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Optimizing Seed Germination and Seedling Growth in Different Kiwifruit Genotypes

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Abstract: The presence of dormant embryos in seeds and nonuniformity in seedling growth are the main limiting factors for kiwifruit propagation. Studies on the germination of hybrid genotypes are limited, especially at different ploidy levels. Low germination percentages and nonuniformity in seedling growth are two of the limiting factors of kiwifruit breeding, especially new and imported germplasm. The effect of cold moist stratification and growing substances on seed germination and seedling growth, respectively, on different kiwifruit seed populations were evaluated in this study. The effect of cold moist stratification (3, 4 and 5 weeks at 4 °C) and growing substrate composition (peat, perlite and coco peat) on seed germination and seedling growth of different *Actinidia* genotypes were assessed. In general, increasing stratification period duration often increased germination speed and uniformity. It was also found that increasing the ratio of peat moss improved the vegetative growth parameters of all kiwifruit seedlings. Stratification treatment, light and temperature fluctuation and consequently suitable substrate can accelerate and increase the production of seedlings and reduce seedling losses. It was also found that peat–perlite (1:1) medium was the best medium for kiwifruit seedling growth.

Keywords: *Actinidia* spp.; cold stratification; dormancy; seed germination; soilless medium



Citation: Maghdouri, M.; Ghasemnezhad, M.; Rabiei, B.; Golmohammadi, M.; Atak, A. Optimizing Seed Germination and Seedling Growth in Different Kiwifruit Genotypes. *Horticulturae* **2021**, *7*, 314. <https://doi.org/10.3390/horticulturae7090314>

Academic Editor: Xiaohong Yao

Received: 8 August 2021

Accepted: 6 September 2021

Published: 16 September 2021

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1. Introduction

Kiwifruit, belonging to the family Actinidiaceae and the genus *Actinidia*, is a dicotyledonous, perennial, deciduous plant that includes a number of important commercial species [1]. In Iran, kiwifruit is one of the most important horticultural and export products that can be cultivated only in Northern provinces such as Mazandaran, Guilan and a small part of Golestan province [2]. Iran ranks fourth in the world for kiwifruit production after China, Italy and New Zealand [3]. The most common kiwifruit cultivar grown in Iran is ‘Hayward’ with green flesh. However, a small number of kiwifruit orchards in Iran contain red-fleshed cultivars. Therefore, for sustainable production and export, the kiwifruit industry needs to introduce novel kiwifruit cultivars with different flesh colors and flavors. To achieve this goal, a population of seed plants with natural or controlled crosses should be available [4]. The presence of dormant embryos in seeds and nonuniformity in seedling growth usually increase the losses of kiwifruit seed populations. A production model that is dependent on only one cultivar of a species decreases the interest in that cultivar over time, causing its price to decrease and kiwifruit growers to give up its production. This situation hinders sustainable production. As a result of solving the problems related to the production and reproduction of different kiwifruit cultivars for a sustainable production

model and transferring them to kiwifruit growers, their sale at higher prices is allowed. Thus, indirectly, different cultivar demands of consumers will be met, and it will be possible to increase the competitiveness of local kiwifruit growers.

Seeds are an important material both for the propagation of seedlings and breeding new kiwifruit cultivars, but their germination capacity is very low [5]. Additionally, another disadvantage is the impossibility of determining the sex of seedlings and genetic differentiation in generative propagation. Because of the low germination ratio of *Actinidia* seeds, they must be treated with chemicals and kept in damp, aerated conditions, alternated between day and night, under low temperatures to increase their germination rate. According to researchers, stratification under cool and moist conditions and/or some chemical treatments can improve the germination rate of kiwifruit seeds [4,6,7].

Germination of kiwifruit is poor or erratic because of dormancy. Seed dormancy is a physiological condition that reduces or inhibits germination in favorable conditions. Previous studies suggested low temperature and gibberellic acid can overcome kiwifruit seed dormancy [8–11]. Kiwifruit seed dormancy is a physiological condition that restricts or prevents germination in otherwise favorable conditions and determines the range of conditions permissive for germination. Additionally, germination is a complex process that cannot be fully understood by studying one or two factors; thus, it is necessary to adopt a multidimensional strategy, combining the analysis of multiple factors to achieve optimal germination percentages. The intrinsic molecular mechanisms determining dormancy can have an embryo and/or a coat component that can interact to determine the degree of ‘whole-seed’ dormancy [8,10].

Although some studies showed that gibberellic acid can enhance kiwifruit seed germination [4,11,12], other studies indicated that cold moist stratification is more effective than gibberellic acid in breaking kiwifruit seed dormancy [8,9,13]. They also found that vermiculite is not a suitable medium for seed germination. The growing media used for germinated kiwifruit seeds is very important for subsequent seedling growth. Atak and Yalçın [14] reported that different media were effective in rooting in kiwifruit. González-Puelles et al. [15] reported that some germination procedures, including dry stratification and/or lower GA3 concentration, can increase seed germination by 80%. Çelik et al. [4] were able to achieve about 99% germination of seed from ‘Hayward’ fruit using gibberellic acid treatment and bottom heat and peat medium. Hsieh et al. [9] were able to induce 80% germination in freshly harvested kiwifruit seed using alternating temperatures (25/15). Others demonstrated the role of fluctuating and constant temperature and far-red and red light in the germination of stratified ‘Hayward’ seed. They also found a positive effect of fluctuating temperature (20/30) on breaking kiwifruit seed dormancy. However, red light had no role in kiwifruit seed germination, but far-red light had an inhibitory role in germination [10].

