

Season, Fruit Maturity, and Storage Affect on the Physiological Quality of F₁ Hybrid ‘VTM580’ Tomato Seeds and Seedlings

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Physiological qualities of F₁ hybrid tomato seeds affected by the growing season; maturity stage at 40, 45, 50, 60, and 70 days after cross-pollination (DAP), and storage in plastic bags at 0°C for four months were studied. The characteristics of seeds from early harvested fruits (45 DAP) that subsequently ripened at ambient temperature (28 ± 2°C) for ten days (designated as “45+ripening”), were also investigated. Higher light intensity, and a wider range of night and day temperatures and relative humidity (RH), but lower night temperature and RH, were observed in the winter season compared to the rainy season. The winter climate delayed physiological maturity of the seeds by 10 days, indicated by the highest weight of dried seed, percentage germination and germination index which occurred at 60 DAP. On the other hand, in the rainy season physiological maturity occurred at 50 DAP. The proportions of irregularly shaped seeds, abnormal seedlings and weak seedlings were independent of fruit and seed maturity, while the amount of speckled seeds significantly increased with an increase in seed development on either the mother plant or the 45+ripening. Fresh (non-stored seeds) and stored seeds of the fruits harvested at 45+ripening and at 60 DAP showed the same quality. The number of fresh ungerminated seed in the seeds of 45 and 50 DAP was markedly reduced after four months storage at 0°C.

Key Words: fruit ripening, seed and seedling disorder, seed development, seed physiological maturity.

Introduction

F₁ hybrid tomato seeds are used for commercial tomato fruit production all year round due to their uniformity, rapid growth, high yield, high fruit quality, and particularly their resistance to pests and diseases (Dias et al., 2006; Tay, 2002) which results in high consumption of the quality F₁ hybrid seeds. The seeds also need to be produced throughout different climates within a year. Therefore, production is avoided in some periods in places where the climate is undesirable for the growth of tomato plants, fruits, and seeds. An undesirable climate causes both negative and positive effects

on the seeds and seedling quality (Singh et al., 1959). Intensive hand pollination, in order to achieve crossing between two genetically dissimilar parents, is an effective technique for the production of F₁ hybrid tomato seeds, which overall is a costly investment. Some physiological disorders of F₁ hybrid tomato seeds and seedlings that make them unmarketable are often observed and have been reported (Hochmuth et al., 1993). Comprehensively, tomato crop yield and physiological characteristics of a fruit are affected by the environment during plant growth. Fluctuations in temperature affect the rate of developmental stages such as fruit maturation (Hurd and Graves, 1984). Thangam and Thamburaj (2008) stated that the number of fruits per plant was larger in an open field by 50% than in shadow. The seed number per fruit and germination rate of harvested seeds are also important for seed production and they are also affected by temperature during plant growth (Adams et al., 2001). Seedling vigor in tomato seeds is significantly related to the growing environment.

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Recently, many seed production areas, including Thailand, have faced low productivity and unacceptable quality of F_1 hybrid tomato seeds. The main cause of this was unpredictable weather, which included unwanted rainfall that not only markedly damages the plants and fruits, but also enhances serious outbreaks of diseases and leads to insect infestations. There have been outbreaks of bacterial [*Ralstonia solanacearum* (Smith)] and fusarium (*Fusarium oxysporum* Schlecht.) wilts (Blancard et al., 2012; Prior et al., 1998), as well as fruit worm infestations. Serious damage has significantly increased in production areas that have been continuously cultivated with the same single tomato crop for a number of years. Harvesting immature fruits provided seed of low quality, but the seeds could develop during the fruit ripening process. Therefore, harvesting immature fruit at an appropriate stage before damage caused by weather, insects, or diseases could reduce losses and seeds of high quality could be obtained (Akpulu et al., 2008; Kumar et al., 2009; Walck et al., 2011). There have been some reports that berry fruits have been harvested successfully during the green mature stage and subsequently ripened at ambient temperature ($25 \pm 5^\circ\text{C}$) such as tomatoes, peppers, and cucumbers (Barbedo et al., 1999; Dias et al., 2006; Shamsheer Ahmed et al., 2008). This method apparently provided the same seed quality as if the fruits were ripened on mother plants. Kader and Morris (1976) demonstrated that immature seeds could fully develop by absorbing nutrients from fruits during post-harvest ripening. The seed dry matter significantly increased during post-harvest storage due to nutrient transfer from the fruit (Alvarenga et al., 1991; Barbedo et al., 1994). Absciscic acid (ABA) is a component that enhances dry matter accumulation in earlier stages of seed development. It gradually reduces in late stages of maturity leading to high rates of seed germination (Norcini and Aldrich, 2007). In some plants however, seed dormancy is caused by high ABA content in the fully mature seeds.

