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Effect of Harvest Frequency, Seed Extraction Time Point and Post-Harvest Cooling on Organic Tomato Seed Production

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Abstract: In light of the continuous increase in organic agriculture, the availability of organic seeds has gained a lot of importance. Especially since the new EU organic regulation came into force on 1 January 2022, proposing reducing the possibility of using untreated conventional seeds in the absence of organic seeds in the future. At the same time, the breeding of tolerant, resistant and adapted varieties is at the basis of organic production as is research to improve seed production and seed quality. In this study, we investigated seed production of 8 tomato genotypes. The aim was to see whether different fruit harvesting frequencies affect seed quality and germination rate. The hypotheses we tested were (i) whether regularly removing fruits from the field would affect total fruit and seed harvest, (ii) whether storage of fruits and (iii) their ripening stage at harvest, had an impact on seed germination. Our results show that while seed production differs between genotypes and extraction time-points, different harvesting procedures, and with that different fruit maturity levels, did not affect thousand-seed weight and seed germination; these findings benefit both small and larger-scale seed producers.

Keywords: seed production; organic agriculture; tomato; seed extraction; tomato breeding; fruit maturity stage; post-harvest cooling; harvest frequency

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1. Introduction

Leading the European production of vegetables with around 18 million t harvested production in 2021, tomatoes are the most important vegetable crop in the European Union [1]. Although total tomato production is expected to decline slightly until 2030, the share of produce for fresh consumption and higher value-added tomatoes is supposed to increase [2]. Tomato consumption is highly traditional in most European countries and, with its nutritional value and bioactive compounds, can have positive antioxidant, anti-inflammatory and anticancer effects [3–5].

With the European Green Deal and Farm to Fork strategy, the European Union launched an ambitious program to reform the European Food System. With stimulating production and processing, the EU aims to reach 25% of agricultural land to be cultivated under organic conditions. For vegetable fruits, around 39 thousand ha or 5.9%, were under organic production in 2020 [6]. Until 2036, farmers will still be allowed to use untreated conventional seeds in their organic production systems if not enough organically produced seeds are available on the market [7]. With more area under organic production and derogations possibly ending, there will be an increased demand for high-quality and quantity of organic vegetable seeds. However, seed production under organic faces several limitations compared to conventional production systems, e.g., restrictions in fertilizer applications and a limited spectrum of plant protection products available. Moreover, the organic sector requires the combination of several strategies to achieve healthy crops, at the base of which is the choice of varieties resistant to biotic and abiotic stresses [8]; these limitations can lead to reduced yields in organic compared to conventional agriculture, which need to be tackled especially in view of additional challenges posed by climate change [9,10].

Tomato fruits ripen sequentially, therefore needing frequent picking. Due to the intensive labour required by tomato seed production, increasing the effectiveness of the process could increase the amount of organically produced tomato seeds on the market. Simplifying the process by focusing on specific timing or stage to harvest for seeds and therefore reducing the number of seed extractions could decrease labour and make tomato seed production more effective. Cool storage of regularly picked fruits can be easily integrated into regular harvest work and reduce not only fruit deterioration if left longer but also the number of seed extractions. Less frequent harvests lead to fruits with different maturity levels; this could have an impact on seed quality and health. Previous studies could already show the importance of maturity status on the seed quality of tomatoes, which is important for germination and seedling development [11–15]. To achieve high seed vigour with optimal thousand seed weight and germination rates, fruits should be harvested at the red ripe stage [11,14]. Demir et al. showed that maximum seed quality is attained several weeks after mass maturity of the fruits [12], and that delayed harvests (80–90 Days After Anthesis—DAA) can lead to a decline in seed quality [15]. Tetteh and colleagues could not detect a difference in vigour and germination at half-ripe, fully ripe, and rotten stages [13]. Focusing on the potential infection of seeds with plant pathogens, several studies could show that a pathway for seed infection are lesions in tomato fruits. *Clavibacter michiganensis* subsp. *Michiganensis*, causing bacterial wilt and canker of tomato, not only enters systemically via the xylem, but also through tomato fruit lesions [16]. In 2021, a study showed that ripe fruit does not seem to have a reduced immune response when inoculated with three fungal diseases; it seems that rather a factor, pectase lyase (PL), is causing susceptibility in specific genotypes [17].

To shed more light on the relation between tomato harvesting frequency and maturity level on seed quantity and quality in different genotypes, we investigated the effects of two different harvesting frequencies on tomato seed vigour. In the first regime, fruits were harvested on a regular basis twice a week (Frequency 1: freq 1) and analysed for fruit weight and number; this was more labour-intensive, but fruits were harvested at a specific maturity level. An additional hypothesis we had was that this procedure could lead to fewer infected fruits and with that less rejected fruits and higher sanitary quality of the seeds. In the second regime, fruits were harvested after three weeks, which is less labour intensive (Frequency 2: freq 2). The frequency of harvest for tomato seed production varies from one producer to the other and also depends on the size of the exploitation. Seed producers dealing with niche varieties cultivated on small surfaces would harvest frequently and store the fruits in a cool place for a few days in order to pool the fruits with the next harvests and extract the seeds altogether. We were therefore also interested to see whether cold storage would affect the quality of seeds such as by reducing their germination or increasing the proportion of abnormal seeds. In contrast to more frequent harvests, for seed production on a larger scale, a combined harvest not resulting in a loss of quality would be more labour and cost efficient.