In this study, we attempted to determine the best germination and development conditions for kiwifruit seeds belonging to different *Actinidia* species as a result of some applications. The effect of cold moist stratification and growing substances on seed germination and seedling growth, respectively, was investigated on different kiwifruit seed populations.

2. Materials and Methods

2.1. Plant Materials

Seeds of 16 kiwifruit populations from *Actinidia chinensis* var. *deliciosa* (DA), *Actinidia chinensis* var. *chinensis* (CK) and *Actinidia arguta* (AA) and a hybrid of *A. chinensis* var. *deliciosa* × *A. chinensis* var. *chinensis* (according to Table 1) were obtained and transferred to the lab at the University of Guilan, Rasht, Iran. After the harvest, fruits were stored at 23 °C, and the ripening stage was controlled every week. Seed extraction was carried out to ensure the fruit was ripe and soft. The fruit pulp was extracted in a juice extractor. The seeds were washed with water and dried and stored at 4 °C until required for germination studies.

Table 1. List of kiwifruit genotypes and their characteristics.

Symbol	Flesh Color	Ploidy Level	Species
CK1	Red	-	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK2	Red	4x	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK3	Red	2x	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK4	Red	2x	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK5	Red	2x	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK6	Red	-	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK7	Gold	4x	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK8	Gold	2x	<i>Actinidia chinensis</i> var. <i>chinensis</i>
CK9	Gold	4x	<i>Actinidia chinensis</i> var. <i>chinensis</i>
DA1	Green	6x	<i>Actinidia chinensis</i> var. <i>deliciosa</i>
DA2	Green	6x	<i>Actinidia chinensis</i> var. <i>deliciosa</i>
DA3	-	-	<i>Actinidia chinensis</i> var. <i>deliciosa</i>
DA4	Green	6x	<i>Actinidia chinensis</i> var. <i>deliciosa</i>
DA5	Green	6x	<i>Actinidia chinensis</i> var. <i>deliciosa</i>
Hybrid	Green	4x	<i>Actinidia chinensis</i> var. <i>deliciosa</i> X <i>Actinidia chinensis</i> var. <i>chinensis</i>
AA	Green	4x	<i>Actinidia arguta</i>

2.2. Treatments

For disinfection, the seeds were soaked in a solution of 2 mg L⁻¹ fungicide (Maxim XL 035) for 1 min and immediately transferred onto paper cloths to remove excess water at room temperature. In the first experiment, 300 disinfected hybrid seeds of each combination divided into three replications were placed into Petri dishes with moist Whatman No. 1 filter paper and incubated at 4 °C for three, four and five weeks. After that, the stratified seeds were placed in a chamber with alternating temperature and light at 24 °C (16 h)/5 °C (8 h) and then transferred to a growth chamber with constant temperature (20 °C) and continuous light for germination. Here, their germination was followed for 8 weeks. This experiment was performed as a factorial experiment in a completely randomized design with three replications.

In the second experiment, the germinated seeds were transferred to greenhouse conditions with 80–90% relative humidity and 25–30 °C temperature with supplemental light and sown in 45 cell trays. The growing media contained different ratios of coco-peat (salinity = 500 mS, pH = 5.8–6.5), perlite (in horticultural size 1–3) and peat moss (pH = 5.5–6.5, salinity = 1 mS/cm, moisture content = 40–60%) with cocopeat–perlite (1:1), cocopeat–peat–perlite (1:1:1), peat–perlite (1:1) and peat–perlite (1:2). The experiment was carried out in a completely randomized design with three replications.

2.3. Data and Statistical Analysis

Daily seed germination was processed using the Germin program, which is based on the linear interpolation of cumulative germination points over time [16,17]. Using this program while measuring the germination percentage (GP), two important traits, including seed germination rate (R50) and germination uniformity (GU), by calculating the time (days) required for germination of 10% (D10), 50% (D50) and 90% (D90) of seeds according to the following formulae were determined. It should be noted that the shorter the germination time, the more uniform the seed germination.

$$GP = \frac{\sum n_i}{N} \times 100\%$$

where N is the total number of seeds, and n is the number of germinated seeds at the end of the experiment.

$$R50 = \frac{1}{D50}$$

$$GU = D90 - D10$$

To determine the effect of substrate composition on seedling growth at the end of two months, the vegetative growth characteristics, including leaf number, stem diameter (using digital caliper) and seedling height (using ruler), were measured.

The obtained data were analyzed using Proc GLM of SAS statistical software version 9.1 [18], and means of treatments were compared with Tukey's test at the 5% probability level.

3. Results

3.1. Germination Characteristics

The results showed that the maternal genotype and the stratification period and their interaction had a significant effect on the seed germination characteristics of all three combinations, DA, CK and AA and the DA × CK hybrid (Table 2).