In some berry fruits, seeds harvested at the green mature stage, especially F_1 hybrid tomato seeds, showed low germination due to immature seeds and balance of hormones (Ramirez-Rosales et al., 2002). Another undesirable characteristic of prematurely harvested seeds is the fact that phenotypes of some F_1 hybrid tomato seedlings can exhibit imbalanced seedling development (including weak radicle or hypocotyl development) and seedlings with seed coats that are less than 50% removed from the cotyledons. Such phenotypes can frequently occur in some parent lines used for F_1 hybrid seed production and can be enhanced by changes in climate. Even though the fact that maternal plants dominate the physical appearance of F_1 hybrid seeds is well documented (Pandey et al., 2013; Weiss et al., 2013; Xu et al., 2014), the microclimate surrounding the plants, the stage of fruit maturity, and post-harvest ripening

may cause changes in the physiological attributes of seeds and seedlings. The objective of this research was to study the effect of season, fruit maturity, and storage on the physiological quality of F_1 hybrid tomato seeds and seedlings.

Materials and Methods

Plant materials

F_1 hybrid tomato seeds were produced by Sakata Siam Seed (Thailand) Co., Ltd. for 2 crops in Phetchabun Province, Thailand. The maternal line was a determinate type and the fruits were used for processing. The parental lines (120 male plants and 400 maternal lines plants) of the 'VTM580' line were grown by transplanting one-month old seedlings (about 20 cm height) with 70 cm spacing between the rows and 50 cm spacing between the plants. Before planting, dolomite (50 kg/1200 plants), compost (500 kg·ha⁻¹), and NPK (13:13:21 kg·ha⁻¹) were applied. Approximately 1–2 liters of water was supplied by a drip irrigation system to adjust the soil water content. We regularly checked the soil water content by squeezing the soil in the palm of our hand every day before supplying water. An insecticide was applied at 5-day intervals until harvesting was finished. When the flowers bloomed, pollen from fully open flowers of the male parent line was collected and then transferred to female stigmas in which petals had turned chartreuse. Second to fifth clusters of each plant were pollinated. Each flower was tagged on the day of pollination (95% of fruits were set after pollination by hand). The temperature, relative humidity (RH), and light intensity were measured daily, every three hours from the blooming stage to the final harvesting time by a data logger recorder (Nature Pro, Nature Eye; Thai Victory Co., LTD., Bangkok, Thailand) and depicted in Figure 1.

Seed preparation

The F_1 hybrid fruits of this line were commercially harvested at 55–60 DAP (days after pollination) (turning red to red stage) for effective and high seed yield, according to the alliance company. The cross-pollinated fruits of this line in this research were randomly harvested 45 (light green stage), 50 (breaker stage), 60 (red or ripening), and 70 (over ripening) DAP in winter and 40 (light green stage), 50 (light red stage), 60 (red or ripening), and 70 (over ripening) DAP in the rainy season from the second to fifth clusters of tomato plants. The seeds were immediately extracted by hand. In addition, fruits harvested at 45 DAP were held at ambient temperature ($28 \pm 2^\circ\text{C}$ and 70%–85% RH recorded by a data logger recorder) in a laboratory room until they ripened (45+ripening). Subsequently, ripening determined by fruit color change and lack of defects took about ten days. The tagged fruits harvested at each developmental stage from different clusters or plants involved 50 fruits/day in each of four replicates (day/

