Jiao, Y.; Li, Z.; Xu, K.; Guo, Y.; Zhang, C.; Li, T.; Jiang, Y.; Liu, G.; Xu, Y. Study on Improving Plantlet Development and Embryo Germination Rates in in Vitro Embryo Rescue of Seedless Grapevine. *N. Z. J. Crop Hortic. Sci.* **2017**, *46*, 39–53. [CrossRef]
22. Li, T.; Li, Z.; Yin, X.; Guo, Y.; Wang, Y.; Xu, Y. Improved in Vitro *Vitis vinifera* L. Embryo Development of F1 Progeny of 'Delight' × 'Ruby Seedless' Using Putrescine and Marker-Assisted Selection. *Vitr. Cell. Dev. Biol.-Plant* **2018**, *54*, 291–301. [CrossRef]
23. Zhu, P.; Gu, B.; Li, P.; Shu, X.; Zhang, X.; Zhang, J. New Cold-Resistant, Seedless Grapes Developed Using Embryo Rescue and Marker-Assisted Selection. *Plant Cell Tissue Organ Cult.* **2019**, *140*, 551–562. [CrossRef]
24. Kebeli, N.; Boz, Y.; Özer, C. Studies on the Applying of Embryo Culture in Breeding New Hybrids by Crossing Seedless Grape Cultivars. *Acta Hortic.* **2003**, *625*, 279–281. [CrossRef]
25. Valdez, J.G.; Ulanovsky, S.M. In Vitro Germination of Stenosperrnie Seeds from Reciprocal Crosses (*Vitis vinifera* L.) Applying Different Techniques. *Vitis* **1997**, *36*, 51–58.
26. Yamashita, H.; Horiuchi, S.; Taira, T. Development of Seeds and the Growth of Triploid Seedlings Obtained from Reciprocal Crosses between Diploids and Tetraploids Grapes. *J. Jpn. Soc. Hortic. Sci.* **1993**, *62*, 249–255. [CrossRef]

27. Doligez, A.; Adam-Blondon, A.F.; Cipriani, G.; Di Gaspero, G.; Laucou, V.; Merdinoglu, D.; Meredith, C.P.; Riaz, S.; Roux, C.; This, P. An Integrated SSR Map of Grapevine Based on Five Mapping Populations. *Theor. Appl. Genet.* **2006**, *113*, 369–382. [CrossRef]
28. Bennici, S.; Di Guardo, M.; Distefano, G.; La Malfa, S.; Puglisi, D.; Arcidiacono, F.; Ferlito, F.; Deng, Z.; Gentile, A.; Nicolosi, E. Influence of the Genetic Background on the Performance of Molecular Markers Linked to Seedlessness in Table Grapes. *Sci. Hortic.* **2019**, *252*, 316–323. [CrossRef]
29. Bennici, S.; Las Casas, G.; Distefano, G.; Guardo, M.D.; Continella, A.; Ferlito, F.; Gentile, A.; La Malfa, S. Elucidating the Contribution of Wild Related Species on Autochthonous Pear Germplasm: A Case Study from Mount Etna. *PLoS ONE* **2018**, *13*, e0198512. [CrossRef]
30. Emanuelli, F.; Lorenzi, S.; Grzeskowiak, L.; Catalano, V.; Stefanini, M.; Troggio, M.; Myles, S.; Martinez-Zapater, J.M.; Zyprian, E.; Moreira, F.M.; et al. Genetic Diversity and Population Structure Assessed by SSR and SNP Markers in a Large Germplasm Collection of Grape. *BMC Plant Biol.* **2013**, *13*, 39. [CrossRef]
31. Bianchi, D.; Brancadoro, L.; Lorenzis, G. De Genetic Diversity and Population Structure in a *Vitis* spp. Core Collection Investigated by SNP Markers. *Diversity* **2020**, *12*, 103. [CrossRef]
32. Laucou, V.; Launay, A.; Bacilieri, R.; Lacombe, T.; Adam-Blondon, A.F.; Bérard, A.; Chauveau, A.; De Andrés, M.T.; Hausmann, L.; Ibáñez, J.; et al. Extended Diversity Analysis of Cultivated Grapevine *Vitis vinifera* with 10K Genome-Wide SNPs. *PLoS ONE* **2018**, *13*, e0192540. [CrossRef] [PubMed]