Table 2. Analysis of variance for effect of genotype and stratification duration on kiwifruit seed germination characteristics.

Source of Variation	df	Mean Square		
		Germination Percentage	Germination Rate	Germination Uniformity
Genotype (A)	15	4224.5292 **	0.3935 **	554.6365 **
Stratification duration (B)	2	6249 **	0.0995 **	361.0304 **
A × B	30	527.0667 **	0.0211 **	143.6298 **
Error	96	7.7917	0.0003	0.7035

** Significant at 1% probability level.

There is a significant difference between kiwifruit genotypes and stratification period (Table 3). The seed germination percentage of the genotypes belonging to DA was higher than the CK genotypes. Among the nine genotypes belonging to CK, the highest germination percentage, 86%, was found in CK2 after four weeks' stratification, and the lowest value, 1%, belonged to CK7 and CK9 after three weeks' stratification. The longer the duration of stratification, the higher the germination percentage. In DA genotypes, the highest and lowest germination percentages belonged to DA2 (99%) and DA4 (34%) after four weeks' cold moist stratification, respectively. The germination percentage in AA ranged from 8% to 67% after 3–5 weeks, respectively (Table 3).

The results showed that the germination rate of kiwifruit genotypes in stratification periods was different. The highest germination rate of 0.55 in CK genotypes belonged to CK4, and the lowest value of 0.03 was found in CK3, CK9 and CK7. However, the highest germination rate in DA genotypes was observed in the DA3 genotype (0.96) and the lowest in DA2 and DA4 genotypes (0.1). The hybrid genotype also had the lowest germination rate (0.03). In hybrid and CK9 genotypes, changes in stratification duration had no effect on germination rate, but in DA1 and DA3, increasing the stratification period increased the seed germination rate. However, the highest germination rate in DA5 was observed in the four-week treatment. In CK1, 3 and 5 weeks' cold stratification showed a higher germination rate than the 4-week treatment. However, the highest germination rate was found in CK2, CK3 and CK4 genotypes after 5 weeks. In CK6 and CK8, the germination rate increased with increasing stratification period, but in CK5, 4 weeks' stratification treatment resulted in faster germination compared to other treatments (Table 3).

Higher germination uniformity of 0.12 was found in the CK7 genotype after 3 weeks' cold moist stratification. Thereafter, the DA3 genotype varied with 1.69 to 2.27 in all three stratification treatments and DA2 with 2.37 after 4 weeks' stratification. The lowest seed germination uniformity was found in CK9 (41.2) and CK3 (39.2) genotypes both after 3 weeks and CK1 (32.97 and 29.1) after 3 and 4 weeks, respectively (Table 3).

Table 3. Genotype and stratification interaction effects on germination percentage, germination rate and germination uniformity of kiwifruit seeds.

Genotype	Stratification Period (Week)	Germination Percentage	Germination Rate	Germination Uniformity
CK1	3	60 i	0.26 g	32.97 b
CK1	4	59 i	0.1 m–q	29.1 b, c
CK1	5	67 h	0.28 f, g	24.96 c–e
CK2	3	52 k, l	0.11 m–p	28.99 b, c
CK2	4	86 d, e	0.15 j–l	7.42 p, q
CK2	5	54 k, j	0.26 g	15.32 j–n
CK3	3	13 q	0.04 s, t	39.2 a
CK3	4	37 o	0.09 o–r	25.6 c, d
CK3	5	27 p	0.13 k–n	14.42 k–n
CK4	3	36 o	0.09 o–r	19.5 f–j
CK4	4	34 o	0.09 o–r	19.16 f–j
CK4	5	62 i	0.55 c	19.13 f–j
CK5	3	62 i	0.11 m–p	13.68 l–o
CK5	4	69 g, h	0.16 j, k	15.67 j–m
CK5	5	68 h	0.11 m–p	11.25 m–p
CK6	3	77 f	0.09 o–r	14.49 k–n
CK6	4	76 f	0.15 j–l	11.51 m–p
CK6	5	60 i	0.28 f, g	8.69 p
CK7	3	1 s	0.03 t	0.12 r
CK7	4	26 p	0.03 t	22.17 d–h
CK7	5	57 i, j	0.04 s, t	23.15 d–f
CK8	3	48 l, m	0.11 m–p	20.76 e–i
CK8	4	59 i	0.13 k–m	21.34 d–h
CK8	5	61 i	0.18 i, j	16.22 i–l
CK9	3	1 s	0.03 t	41.2 a
CK9	4	52 k, l	0.03 t	16.45 i–l
CK9	5	45 n, m	0.04 s, t	18.1 g–l
DA1	3	69 g, h	0.15 j–l	9.84 o, p
DA1	4	82 e	0.21 h	7.31 p, q
DA1	5	92 b, c	0.44 d	3.9 q, r
DA2	3	92 b, c	0.1 n–q	7.08 p, q
DA2	4	99 a	0.2 h, i	2.37 r
DA2	5	95 a, b	0.17 i, j	7.47 p, q
DA3	3	57 k–m	0.84 b	2.27 r
DA3	4	74 f	0.93 a	1.71 r
DA3	5	85 e	0.96 a	1.69 r
DA4	3	42 n	0.1 m–p	17.56 h–l
DA4	4	34 o	0.08 p–r	14.11 l–o
DA4	5	49 k–m	0.12 l–o	10.83 n–p
DA5	3	43 n	0.3 f	19.46 f–j
DA5	4	73 f, g	0.4 e	7.29 p, q
DA5	5	90 d, c	0.22 h	8.79 p
Hybrid	3	10 q, r	0.03 t	20.4 f–i
Hybrid	4	13 q	0.04 s, t	19.48 f–j
Hybrid	5	36 o	0.06 r–t	20.68 f–i
AA	3	8 r	0.14 k–m	11.33 m–p
AA	4	50 k–m	0.07 q–s	22.73 d–g
AA	5	67 h	0.08 p–r	18.73 f–k