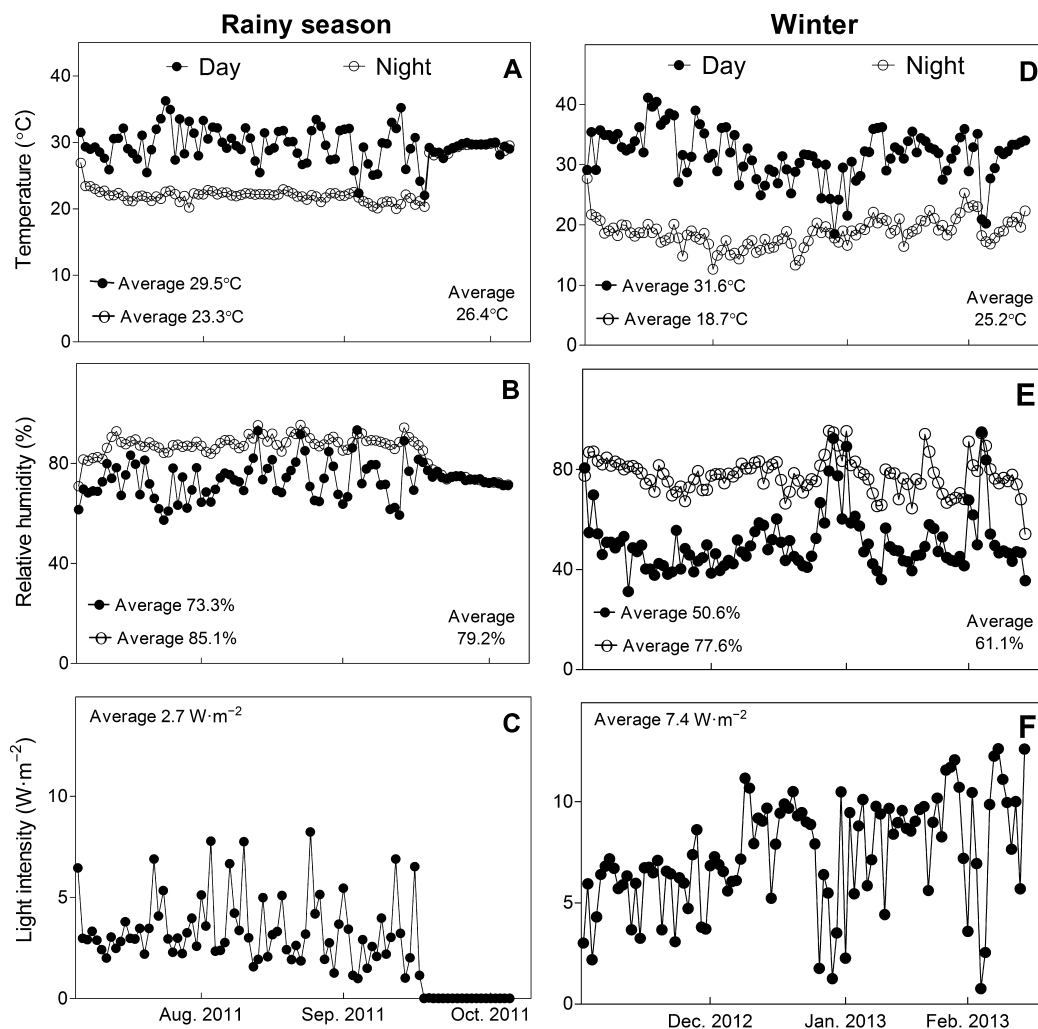


Fig. 1. Day (9:00 am–6:00 pm) (●) and night (9:00 pm–6:00 am) (○) temperatures (A and D), relative humidity (B and E) and light intensity at 9:00 am–6:00 pm (C and F) of F_1 hybrid tomatoes growing in a plastic house in rainy and winter seasons were recorded every three hours, and shown as average daily values. In the rainy season, the sun is usually shining between 6:03 am–6:17 pm (12 h 14 min) whereas in winter, it is usually shining between 6:00 am–5:42 pm (11 h 42 min) from Thai Astronomical Society (<http://thaiastro.nectec.or.th/skyevnt/sunmoon/riseset.html>).

replication) that were used. The fruits were manually cut and then the slurry containing the seeds was removed and allowed to ferment naturally for one night at room temperature (rainy season at 23.3°C and winter at 18.7°C). The seeds were separated by washing with tap water to remove the mucilaginous seed coat and the immature seeds floating on the surface. The seeds were dried under the sun (rainy season at 29.5°C and winter at 31.6°C) for one to two days until they reached 6.5%–8.0% moisture content (fresh or non-stored seed) as per the recommended method of the alliance company. The mature seeds were either assessed immediately or packed in sealed polyethylene plastic bags (110 μm thickness) and then stored at 0°C and 60%–65% RH for four months (stored seeds). Fresh (non-stored) and stored seeds were used for seed quality measurement.

The number and weight of mature seeds per fruit

The number of mature seeds per fruit was counted from ten fruits per replicate (four replications per treatment) and expressed as the mean. Dried seed samples were weighed using an electronic balance (ADVENTURER™ Electronic Balance, ARC 120; OHAUS, Ontario, Canada).