Fruit extraction took place 4 weeks after first fruit picking for both harvesting regimes, with the two treatments including different maturity levels. In comparison to freq 1, the fruits harvested in freq 2 were of mixed maturity level (from just ripe to very ripe), which could go along with a potentially higher infection and reject risk and decrease in seed vigour. In addition to the effect of different harvesting frequencies, we investigated in two genotypes, whether cooling of fruits prior to seed extraction alters seed vigour. Therefore, we stored fruits harvested in freq 1 in a cool chamber (3 °C) for up to 3 weeks until seed extraction time point. If storage of fruits would not have a significant impact on seed parameters, with this option one could combine the positive effect of the ideal maturity level of freq 1 and the reduced work load of freq 2, which would consist of much fewer seed extraction events compared to regular harvest and extraction.

2. Materials and Methods

2.1. Technical Data of Tomato Cultivation

The experiment was set up in 2019 and 2020 in a polytunnel at the FiBL-Station in Frick, Switzerland. The soil at the site is loamy with 2.5% organic matter (touch test) and a pH of 7.7. Soil analysis indicated the following available nutrient levels in fresh soil: 3.1 mg P/kg soil, 48.3 mg K/kg soil, 411.5 mg Ca/kg soil and 109.3 mg Mg/kg soil. Fertilization was applied following official Swiss organic guidelines and Swiss basics for fertilization respecting available nutrient stock in soil [18,19]. In total 170 kg N/ha, 96 kg P_2O_5/ha, 272 kg K_2O/ha and 78 kg MgO/ha have been applied and incorporated two weeks before planting. Drip-irrigation was installed and the quantity of water given was adapted to the weather conditions. The tomato plants were cultivated in double-rows with 35 cm spacing in-between plants and 80 cm spacing between rows. In cultivation year 2019, blossom end rot occurred and yield was significantly lower than in 2020. Additionally, single plants of the trial had to be removed due to impurity within some genotypes; this resulted in many missing values in the dataset of 2019. Furthermore, the seed extraction process is complex and needs experience. The trial of 2019 was used as a learning experience, especially regarding seed extraction. The uniformity of the extraction process is estimated to be more comparable between different extractions of the year 2020. We will therefore focus the results on the data for 2020. Therefore, data from the two different years were not combined for analysis.

2.2. Description of Genotypes

Seeds from eight tomato genotypes—some of them with a known seed production level—were procured from seed producers: G1-Amish Pasta, G2-Noire de Crimée and G3-Berner Rose from organic seed producer Sativa Rheinau; and G4-De Penjar "Moradeta" from University of Valencia, G5-Pera d'Abbruzzo, ISI Sementi, G6-Pilu, Bingenheimer Saatgut, G7-Byelsa F1 and G8-fedele F1 from Semillas Fito (Table 1). Sativa Rheinau AG uses the following classification of varieties regarding seed production: low-producing, <20 g seeds/m^2; medium-producing, 20–50 g seeds/m^2 and high-producing, >50 g seeds/m^2. The quantitative classification of other seed producers is not acquainted.

Table 1. Variety name, origin, type, and estimated seed set of examined genotypes.

Genotype	Variety	Origin	Type	Seed Set
G1	Amish Pasta	Sativa Rheinau	Pelati	low
G2	Noire de Crimée	Sativa Rheinau	Beefsteak	medium
G3	Berner Rose	Sativa Rheinau	Beefsteak	high
G4	De Penjar "Moradeta"	University of Valencia	Cocktail	medium
G5	Pera d'Abbruzzo	ISI Sementi	Oxheart	medium
G6	Pilu	Bingenheimer Saatgut	Standard	-
G7	Byelsa F1	Semillas Fito	Plum	high
G8	Fedele F1	Semillas Fito	Cherry	-

2.3. Experimental Set-Up

The experiment was set up in a randomized split block design with three repetitions (blocks) per genotype and treatment. Treatments consisted of a regular harvest frequency (twice a week; freq 1), and second frequency where fruits were harvested every 3 weeks (freq 2). Each repetition consisted of 12 plants per genotype, of which each six plants were assigned to frequency 1 (freq 1) and 6 to frequency 2 (freq 2). Seeds from the eight genotypes were sown into growing trays on 19 March 2019 and 11 March 2020 and transplanted to 13 cm pots after three weeks in 2019 and two weeks in 2020. Seedlings were planted under the polytunnel 8 weeks and 7 weeks after seeding in 2019 and 2020, respectively. Harvest started for both years in July, 10 weeks after planting (WAP) and ended in September (20 WAP) after the last seed extraction. Each harvest was weighed, and fruits were classified as marketable or rejects according to regular farming practices, in particular in the presence of clear symptoms of the disease on the fruits. Plant development has been assessed at 8 and 12 weeks after planting (WAP). Plant health has been assessed at 12, 15 and 19 weeks after planting.