For each column, values with similar letters are not significantly different ($p < 0.01$).

3.2. Seedling Growth Parameters

The seedling growth characteristics significantly depended on kiwifruit genotypes and growing media (Table 4). The highest seedling stem diameter belonged to CK1, CK5, CK6 and DA1 genotypes in peat–perlite (1:1) and CK2 and DA3 in peat–perlite (1:2). In contrast, cocopeat–perlite (1:1) showed the lowest seedling stem diameter. However, CK2, hybrid, DA2 and DA3 did not show a significant difference between peat–perlite (1:1) and (1:2) medium for stem diameter. In the other eight genotypes, stem diameter in the peat–perlite (1:1) reached about 3 to 4.5 mm after two months of germination. In the case of DA4, the largest stem diameter was observed in the peat–perlite (1:2) mixture (Table 4).

Table 4. Genotype and media interaction effects on crown diameter, leaf number and height of kiwifruit seedlings.

Genotype	Media	Crown Diameter	Leaf Number	Height of Seedling
CK1	Cocopeat–Perlite (1:1)	2.04 k–m	7.3 d	8 i
CK1	Cocopeat–Peat–Perlite (1:1:1)	3.82 b–f	5.2 f	5 l, m
CK1	Peat–Perlite (1:1)	4.63 a	5 f	9 h
CK1	Peat–Perlite (1:2)	0	0	0
CK2	Cocopeat–Perlite (1:1)	0	0	0
CK2	Cocopeat–Peat–Perlite (1:1:1)	1.74 l–n	3.5 h	1.65 r–t
CK2	Peat–Perlite (1:1)	3.92 b–f	5.31 f	11.41 g
CK2	Peat–Perlite (1:2)	4.21 a–d	5.33 f	15.05 d
CK3	Cocopeat–Perlite (1:1)	0	0	0
CK3	Cocopeat–Peat–Perlite (1:1:1)	1.25 n–p	3.6 h	0.75 u–w
CK3	Peat–Perlite (1:1)	0	0	0
CK3	Peat–Perlite (1:2)	0	0	0
CK4	Cocopeat–Perlite (1:1)	1.34 n, o	3.23 h	1.25 s–v
CK4	Cocopeat–Peat–Perlite (1:1:1)	2.6 h–j	4.33 g	4 n, o
CK4	Peat–Perlite (1:1)	2.26 i–k	4.13 g	3.75 n–p
CK4	Peat–Perlite (1:2)	0	0	0
CK5	Cocopeat–Perlite (1:1)	1.3 n, o	3.41 h	1.3 s–u
CK5	Cocopeat–Peat–Perlite (1:1:1)	1.24 n–p	3 h	1.32 s–u
CK5	Peat–Perlite (1:1)	4.31 a, b	6 e	18.8 a
CK5	Peat–Perlite (1:2)	3.52 e–g	5.33 f	16.6 b
CK6	Cocopeat–Perlite (1:1)	1.6 n, m	4 g	2.1 r
CK6	Cocopeat–Peat–Perlite (1:1:1)	1.04 o, p	3 h	1.5 r–u
CK6	Peat–Perlite (1:1)	4.26 a–c	4 g	16.9 b
CK6	Peat–Perlite (1:2)	3.58 e, f	5 f	17 b
CK7	Cocopeat–Perlite (1:1)	0	0	0
CK7	Cocopeat–Peat–Perlite (1:1:1)	2.57 h–j	4.6 g	3.25 p, q
CK7	Peat–Perlite (1:1)	3.85 b–f	5 f	12.8 f
CK7	Peat–Perlite (1:2)	3.06 g, h	5 f	7.2 j
CK8	Cocopeat–Perlite (1:1)	0	0	0
CK8	Cocopeat–Peat–Perlite (1:1:1)	1.68 l–n	3.34 h	0.83 u–w
CK8	Peat–Perlite (1:1)	3.97 b–e	6.1 e	12.8 f
CK8	Peat–Perlite (1:2)	0	0	0
CK9	Cocopeat–Perlite (1:1)	0	0	0
CK9	Cocopeat–Peat–Perlite (1:1:1)	1.26 n–p	3 h	2.2 r
CK9	Peat–Perlite (1:1)	3.76 c–f	5.33 f	6.75 j
CK9	Peat–Perlite (1:2)	2.56 h–j	3 h	3.4 o–q
DA1	Cocopeat–Perlite (1:1)	0.77 p, q	8 c	0.5 v, w
DA1	Cocopeat–Peat–Perlite (1:1:1)	1.03 o, p	3.63 h	1 t–v
DA1	Peat–Perlite (1:1)	4.24 a–c	6.14 e	11.38 g
DA1	Peat–Perlite (1:2)	3.71 d–f	7.53 d	11.63 g
DA2	Cocopeat–Perlite (1:1)	0	0	0
DA2	Cocopeat–Peat–Perlite (1:1:1)	1.52 n, o	4.3 g	2 rs
DA2	Peat–Perlite (1:1)	3.68 d–f	7.24 d	11.2 g
DA2	Peat–Perlite (1:2)	3.97 b–e	6 e	11.6 g
DA3	Cocopeat–Perlite (1:1)	0	0	0
DA3	Cocopeat–Peat–Perlite (1:1:1)	3.39 f, g	6.2 e	8.2 i
DA3	Peat–Perlite (1:1)	3.96 b–e	6.18 e	14.6 d
DA3	Peat–Perlite (1:2)	4.29 a–c	6 e	13.6 e
DA4	Cocopeat–Perlite (1:1)	0	0	0
DA4	Cocopeat–Peat–Perlite (1:1:1)	0.76 p, q	2 i	0.5 u–w
DA4	Peat–Perlite (1:1)	0	0	0
DA4	Peat–Perlite (1:2)	3.48 e–g	6 e	6 k
DA5	Cocopeat–Perlite (1:1)	0	0	0
DA5	Cocopeat–Peat–Perlite (1:1:1)	2.71 h, i	5 f	5.2 l
DA5	Peat–Perlite (1:1)	3.86 b–f	6.3 e	15.9 c
DA5	Peat–Perlite (1:2)	3.69 d–f	6.25 e	9.6 h
Hybrid	Cocopeat–Perlite (1:1)	0.54 q, r	2 i	0.75 u, v
Hybrid	Cocopeat–Peat–Perlite (1:1:1)	2.14 j–l	4.61 g	3.2 p, q
Hybrid	Peat–Perlite (1:1)	3.59 ef	5 f	5.4 k
Hybrid	Peat–Perlite (1:2)	3.7 d–f	4.23 g	4.4 m, n
AA	Cocopeat–Perlite (1:1)	0	0	0
AA	Cocopeat–Peat–Perlite (1:1:1)	2.43 i–k	9.1 b	8 i
AA	Peat–Perlite (1:1)	2.6 h–j	10 a	2.9 q
AA	Peat–Perlite (1:2)	0	0	0