Percentage of speckled and irregularly shaped seeds

A sample of one hundred seeds from each of the four replicates was randomly selected from the prepared seeds from each treatment and observed by the naked eye. Data were calculated and reported as percentages. For irregularly shaped seed measurement, one hundred seeds were randomly sampled and classified into two groups: normally shaped and irregularly shaped seeds. In the case of speckled seeds, one hundred seeds were divided into two groups: normal and speckled seeds.

Percentage of standard germination

The between paper method was used for the germination test (International Seed Testing Association, 2007). Seeds were placed on paper towels saturated with water (pH: 6.5–7.5 and Electrical Conductivity (EC): 0.2–0.4 mS·cm⁻¹). The paper towels were kept in a basket covered with a plastic bag and incubated at 30°C during the day and 20°C at night. Four replications, each of one hundred seeds, were examined. Classification of seedlings followed the International Seed Testing Association (2007) with a minor modification regarding commercial preferences (Fig. 2). Ungerminated seeds were categorized as decayed and fresh ungerminated seeds. In the stored seeds, fresh ungerminated seeds were further classified by a tetrazolium chloride test (TZ test) for their viability. In this experiment, the percentage of germination precluded weak seedlings.

Germination index (GI)

GI was determined by germinating the seeds on the top of a paper saturated with tap water in a plastic box. The germination box was incubated at 30°C during the day and 20°C at night. Normal seedlings were classified using the same criteria as the percentage of standard germination that were recorded daily until the germination time was completed (fourteen days), according to the ISTA rules (International Seed Testing Association, 2007). A high GI indicated a high uniformity of seed

germination. GI was calculated as follows:

$$GI = \Sigma(n/d)$$

n = number of normal seedlings on day “d”

d = days after planting

TZ test

Fresh ungerminated seeds from the storage treatments were washed. Subsequently, the middle of the seed coat was punctured with a needle. The seeds were then soaked in 0.5% solution of 2,3,5-triphenyltetrazolium chloride and incubated at 37°C for four hours in an incubator (Model UNE 550; Memmert, Germany), in a dark environment. Soaked seeds were washed twice with deionized water and then longitudinally dissected with a razor blade. The embryo was carefully classified under stereomicroscopy according to the International Seed Testing Association (2007).

Potato dextrose agar (PDA) and total plate count (TPC) blotter methods

The PDA and TPC blotter methods following Lane et al. (2012) were employed with a slight modification. Briefly, both powder of potato dextrose agar (39 g) or plate count agar (23.5 g) (HIMEDIA; Himedia Laboratories Pvt. Ltd., Mumbai, India) were suspended in 1000 mL of distilled water. The suspensions were boiled with frequent agitation until dissolved completely. The medium was sterilized in an autoclave before pouring into sterile plates. The seed surface was sterilized with 5% sodium hypochlorite and then rinsed twice in an excess of sterile water. Twenty-five sterilized seeds in each of four replicates were placed on a medium plate agar and incubated at room temperature (28°C–30°C) for five to seven days. Detection of microbial growth was performed visually.

Statistical design and analysis

The experiment was arranged as a randomized complete block design (RCBD). Analysis of variance (ANOVA) and significant differences among means were determined by Fisher's Least Significant Difference (LSD) test with the SPSS Statistics Base 17.0 system (Amos Development Corporation, Chicago, IL, USA) to separate the treatment means at $P \leq 0.05$ probability. Data given in percentages were subjected to arcsine transformation before ANOVA.

Results

Light intensity and the differences in temperature and RH were lower in the rainy season than in the winter (rainy season: 2.7 W·m⁻², 29.5°C at day, 23.3°C at night, 73.3% RH at day, 85.1% RH at night, winter season: 7.4 W·m⁻², 31.6°C at day, 18.7°C at night, 50.6% RH at day and 77.6% RH at night) (Fig. 1A–F). Different climates in the rainy and winter seasons during the growth of tomato fruits significantly affected ripening

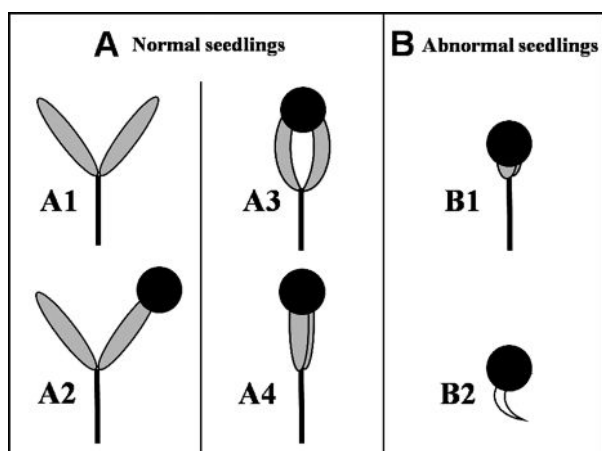


Fig. 2. The structure of normal (A) and abnormal (B) seedlings.