We applied two different harvesting regimes: In freq 1, mature fruits were regularly harvested twice a week, counted, weighed, and afterwards discarded (G3–G8) or cool stored at 3 °C (G1 and G2). After three weeks, seeds from mature fruits harvested on extraction day (G1–G8) and from accumulated cooled fruits were extracted (G1 and G2). In freq 2, fruits with a mixed ripe maturity level were harvested after three weeks on extraction day only, counted, weighed and seeds were extracted (Figure S3). Extractions took place every three weeks and in total three times.

For seed yield extrapolation of "freq 1 cooled", fruit yield of freq 1 of the corresponding genotype was used. In the results, we treat the comparison of harvest frequencies (freq 1, freq 2), and extraction time point for genotypes G1–G8 separately from the comparison of the cool storage (harvest frequencies: freq 1, freq 2, freq 1 cooled; extraction time points) of G1 and G2.

2.4. Seed Extraction

Tomato seeds were extracted at three-time points per season. For each extraction, a representative subsample of 1–2 kg fruits treatment, genotype and repetition were squeezed and mixed with approximately 10% water. For around 7% of the samples of 2020, the extraction was done on less than 1 kg fruit; this was particularly the case for samples from Genotype 1 and 3 in the freq 1 treatment; this is understandable as in freq 1, only the fruits of the day's harvest were available for extraction. Therefore, for this treatment, in most cases all fruits of the harvest were used, whereas for frequency 2 mostly a subsample was used, as described above. The mixture was then fermented at 25 °C for three days and stirred three times a day to facilitate the fermentation process. After three days, seeds were washed out of the fermented tomato mixture by rinsing the ferment thoroughly and collecting the seeds in sieves. Seeds were air-dried on filter paper. Dry seeds were separated, weighed, and counted with the seed-counting machine (DATA count S-25 PLUS, Kibbutz Tzora, 9980300, Israel and Elmor C1 seed counter, 6430 Schwyz, Switzerland), which were calibrated for each genotype.

2.5. Seed Germination Trial

The seed lots of three repetitions per treatment and genotype were pooled and were re-divided in three repetitions per treatment and extraction. The germination test was carried out in the FiBL-Greenhouse, using Floradur Bio Block substrate from Floragard, and irrigated manually. Germination of 50 seeds per repetition was assessed at 4, 7, 14 and 21 days after seeding, according to the International rules for seed testing (ISTA). Seedling evaluation was carried after the protocol of the ISTA handbook of seedling evaluation [20]. For comparison of seed emergence, the germination rate index has been calculated as follows:

$$\mathrm{GRI} = \frac{\mathrm{G1}}{1} + \frac{\mathrm{G2}}{2} + \cdots + \frac{\mathrm{Gx}}{\mathrm{x}}$$

G1 = Germination percentage × 100 on the first day after sowing, G2 = Germination percentage × 100 at the second day after sowing [21]. A higher GRI value indicates higher and faster germination.

2.6. Statistical Design and Data Analysis

Data was analysed using the open-source software R for Statistical Computing (Version 4.0.3) [22]. Analysis of variance (Anova) was performed on a mixed model, with a fixed effect of treatment (and extraction) and the random effect of the plants. For post-hoc test, the estimated marginal means were calculated using the R package "emmeans" or using the Tukey-HSD Test [23]. The normality of the residuals was tested visually and if needed, data was transformed prior to the statistical testing (square root for all variables except germination rates which were transformed using the arcsin function). Visualization of data was realized either with Microsoft Excel (2016) or with the R package "ggplot2" [24,25].

For seed yield extrapolation of "freq 1 cooled", fruit yield of freq 1 of the corresponding genotype was used. In the results, we treat the comparison of harvest frequencies (freq 1, freq 2) and extraction time point for genotypes G1-G8 separately from the comparison of the cool storage (harvest frequencies: freq 1, freq 2, freq 1 cooled; extraction time points) for G1 and G2.

3. Results

3.1. Plant Development

In 2020, G1, G2, G3, G4 and G5 developed similarly forming 2–3 trusses until week 8 after planting and 5–6 trusses until week 12 after planting (Figure 1). Genotypes G6, G7 and G8 produced 6–8 trusses by week eight after planting and had a total of 8–11 trusses per plant in week 12 after planting. The harvest frequency did not alter truss formation until WAP 12.