For each column, values with similar letters are not significantly different ($p < 0.01$).

The highest seedling height was observed in CK5 when planted in the peat–perlite (1:1) mixture. In CK2 and DA4 genotypes, the highest seedling height was produced in peat–perlite (1:2) and in CK3 and AA in cocopeat–peat–perlite (1:1:1). In the 12 other genotypes, plants showed higher height in peat–perlite (1:1) medium, although CK6 and DA2 genotypes did not show a statistically significant difference between peat–perlite (1:1) and peat–perlite (1:2) (Table 4).

The maximum leaf number belonged to the AA genotype in peat–perlite (1:1) medium. In total, peat–perlite (1:1) was the best growing medium, so the highest leaf number for most kiwifruit genotypes was found in peat–perlite (1:1), while cocopeat–perlite (1:1) medium showed the lowest vegetative growth characteristics (Table 4). Furthermore, the results showed that with increasing peat in the growing medium, vegetative growth parameters of the kiwifruit seedlings, especially stem diameter and seedling height, increased.

4. Discussion

The results showed that seed germination characteristics such as germination percentage, rate and uniformity were significantly dependent on kiwifruit genotypes and cold moist stratification periods.

According to the results, the germination percentage of kiwifruit genotypes was significantly higher than in previous studies. The difference between kiwifruit genotypes (DA, CK, AA and Hybrid) for germination characteristics was mostly due to differences in the chilling requirement period. Sekhukhune et al. [13] reported that 37 days of stratification at 4 °C for CK seeds in in vitro culture induced 16% germination but had no effect on germination of AA genotypes. Zhao et al. [19] also found that the reason for differences between kiwifruit genotypes in terms of seed germination could be due to differences in their ploidy. In this study, we found that DA hexaploid genotypes need a longer stratification period for maximum germination percentage. In contrast, in most CK genotypes that were diploid, 4 weeks' stratification was enough for maximum germination percentage. Asakuro and Hoshino [20] reported that the hybrid seeds of *Actinidia* species with different ploidy levels had different viability rates. They reported that the viability and germination rates of hybrid seeds obtained from the combinations at different ploidy levels were quite low.

Lastuvka et al. [21] evaluated the functional relationships between changes in the sensitivity of seed population to different cycle-doses of fluctuating temperature and dormancy alleviation under different times and stratification temperatures. Their results showed that stratification at cool temperatures produced higher rates of change than stratification at warmer temperatures. Similar to our study, stratification at cold temperatures can use an important tool to overcome dormancy, resulting in more efficient and faster production of kiwifruit seedlings or rootstocks.