Normal seedlings (A). There were two types of normal seedlings, (A1) and (A2): the seedlings with 100% all of their essential structures well-developed, complete and healthy with seed coats that were more than 75% removed from the cotyledons were classified as normal seedlings. (A3) and (A4): the seedlings with complete root and hypocotyl with seed coats only 50%–75% removed from the cotyledons were classified as weak seedlings. Abnormal seedlings (B). Abnormal seedlings were classified into two groups: (B1) deformed seedlings which were seedlings with complete root and hypocotyls, but with seed coats that were less than 50% removed from the cotyledons; (B2) imbalanced seedlings which were seedlings that had only the radicle or seedlings with the radicle, but only short hypocotyls without seed coat removal.

of both fruits, as well as physiological quality of F_1 hybrid tomato seeds and seedling vigor. In regard to the characteristics of these parental lines and our observations, the ripening stage observation based on the fruit color showed that the breaker stage occurred at 50 DAP (L^* : 42.4; a^* : 18.7) and the fruits entered the light red stage at 60 DAP (L^* : 37.3; a^* : 28.2) in winter. The rainy season, however, had a greater efficiency in expediting fruit maturity by ten days. The breaker and light red stages were at 40 (L^* : 42.6; a^* : 19.9) and 50 DAP (L^* : 38.9; a^* : 27.0), respectively.

Effect of season on the physiological quality of seeds

The number of mature seeds per fruit and the weight of dried seeds (6.5%–8.0% moisture content) from fruits growing in the rainy season were not significantly different compared to the winter ones at all mature stages (Table 1), except the number of mature seeds per fruit harvested at 60 DAP. The different climate in the rainy and winter seasons during plant and fruit development significantly affected the percentage of speckled and irregularly shaped seeds (Table 1). In the rainy season, all fruit stages had a higher percentage of speckled and irregularly shaped seeds compared to winter (Fig. 3C, G; Table 1).

Effect of fruit maturity on the physiological quality of seeds

Maturity stages significantly affected the number of mature seeds per fruit and the weight of dried seeds. In the rainy season, both values were the lowest for the fruits harvested at 40 DAP, but the highest value was observed in the fruits at 50–70 DAP (Fig. 3A, B). In the winter season, the values of these characteristics for the tomato fruits harvested at 60 DAP and 45+ripening

were higher than in other treatments (Fig. 3E, F). The values for speckled seeds in the rainy season were lower for the fruits harvested at 40 and 50 DAP than for those harvested at 60 and 70 DAP (Fig. 3C). The speckled seeds of tomato fruit harvested at 45 DAP in winter was significantly the lowest (Fig. 3G). Our observations using PDA blotter and TPC methods indicated that there was no incidence of microbial growth (data not shown). Hence, harvesting a tomato fruit at 45 DAP prior to ripening at ambient temperature (45+ripening) would not significantly contribute to an increased number of speckled seeds in comparison to those harvested at 50–70 DAP. Maturity did not significantly affect the proportion of irregularly shaped seeds in either season (Fig. 3D, H).

Effect of season on the physiological quality of seedlings

Percentages of normal seedlings or germination and GI of seeds from fruits growing in the rainy season were significantly higher than those in winter at all maturity stages (Table 1). A high germination percentage from fruits growing in the rainy season started at 50 DAP, while in the winter season it started at 60 DAP. This meant that the winter climate delayed seed's physiological maturity by ten days. The percentage of abnormal seedlings and ungerminated seeds from fruits growing in the rainy season was lower than that of fruits grown in winter (Table 1).

Effect of fruit maturity on the physiological quality of seedlings

Germination percentage of the seeds in the rainy season beyond 50 DAP was approximately 80% (Fig. 4A). The GI of the tomato seeds harvested at 50–70 DAP

Table 1. A comparison of the physiological quality of F_1 hybrid tomato seeds and seedlings harvested at 40, 45, 45+ripening (45+R), 50, 60, and 70 days after pollination (DAP) in the difference seasons, before and after storage at 0°C.