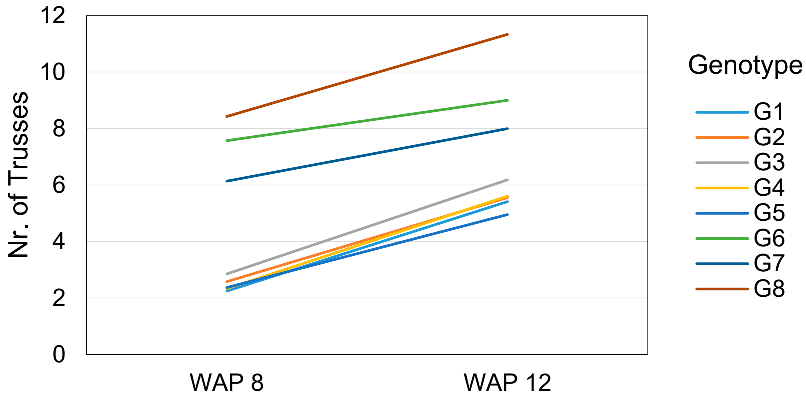

Figure 1. Truss formation of the different genotypes after eight 8 and 12 Weeks After Planting (WAP) in 2020.

3.2. Effect of Harvest Frequency on Fruit and Seed Yield

Throughout the 2020 harvest season, regularly harvested plants (freq 1) produced on average 25% more marketable yield than plants harvested every three weeks (freq 2) ($p < 0.001$, Figure 2). The same trend was observed in the fruit yield of 2019 (Figure S1 in supplementary material). Consequently, first quality fruit yield was significantly lower under the harvest regime of freq 2, ranging from 3–62% depending on genotype (Table 2). A significant interaction on genotype and harvest frequency was observed for marketable fruit yield of G1, G3 and G6 ($p < 0.01$). The share of rejected yield was on average 12% lower under frequently harvested plants (freq 1), with high variation among genotypes (Table 1). Similar to the observed interaction of marketable fruit yield, the share of rejected yield was significantly higher for freq 2 in G1, G3 and G6 ($p < 0.05$). The reason for rejection was mostly due to the bursting of fruits or blossom end rot and therefore might be due to the different irrigation needs of genotypes. Since blossom end rot is mainly due to inadequate irrigation, further results are based on total fruit yield in order not to jeopardize varieties whose irrigation needs could not be individually satisfied and therefore burst or experienced blossom end rot. Harvest frequency had a significant effect on total fruit yield ($p < 0.05$), with more yield harvested under frequency 1. Total fruit yield differed among Genotypes, with G5 and G7 producing significantly more yield than G8 ($p < 0.05$). In 2019, the proportion of rejects was very high, and similarly to 2020, generally higher under the freq 2 regime (Figure S1).

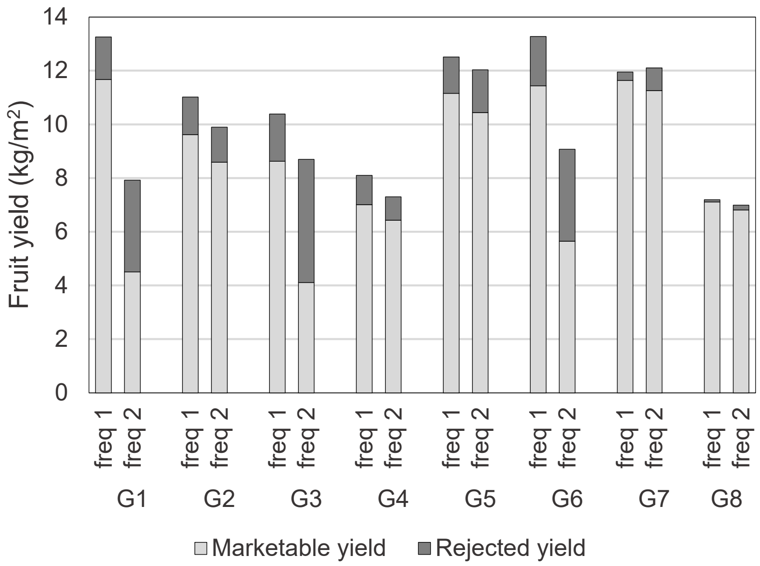

Figure 2. Average marketable and rejected tomato fruit yield by genotype and harvest frequency in 2020.

Table 2. Fruit and seed yield parameters of genotypes and their average at different harvest frequencies (mean ± sd, n = 3 resp. 24 if not indicated differently) in 2020. Estimated seed yield is extrapolated from total fruit yield harvested throughout the season, which includes rejected yield. n = 3 per genotype for marketable and rejected fruit yield as well as seed yield.