In kiwifruit, however, red light does not affect seed germination, but far-red light, even for a short period of time, can strongly inhibit germination. In addition, incubation of stratified seeds at fluctuating temperatures compared to constant ambient temperature significantly increases the germination percentage [10]. Temperature and plant phytochromes are factors that can play an effective role in seed dormancy. Unlike the results of this experiment, the low germination percentages reported by Sekhukhune et al. [13] were because of seed sowing immediately after receiving stratification treatment and no light treatment.

It seems that temperature and light treatments increased seed germination percentage when stratification was complete, which was consistent with the results from Windauer et al. [10]. The results also confirmed the positive effect of stratification period on seed germination percentage in different genotypes. These results were in agreement with previous findings that showed the relationship between stratification treatment periods and germination percentage and rate follows a quadratic [10]. In living systems, the existence of quadratic mathematical relationships is an indication of a density-dependent growth pattern (DDG) that, based on the concentration or period of influence of a critical factor, considers growth to three stages, including stimulation, saturation and inhibition [22]. Increasing the stratification period to 5 weeks increased the seed germination percentage of

both 'Hayward' (DA4) and 'Bruno' (DA5), but the germination rate of seed from 'Hayward' fruit remained significantly lower than that of seed from 'Bruno' fruit. This result is in accordance with the results of Ahmad [23], who reported the positive effect of stratification period prolongation on 'Hayward' and 'Bruno' seed germination. However, his reported germination rate for 'Bruno' was lower than in the present study.

In most genotypes studied, the treatment that resulted in maximum germination rate also had the highest germination uniformity. Thus, the five weeks' stratification treatment with increasing germination rate and seed germination uniformity led to shortening of the germination period in most genotypes. This can be due to the positive effect of the cold period on breaking seed dormancy, and many researchers believe that dormancy is a major factor in prolonging germination time [8]. It has been previously reported that stratification treatment leads to increased germination rate and shortened germination time [9]. Prolonged germination time is an indicator of seed dormancy that can be alleviated by using different mechanisms before seed planting. In general, when seeds break dormancy, the germination rate increases owing to the elimination of dormancy agents, and there can be a high correlation between the duration of stratification treatment and seed germination rate [13].

After seed germination, subsequent plant growth is a key factor for producing healthy and strong seedlings in a short time. In general, healthy and strong seedlings will cause early economic fruiting in vineyard and sustainable production. The results showed that the subsequent growth of most genotypes in the growing media containing cocopeat-perlite (1:1) was very weak due to poor physical and chemical properties. Some germinated seeds died, and seedling growth was poorer than with other substrates. Cocopeat has a high cation exchange capacity [24] and moisture-holding capacity [25], and its pH is neutral to slightly acidic. However, adding cocopeat to perlite reduces the pH of the substrate, increases water holding capacity and thus leads to better absorption of nutrients [24], but the uptake of potassium by cocopeat reduces its level in the nutrient solution, and less nitrogen and potassium uptake occurs in substrates with high cocopeat [26]. In addition, cocopeat substrate has high sodium content [27]. These factors may be possible reasons for the weak growth of kiwifruit seedlings, which are acidophilic and require a lot of potassium and nitrogen. In contrast, the addition of peat causing acidification of the substrate can have a great effect on the growth of kiwifruit seedlings. The genotypes belonging to the three taxa CK, DA and AA showed almost the same behavior in terms of the type of suitable substrate for growth. In most kiwifruit genotypes, peat-perlite (1:1) mixture was selected as the most suitable medium. This substrate can be used as a soilless medium in the early growth stages of most studied genotypes. The results of this study were consistent with the findings of previous reports on the positive effect of peat addition on root growth of kiwifruit cuttings [28]. Sharma et al. [29] reported that GA3 and growing media were effective in germination in their study with the seedlings of the Hayward variety. In addition, the highest germination percentage was obtained with peat moss, as in our study.

The positive effect of peat on kiwifruit seedling growth may be due to the presence of a group of organic compounds called heteroxins in the peat structure and the release of auxin from these compounds during plant growth in the peat medium, which encourages rooting. High cation exchange capacity of peat can also be another benefit [30], which increases the absorption of some nutrients [31]. Additionally, the content of organic matter and the amounts of phosphorus and potassium in peat substrate are high [32]. Due to the kiwifruit requiring light, humus-rich and well-drained soil and preferring slightly acidic soils (pH = 5.5–6.5) for optimum growth and fruiting [33], these factors can help to better absorb the needed plant nutrients.

It is clear that the positive effect of perlite on plant growth can be attributed to its highly porous structure and capillary action. This structure has caused this substrate to hold 3–4 times more water than its weight, and thus, while providing high ventilation in the rhizosphere, sufficient water is provided for plant growth around the roots [31].

However, increasing the perlite ratio in the growing medium reduces moisture retention and availability of nutrients; therefore, it can have a negative effect on plant growth [34]. Overall, the incorporation of peat and perlite in the correct ratio improved the physical and chemical properties of the growing medium and resulted in better seedling growth indices.