Season	Number of mature seeds per fruit (seeds)				Weight of dried seeds per fruit (mg)				Speckled seeds (%)				Irregularly shape seeds (%)							
	40:45 ^y	50	60	70	40:45 ^y	50	60	70	40:45 ^y	50	60	70	40:45 ^y	50	60	70				
Rainy Winter	NS	NS	*	NS	NS	NS	NS	NS	**	**	**	**	**	*	*	**				
Season	Germination ^z (%)				Germination index				Abnormal seedling (%)				Ungerminated seeds (%)							
	40:45 ^y	50	60	70	40:45 ^y	50	60	70	40:45 ^y	50	60	70	40:45 ^y	50	60	70				
Rainy Winter	**	**	**	**	**	**	**	**	NS	**	**	*	*	*	NS	NS				
Season	Germination ^z (%)				Germination index				Abnormal seedling (%)				Ungerminated seeds (%)							
	45	45+R	50	60	70	45	45+R	50	60	70	45	45+R	50	60	70	45	45+R	50	60	70
Winter	*	*	NS	NS	NS	**	NS	*	NS	NS	**	*	*	**	NS	**	NS	**	NS	NS
Winter+storage at 0°C																				

^z The percentage of germination precluded weak seedlings.

^y The data of harvested seeds at 40 DAP in the rainy season compared with 45 DAP in winter.

*, **, and NS indicate significant at $P \leq 0.05$, $P \leq 0.01$, and non-significant difference by Fisher's least significant difference (LSD) test, respectively.