Genotype	Marketable Yield (kg/m^2)		Rejected Yield (% of Total Yield)		Thousand-Seed Weight (g)				Seed Yield (g/m^2)	
	Freq 1	**Freq 2**	**Freq 1**	**Freq 2**	**N**	**Freq 1**	**N**	**Freq 2**	**Freq 1**	**Freq 2**
G1	11.7 ± 4.9	4.5 ± 0.5	12%	36%	9	2.41 ± 0.44	7	2.77 ± 0.42	40 ± 19	24 ± 9
G2	9.6 ± 0.2	8.6 ± 1.2	12%	13%	8	3.1 ± 0.33	8	3.07 ± 0.35	41 ± 9	36 ± 8
G3	8.6 ± 1.1	4.1 ± 2.3	17%	55%	9	2.71 ± 0.33	8	2.75 ± 0.34	62 ± 16	47 ± 22
G4	7 ± 1.6	6.4 ± 0.7	14%	11%	9	3.18 ± 0.37	8	3.31 ± 0.32	43 ± 8	37 ± 8
G5	11.2 ± 3	10.4 ± 2.9	11%	14%	9	2.99 ± 0.6	9	3.14 ± 0.78	26 ± 7	23 ± 6
G6	11.4 ± 4	5.6 ± 1.6	15%	38%	8	2.87 ± 0.43	9	2.86 ± 0.45	44 ± 5	32 ± 14
G7	11.6 ± 1.8	11.3 ± 1.8	3%	7%	9	2.92 ± 0.19	6	3.1 ± 0.19	37 ± 8	19 ± 3
G8	7.1 ± 1	6.8 ± 1	1%	3%	9	1.89 ± 0.09	9	1.96 ± 0.14	46 ± 8	37 ± 3
Ø	9.8 ± 2.9	7.2 ± 2.9	10%	22%		2.75 ± 0.54		2.86 ± 0.57	42 ± 13	32 ± 13
p-value	<0.01		<0.001				0.698		<0.05	

While freshly burst fruits could still be used for seed extraction, secondary infection is likely to be found in fruits when there is a delay in harvest (freq 2).

Throughout the 2020 season, all genotypes produced more than the threshold of <20 g/m^2 for low seed-producing varieties given by the seed producer (Figure 3A). Genotype 3 produced significantly higher seed yield than G1, G5 and G7, which by producers estimation are low (G1) and medium (G5, G7) seed-producing varieties (p < 0.001, Table 1). The classification of G1, G2 and G3 as low, medium, respectively high seed-producing varieties appears to match our observations. Following this classification, we assign G5 as low, G4, G6 and G8 respectively as medium seed-producing genotypes.

If however, seed yield is linked with thousand seed weight by investigating the number of seeds/m^2, a slightly different pattern appears. As G8 seeds are by far the lightest, in contrast to its seed yield the genotype produces very high number of seeds/m^2 and would rather be aligned to G3 as a high seed-producing genotype (Figure 3B). Effect of harvest frequency on number of seeds per m^2 is shown in Figure S4.

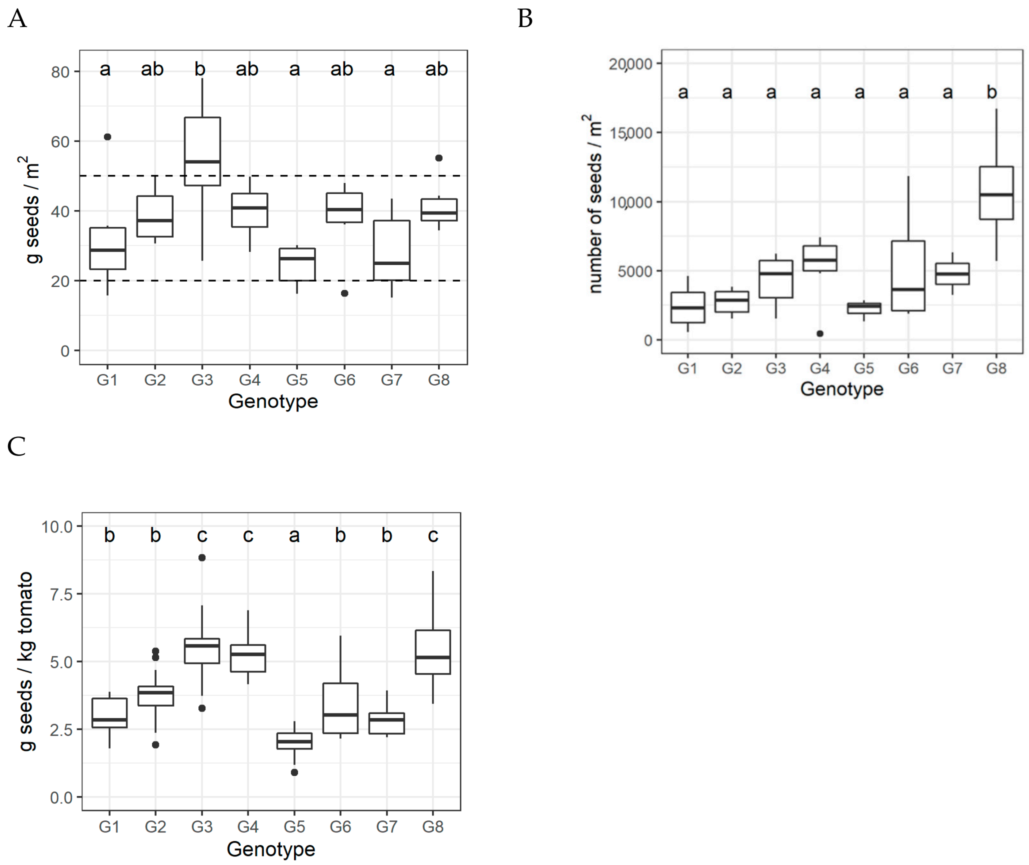

Figure 3. Estimated seed yield with threshold of 20 and 50 g seeds/m^2 (**A**) and the number of seeds (**B**) per genotype produced throughout harvest season 2020, averaged on both treatments, extrapolated on total fruit yield (n = 48). (**C**): Average seed quantity extracted per kg tomato average of all extraction time points on both treatments (n = 134). Different letters indicate statistically significant differences at $p < 0.05$. For genotype names, see Table 1.