33. Mejía, N.; Soto, B.; Guerrero, M.; Casanueva, X.; Houel, C.; de los Ángeles Miccono, M.; Ramos, R.; Le Cunff, L.; Boursiquot, J.M.; Hinrichsen, P.; et al. Molecular, Genetic and Transcriptional Evidence for a Role of VvAGL11 in Stenospermocarpic Seedlessness in Grapevine. *BMC Plant Biol.* **2011**, *11*, 57. [CrossRef] [PubMed]
34. Bergamini, C.; Cardone, M.F.; Anaclerio, A.; Perniola, R.; Pichierri, A.; Genghi, R.; Alba, V.; Forleo, L.R.; Caputo, A.R.; Montemurro, C.; et al. Validation Assay of P3-VvAGL11 Marker in a Wide Range of Genetic Background for Early Selection of Stenospermocarpy in *Vitis vinifera* L. *Mol. Biotechnol.* **2013**, *54*, 1021–1030. [CrossRef] [PubMed]
35. Malabarba, J.; Buffon, V.; Mariath, J.E.A.; Maraschin, F.S.; Margis-Pinheiro, M.; Pasquali, G.; Revers, L.F. Manipulation of VviAGL11 Expression Changes the Seed Content in Grapevine (*Vitis vinifera* L.). *Plant Sci.* **2018**, *269*, 126–135. [CrossRef] [PubMed]
36. Ferlito, F.; Distefano, G.; Gentile, A.; Allegra, M.; Lakso, A.N.; Nicolosi, E. Scion–Rootstock Interactions Influence the Growth and Behaviour of the Grapevine Root System in a Heavy Clay Soil. *Aust. J. Grape Wine Res.* **2020**, *26*, 68–78. [CrossRef]
37. Rana, G.; Katerji, N.; Introna, M.; Hammami, A. Microclimate and Plant Water Relationship of the "Overhead" Table Grape Vineyard Managed with Three Different Covering Techniques. *Sci. Hortic.* **2004**, *102*, 105–120. [CrossRef]
38. Meier, U. *Growth Stages of Mono and Dicotyledonous Plants*; BBCH Monograph; Federal Biological Research Centre for Agriculture and Forestry: Berlin/Braunschweig, Germany, 2001.
39. Emershad, R.; Ramming, D. Somatic Embryogenesis and Plant Development from Immature Zygotic Embryos of Seedless Grapes (*Vitis vinifera* L.). *Plant Cell Rep.* **1994**, *14*, 6–12. [CrossRef]
40. Wang, W.Q.; Song, S.Q.; Li, S.H.; Gan, Y.Y.; Wu, J.H.; Cheng, H.Y. Quantitative Description of the Effect of Stratification on Dormancy Release of Grape Seeds in Response to Various Temperatures and Water Contents. *J. Exp. Bot.* **2009**, *60*, 3397–3406. [CrossRef]
41. Ergenoglu, F.; Tangolar, S.; Gok, S. The Effects of Some Pre-Treatments for Promoting Germination of Grape Seeds. *Acta Hortic.* **1997**, *441*, 207–212. [CrossRef]
42. Doyle, J. DNA Protocols for Plants. In *Molecular Techniques in Taxonomy*; NATO ASI Series; Springer: Berlin, Germany, 1991; Volume 57, pp. 283–293. [CrossRef]
43. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2020.
44. Revelle, W. *Psych: Procedures for Personality and Psychological Research*; R Package Version 2.2.5.; Northwestern University: Evanston, IL, USA, 2020; Available online: https://cran.r-project.org/package=psych (accessed on 1 March 2022).
45. Li, Z.; Li, T.; Wang, Y.; Xu, Y. Breeding New Seedless Grapes Using in Ovulo Embryo Rescue and Marker-Assisted Selection. *Vitr. Cell. Dev. Biol.-Plant* **2015**, *51*, 241–248. [CrossRef]
46. Motosugi, H.; Naruo, T. Production of Triploid Grape Rootstocks by Embryo Culture and Their Growth Characteristics. *J. Jpn. Soc. Hortic. Sci.* **2003**, *72*, 107–115. [CrossRef]