While the germination and development of a very limited number of genotypes or populations were investigated in previous studies, in this study, the germination and subsequent development of a larger number of *Actinidia* hybrid genotypes with different ploidy levels were investigated. The results of the current study showed that the chilling requirement for breaking dormancy and achieving the maximum seed germination and seedling uniformity is significantly dependent on kiwifruit genotypes and ploidy level. We found that the combination of low temperature, light treatment could improve seed germination, seedling growth and uniformity. The best growing medium for the early growth of kiwifruit seedlings was peat and perlite with a 1:1 ratio.

5. Conclusions

Overall, the results of this study showed that seed germination and seedling growth in 16 different kiwifruit genotypes belonging to three species were significantly different in response to stratification treatments and light, temperature conditions and medium composition. In all three species, increasing the duration of stratification increased the germination rate and uniformity. Stratification treatments, light and temperature fluctuation and consequently suitable substrate can accelerate and increase the production of seedlings and reduce seedling losses.

Based on the results of this experiment, peat–perlite (1:1) medium was the best medium for kiwifruit seedling growth due to the presence of heteroxin in the peat structure, high cation exchange capacity, high organic matter, high amount of phosphorus and potassium and pH close to the appropriate pH for kiwifruit, the possibility of high ventilation in the rhizosphere and providing enough water around the plant roots.

Author Contributions: Methodology, M.M., M.G. (Mahmood Ghasemnezhad) and M.G. (Morteza Golmohammadi); data curation; M.M.; writing—original draft preparation, M.M.; Conceptualization, M.G. (Mahmood Ghasemnezhad); Investigation, M.G. (Mahmood Ghasemnezhad); resources, M.G. (Mahmood Ghasemnezhad); writing—review and editing, M.G. (Mahmood Ghasemnezhad); supervision, M.G. (Mahmood Ghasemnezhad); project administration; M.G. (Mahmood Ghasemnezhad); investigation; A.A.; writing—review and editing; A.A.; software, B.R.; formal analysis, B.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by University of Guilan and Iran National Science Foundation.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors will make the results available if requested.

Acknowledgments: The authors appreciate the University of Guilan and the Iran National Science Foundation for their financial support.

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

References

1. Guroo, I.; Wani, S.; Wani, S.; Ahmad, M.; Mir, S.; Masoodi, F. A review of production and processing of kiwifruit. *J. Food. Proc. Technol.* **2017**, *8*, 1–6. [CrossRef]
2. Ministry of Agriculture Jihad. *Agricultural Production Statistics*; Ministry of Agriculture Jihad: Tehran, Iran. Available online: <https://irandataportal.syr.edu/ministry-of-agriculture> (accessed on 5 August 2021). (In Persian).
3. FAOSTAT (Food and Agriculture Organization of the United Nations). Statistics Database. Available online: <http://www.fao.org/faostat/en/#data> (accessed on 2 August 2021).
4. Çelik, H.; Zenginbal, H.; Özcan, M. Enhancing germination of kiwifruit seeds with temperature, medium and gibberellic acid. *Hortic. Sci.* **2006**, *33*, 39–45. [CrossRef]
5. Strik, B.; Cahn, H. *Growing Kiwifruit*, EC 1464; Oregon State University: Corvallis, OR, USA, 1994.