The amount of seeds (g) which is extracted per kg tomato which classifies G3, G4 and G8 into high, G1, G2, G6 and G7 in to medium and G5 into low producing genotypes (Figure 3C).

3.3. Effect of Harvest Frequency and Extraction Time Point on Seed Yield

In 2020, Thousand-seed weight was highest in the first extraction and decreased significantly with the second ($p < 0.001$) and again with the third extraction ($p < 0.001$, Figure 4A). Harvest frequency had no effect on thousand-seed weight, and no interaction was observed.

The harvesting schedule did not have a significant effect on the seed yield ($p = 0.0519$, Figure 4B). Seed yield varied significantly between extraction time points, at second extraction significantly more seeds were extracted compared to the first and third extraction ($p < 0.001$, $p < 0.01$). A similar trend is observed with total fruit yield, which results in being significantly higher during the period of the second extraction compared to the fruit yields obtained until the first, respectively third extraction (both $p < 0.001$). Harvest frequency had a significant effect on the total fruit yield of the last extraction, where regular harvested plants produced higher yields ($p < 0.05$). Regularly harvested plants thus produced more seeds in total than plants from which fruits were harvested under freq 2 ($p < 0.05$), due to a correlation of seed yield with fruit yield (Figure 4B,C). The amount of seeds/kg tomato was lower in the first extraction (3.71 $\pm$ 0.26 g/kg fruit) compared with second extraction (4.32 $\pm$ 0.26 g/kg fruit, $p < 0.05$) and third extraction (4.26 $\pm$ 2.17 g/kg fruit). Harvest frequency did not have an effect on amounts of seed extracted per kg fruit.

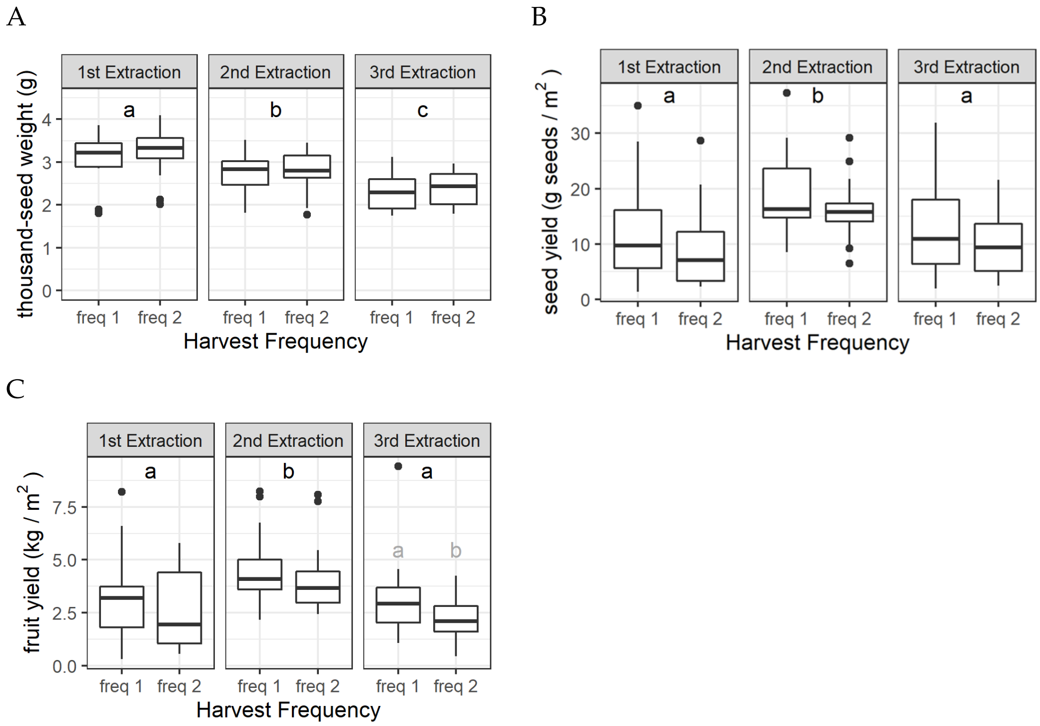

Figure 4. (**A**) Thousand-seed weight by extraction (beginning, middle and end of the season) and harvest regime (freq 1 = regular, freq 2 = every three weeks) in 2020. Extraction time point had a significant effect on thousand-seed weight ($p < 0.001$). (**B**) Effect of Extraction date ($p < 0.001$) and harvesting treatment ($p < 0.05$) on seed yield. (**C**) Effect of harvest frequency ($p < 0.05$) and extraction time point ($p < 0.001$) on total fruit yield. Different letters indicate a significant difference between extractions (black) and harvest frequency (grey) at a level of $p < 0.05$. Genotype 7 is excluded from Figures and corresponding statistical analysis, as no data points are available for freq 2 of third extraction.