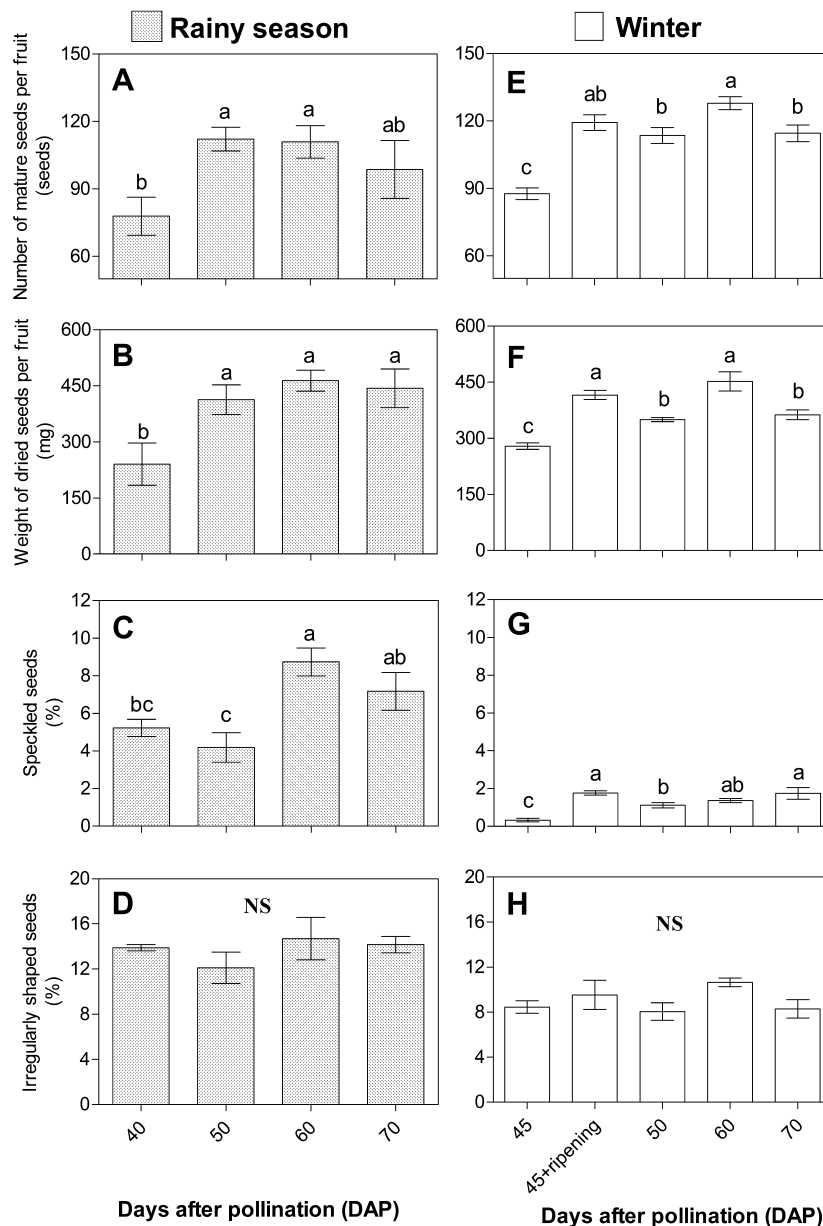


Fig. 3. Number of mature seeds per fruit (A and E; $n = 40$), weight of dried seeds per fruit (B and F; $n = 4$), percentage of speckled seeds (C and G; $n = 4$ with one hundred seeds per replicate), and percentage of irregularly shaped seeds (D and H; $n = 4$ with one hundred seeds per replicate) of F_1 hybrid tomato seeds from fruits growing in rainy and winter seasons harvested at 40, 45, 50, 60, and 70 days after pollination (DAP) and at 45 DAP with subsequent ripening at ambient temperature ($28 \pm 2^\circ\text{C}$) for ten days (45+ripening). Different small letters and NS indicate significant and non-significant difference at $P \leq 0.05$ by Fisher's least significant difference (LSD) test.

was not significantly different, while the GI of the seeds harvested at 40 DAP was significantly the lowest in the rainy season (Fig. 4B). Significantly higher germination and GI occurred in the seeds harvested at 45+ripening, 60, and 70 DAP in winter (Fig. 4E, F). No mature stages significantly affected the percentage of weak seedlings in either season and it was observed to be below 7.0% of the population in all treatments. The values of germination and GI, seeds harvested beyond light green stages as well as the light green stage with post-harvest ripening (45+ripening), were higher than those of the light green stages (45 DAP) in winter. The

effects of maturity on abnormal seedlings and ungerminated seeds in the rainy and winter seasons were different (Fig. 4C, D, G, H). The seeds from the young fruits at 40 DAP in rainy season had significantly the highest percentage of abnormal seedlings. After this stage (40 DAP) these incidences significantly decreased (Fig. 4C). In winter, the abnormal seedlings from the 45 DAP were significantly lower than with other treatments (Fig. 4G).

The proportion of ungerminated seeds in the seeds from the light green stages (40 and 45 DAP) was significantly higher compared to the later mature stages in