6. Sale, R.P. *Kiwifruit Culture*; V.R. Ward Government Printer: Wellington, New Zealand, 1985.
7. Kim, I.S.; Hwang, J.L.; Han, K.P.; Lee, K.E. Studies on the germination of seeds in native *Actinidia* species. *Hortic. Abstr.* **1998**, *58*, 7336.
8. Baskin, C.C.; Baskin, J.M. Germination ecophysiology of herbaceous plant species in a temperate region. *Am. J. Bot.* **1988**, *75*, 286–305. [[CrossRef](#)]
9. Hsieh, T.; Nee, C.; Chien, C. Seed germination of Taiwanese *Actinidia latifolia* (Gardn. and Champ.). *Merr. Taiwan J. For. Sci.* **2004**, *19*, 173–176.
10. Windauer, L.B.; Insausti, P.; Biganzoli, F.; Benech-Arnold, R.; Izaguirre, M.M. Dormancy and germination responses of kiwifruit (*Actinidia deliciosa*) seeds to environmental cues. *Seed Sci. Res.* **2016**, *26*, 342–350. [[CrossRef](#)]
11. Zhang, Y.; Zhang, H.; Kong, L.; Tang, H. Effects of different treatment methods on seed germination of kiwifruit. *IOP Conf. Ser. Mater. Sci. Eng.* **2018**, *394*, 1–6. [[CrossRef](#)]
12. Bishwas, K.; Amit, K.; Santosh, S.; Raj, K.K.; Dipendra, R. Effect of GA3 on germination parameters of different varieties of kiwi. *Curr. Investig. Agric. Curr. Res.* **2018**, *165*, 338–341. [[CrossRef](#)]
13. Sekhukhune, M.K.; Maila, M.Y.; Nikolova, R.V.; Mphosi, M.S. Preliminary studies on in vivo germination of *Actinidia arguta* and *Actinidia chinensis*. *Acta Hortic.* **2016**, *1204*, 123–131. [[CrossRef](#)]
14. Atak, A.; Yalçın, T. Effects of different applications on rooting of *Actinidia deliciosa* ‘Hayward’ hardwood and softwood cuttings. *Acta Hortic.* **2015**, *1096*, 117–126. [[CrossRef](#)]
15. González-Puelles, J.E.; Landin, M.; Gallego, P.P.; Barreal, M.E. Deciphering kiwifruit seed germination using neural network tools. *Acta Hortic.* **2018**, *1218*, 359–365. [[CrossRef](#)]
16. Inglis, G.J.; Waycott, M. Methods for assessing seagrass seed ecology and population genetics. In *Global Seagrass Research Methods*; Short, F.T., Coles, R.G., Eds.; Elsevier: Amsterdam, The Netherlands, 2001; pp. 123–140.
17. Soltani, A.; Madah, V. *Simple Applied Programs for Education and Research in Agriculture*; Niac Press Publication: Dublin, Ireland, 2009; p. 80.
18. SAS. *Statistical Analysis System. SAS Release 9.1 for Windows*; SAS Institute Inc.: Cary, NC, USA, 2003.
19. Zhao, T.; Li, D.; Li, L.; Han, F.; Liu, X.; Zhang, P.; Chen, M.; Zhong, C. The differentiation of chilling requirements of kiwifruit cultivars related to ploidy variation. *Hortic. Sci.* **2017**, *52*, 1676–1679. [[CrossRef](#)]
20. Asakura, I.; Hoshino, Y. Interspecific hybridization using miyama matatabi (*Actinidia kolomikta*), a japanese indigenous wild kiwifruit relative. *Hortic. J.* **2018**, *84*, 481–489. [[CrossRef](#)]
21. Lastuvka, M.; Benech-Arnold, R.; Windauer, L. A stratification thermal time-based model as a tool for de-signing efficient methodologies to overcome seed dormancy constraints to kiwifruit seedling production. *Sci. Hortic.* **2021**, *277*, 109776. [[CrossRef](#)]
22. Salisbury, F.B.; Ross, C.W. *Plant Physiology, Hormones and Plant Regulators: Auxins and Gibberellins*, 4th ed.; Wadsworth Publishing: Belmont, CA, USA, 1992; pp. 357–381.
23. Ahmad, M.F. Enhancement of seed germination in kiwi fruit by stratification and gibberellic acid application. *Ind. J. Hortic.* **2010**, *67*, 34–36.
24. Rezaee, A.; Mobli, M.; Etemadi, N.; Baninasab, B.; Khoshgoftarmanesh, A.H. Effect of different growing substrates on the yield and cut flower quality of rose cv. *Maroussia*. *J. Sci. Technol. Greenh. Cult.* **2013**, *4*, 105–114.
25. Torkashvand, A.M.; Seyedi, N. To evaluate influence of Ca concentration in nutrient solution and growth medium on the quantitative and qualitative yield of Lilium (Asiatic hybrid lilium). *Ir. J. Hortic. Sci. Tech.* **2016**, *46*, 637–647. [[CrossRef](#)]
26. Savvas, D.; Samantouros, K.; Paralemos, D.; Vlachakos, G.; Stamnatakis, M.; Vassilatos, C. Yield and nutrient status in the root environment of tomatoes (*Lycopersicon esculentum*) grown on chemically active and inactive inorganic substrates. *Acta Hortic.* **2004**, *644*, 377–383. [[CrossRef](#)]
27. Treder, J. The effects of cocopeat and fertilization on the growth and flowering of oriental lily ‘star gazer’. *J. Fruit Ornament. Plant Res.* **2008**, *16*, 361–370.
28. Ozenc, D.B.; Ozenc, N. The effect of hazelnut husk compost and some organic and inorganic media on root growth of kiwifruit (*Actinidia deliciosa*). *J. Agron.* **2007**, *6*, 113–118. [[CrossRef](#)]
29. Sharma, A.; Devkota, D.; Thapa, M.; Bista, B. Improving germination and stand establishment of kiwifruit (*Actinidia deliciosa* cv. Hayward) seed through media selection and hormonal use in Dolakha, Nepal. *Trop. Agrobiodiversity* **2021**, *2*, 16–21. [[CrossRef](#)]
30. Verdonck, O.D.; de Vleeschauwer, D.; de Boodt, M. The influence of the substrate to plant growth. *Acta Hortic.* **1981**, *126*, 251–258. [[CrossRef](#)]
31. Şirin, U.; Ertan, E.; Ertan, B. Growth substrates and fig nursery tree production. *Sci. Agric.* **2010**, *67*, 633–638. [[CrossRef](#)]
32. Gungor, F.; Yildirim, E. Effect of different growing media on quality, growth and yield of pepper (*Capsicum annuum* L.) under greenhouse conditions. *Pak. J. Bot.* **2013**, *45*, 1605–1608.
33. Morton, J.F. *Fruits of Warm Climates*; Creative Resource Systems Inc.: Winterville, NC, USA, 1987; pp. 293–300.
34. Oki, L.R.; Lieth, J.H. Effect of changes in substrate salinity on the elongation of *Rosa hybrida* L. ‘Kardinal’ stems. *Sci. Hortic.* **2004**, *101*, 103–119. [[CrossRef](#)]