In 2019, the extraction time point had a significant effect on thousand-seed weight ($p < 0.001$). Interaction was observed at the first extraction, where a significantly higher thousand-seed weight was obtained in the freq 2 regime (Figure S2A in the supplementary material). The extraction date and harvesting treatment had an effect on seed yield ($p < 0.001$ and $p = 0.084$, respectively; Figure S2B). Harvest frequency and extraction time point had an effect on total fruit yield ($p = 0.176$ and $p < 0.001$, respectively; Figure S2C). Over both seasons, except for the thousand seed weights in the first extraction, there was no difference between freq 1 and freq 2.

Cooled fruits from genotype 1 and 2 of regular harvest did not differ in thousand-seed weight or gram seeds extracted per kg tomato from values obtained from freq 1 (i.e., same maturity level of fruits compared to cooled) or freq 2 (different fruit maturity but similar mix of tomato stages included).

3.4. Effect of Harvest Frequency, Extraction Time Point and Cool Storage on Seed Germination

Based on the data of 2020, at 14 days after seeding (or days after planting DAP), seeds from the second and third extraction showed a significant higher germination rate of healthy seedlings (87% and 88%, respectively) than seeds obtained from the first extraction (78%, $p < 0.01$ resp. $p < 0.001$). Significantly higher germination rates were also observed for seeds of second extraction (93%) and third extraction (92%) 21 days after seeding when compared with first extraction (86%, $p < 0.001$ and $p < 0.01$, respectively; Figure 5A). Significant effects of genotype and extraction, as well as their interaction "genotype x extraction" were observed (Table S1).

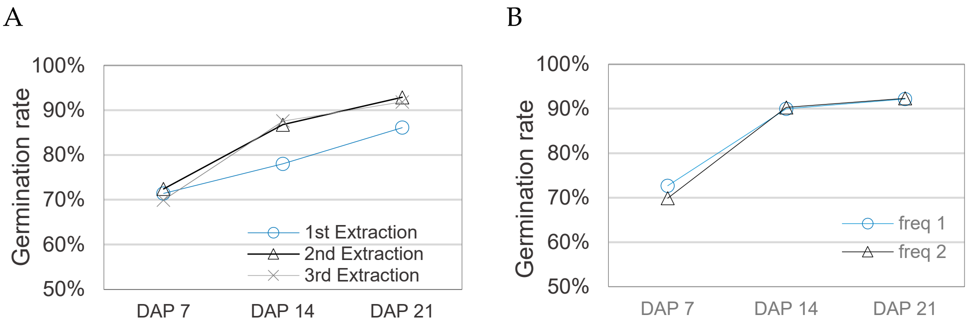

Figure 5. Seed germination rate at 7, 14 and 21 days after planting (DAP) of all genotypes by extraction date (**A**) and harvest frequency (**B**) in 2020.

No significant difference in germination was found between regularly harvested fruits and periodically harvested fruits (Figure 5B). Harvest frequency and extraction time point were not found to affect Germination Rate Index.

The cooling of fruits had no significant effect on the germination rate. The average germination rate over all three extractions of seeds extracted from freq 1 fruits was lower than those which were cool stored or harvested with freq 2 (Table 3). The lower germination rate of freq 1 was not significant and can be explained by the high variance of germination rates in genotype 1.

Table 3. Germination rate of healthy seedlings at 7, 14 and 21 day after seeding by genotype, harvest frequency and optional cooling treatment in 2020.

Genotype	Days after Seeding	Harvest Frequency + Treatment		
		Freq 1	**Freq 1 Cooled**	**Freq 2**
G1	7	0.72 ± 0.23	0.71 ± 0.13	0.66 ± 0.28
	14	0.82 ± 0.11	0.81 ± 0.11	0.78 ± 0.14
	21	0.86 ± 0.11	0.87 ± 0.06	0.9 ± 0.06
G2	7	0.58 ± 0.32	0.84 ± 0.1	0.73 ± 0.2
	14	0.69 ± 0.23	0.86 ± 0.15	0.83 ± 0.1
	21	0.8 ± 0.14	0.91 ± 0.07	0.9 ± 0.06

4. Discussion

In this study, no difference in seed production/kg fruit was found between frequently (freq 1) and infrequently (freq 2) harvested fruits, nor was a difference found with frequently harvested fruits which were stored at cool temperature until seed extraction. Plants from which fruits were regularly harvested produced in total a higher fruit yield and therefore a higher seed yield per square meter; this feature is known for bush (dwarf) beans and has already been observed in our green beans trials where a production for grains—without harvesting the green pods—resulted in a significantly lower pod production compared to when regularly harvesting pods (FiBL). Tijskens and colleagues showed that thinning can affect the diameter of tomatoes [26]. The authors hypothesize that the increase in diameter could be related to assimilating supply, which could be caused by a decreased sink strength [27,28]. By harvesting fruits regularly and therefore removing assimilate sinks from the plant, there could be a beneficial effect on further developing fruits on the same plant. The gained fruit yield can add value to parallel fruit harvest and seed multiplication.

Freq 1 and freq 2 fruits differed in their maturity stage, where freq 1 fruits were harvested as soon as they were ready (i.e., based on the usual tomato harvest for consumption), and freq 2 contained a mixture of fruits from different maturity levels, ranging from ripe with some overripe. The fruits of freq 1 used for the seed extraction comprised fruits from one harvest and therefore had a low diversity of cluster/inflorescence origin or plant developmental stage. The fruits of freq 2 and freq 1-cooled—while differing from each other in fruit maturity—included the same diversity of plant stage or cluster origin. Our results indicate that fruit maturity level (ripe to overripe), as well as the difference in TSW of different genotypes, did not affect seed germination. Therefore, the two strategies of harvest can offer benefits to different seed multipliers. Larger seed producers can leave the fruits up to 3 weeks after they would be ready for harvest, reduce labour in fruit harvesting and increase seed production efficiency without a significant loss of seeds. Our results show that even the extraction of freshly burst fruits does not compromise seed quality. However, diseases must be monitored as—according to our results—secondary infection is likely to be found in fruits when there is a delay in harvest; this is particularly important as studies have shown that lesions in tomato fruits can be a pathway to the infection of fruits with *Clavibacter michiganensis* subsp. *Michiganensis* [16]. Therefore, as long as infected fruits are excluded, an infrequent harvest does not compromise seed quality. On the other hand, small seed producers can produce seeds in parallel to fruit production, harvesting and storing at a cool temperature a proportion of fruits and pooling these fruits from different harvests to save time by extracting them at once. Following freq 1, small seed producers can use available infrastructure as cooled storage facilities to reduce the number of seed extractions.

For several varieties, we found a tendency for a higher Thousand Seed Weight (TSW) of seeds extracted from fruits of infrequently harvested plants (freq 2) compared to that of seeds extracted from freq 1 fruits; this can be explained by the fact that while in the ideal stage for marketing, some fruits of freq 1 may not have reached the full ripeness regarding seed weight gain [15]. However, overall TSW was not significantly different between the two groups. In contrast to harvesting frequency, our results showed differences between different extraction time-points in TSW, seed yield and germination rate. The first seed extraction which consisted of fruits from early (1st and second in most cases) clusters resulted in seeds with the lowest seed germination rates. However, also seeds from the first seed extraction met the minimum germination requirements of 75%, which are set within the Council Directive 2002/55/EC on the marketing of vegetable seed [29]. The second extraction which corresponds to the middle of the harvest season, where more fruits and therefore more seeds were produced, resulted in a significantly higher germination rate compared to the first extraction; this corresponds to approximately the third to seventh truss depending on variety and could be taken as an optimal period for seed production. However, although seed yield in the third extraction was smaller compared to extraction time-point 2, the overall germination rate was comparable to the one of extraction time-point 2. For further studies, it will be interesting to link seed production and germination rate with the fruits of specific developmental stages or trusses.

The main difference in seed yield g/m^2 was due to the differences in fruit yield between the genotypes. Balcha et al. found a significant of variety choices on yield components and between fruit yield and seed weight [30]. Furthermore, a positive, hence non-significant, correlation between fruit and seed yield was also shown in a previous study conducted with 120 tomato genotypes [31]. The seed production yield we found was in general in line with the classification of seeds producers who provided these genotypes, except for G5 which was indicated as a medium but after our trial found a low producer; this increases the confidence that this trial truly reflects production conditions. However, we also see that differences in seed yield in g/m^2 were not necessarily indicative of the number of plants yielded when seeds germinated. Different TSWs were not reflected in different germination capacities. On the contrary, seeds extracted from the first extraction had overall higher TSW compared to the second and third extraction, which showed higher seed germination rates. Therefore, the number of seeds can be a better indication of the seed production potential of a variety; this is also relevant for the packaging of the seeds.

5. Conclusions

At a time when the market for organically produced food is expected to grow [32,33], and new as well as old varieties adapted to changing climates and the conditions of low-input farming are needed [9,10], different harvesting frequencies can be followed without losses in seed yield per kg fruit or quality. To conclude, the harvest of tomato fruits for seed production can be (i) regularly done and fruits stored for a pooled extraction or (ii) harvested every three weeks and extracted after exclusion of infected fruits; these results can support small and larger seed producers in their strategy to multiply seeds under organic conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su141811575/s1, Figure S1: Marketable and rejected fruit yield of 2019 season. Figure S2 ABC: Thousand-seed weight, seed yield and fruit yield per extraction and harvest frequency in 2019. Figure S3: harvest frequency scheme; Figure S4: Estimated number of seeds produced per m^2 by genotype and harvest frequency; Table S1: Germination rate of healthy seedlings at 7, 14 and 21 day after seeding by genotype, harvest frequency and optional cooling treatment; Tables S2–S4: Seasonal summary, Extraction data and Germination, respectively.

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