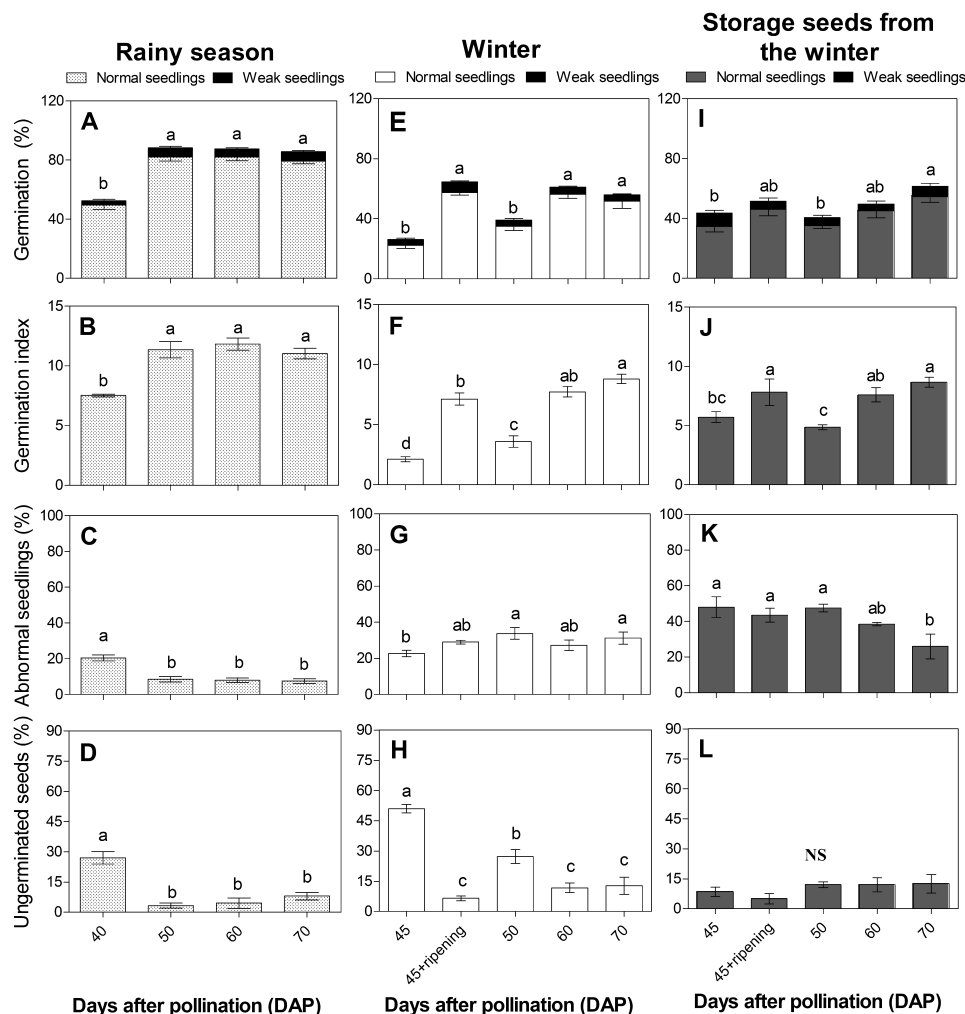


Fig. 4. Germination (A, E, and I), germination index (B, F, and J), abnormal seedlings (C, G, and K), and ungerminated seeds (D, H, and L) of fresh F_1 hybrid tomato seeds from fruits harvested at 40, 45, 50, 60, and 70 days after pollination (DAP) and at 45 DAP with subsequent ripening at ambient temperature ($28 \pm 2^\circ\text{C}$) for 10 days (45+ripening) growing in rainy and winter seasons, and stored seeds from winter (at 0°C for four months) ($n = 4$ from one hundred seeds per replicate). Different small letters and NS indicate significant and non-significant difference at $P \leq 0.05$ by Fisher's least significant difference (LSD) test.

both seasons (Fig. 4D, H). The proportion of ungerminated seeds in the rainy season was significantly lower in the fruits from 50–70 DAP (Fig. 4D).

Effect of storage at 0°C on the physiological quality of seedlings

Changes in seed quality after storage at 0°C for four months were closely investigated to confirm the effects of maturity on the fresh (non-stored) seeds and to further determine the proportions of abnormal seedlings and ungerminated seeds in the various treatments in the winter. Germination of fresh and stored seeds showed the same patterns, and indicated that germination of the tomato seeds harvested at 45+ripening was as high as that at 60 and 70 DAP (Fig. 4E, I). The proportion of weak seedlings was below 7.0%, except for the seeds at 45 DAP. The GI of tomato seeds harvested at 45+ripening, 60, and 70 DAP was not different after storage for four months. Their GI values were higher than those at

45 and 50 DAP (Fig. 4J). This is consistent with the initial GI values (Fig. 4F). Storage caused an increase in the proportion of abnormal seedlings with the exception of the seeds harvested at 70 DAP (Table 2). The ungerminated seeds of storage seeds were not significantly different at any stages (Fig. 4I). The proportion of deformed seedlings was significantly the highest in the seeds of 45+ripening compared to other treatments. However, the proportions of imbalanced seedlings in the seeds of 45+ripening and 70 DAP were significantly the lowest (Table 2). The proportions of fresh ungerminated and decayed seeds in each treatment were not significantly different after storage at 0°C for four months (Fig. 4L; Table 2). The fresh (non-stored) seeds from the 45 and 50 DAP treatments of ungerminated seeds subsequently declined after storage (Fig. 4H, L). In contrast, the fresh and stored seeds at 45+ripening, 60, and 70 DAP treatments had almost the same proportions of ungerminated seeds (Fig. 4H, L), meaning

Table 2. Percentage of abnormal seedlings and ungerminated seeds of F₁ hybrid tomato seeds from fruits harvested at 45, 50, 60, and 70 days after pollination (DAP) and at 45 DAP with subsequent ripening at ambient temperature ($28 \pm 2^\circ\text{C}$) for ten days (45+ripening) growing in the winter season after storage in plastic bags at 0°C for four months (stored seeds).

DAP	Abnormal seedlings (%)		Ungerminated seeds (%)		
	Deformed	Imbalanced	Fresh ungerminated	Dead	Decayed
45	29.5 ab	18.5 a	2.0	2.5	4.0
45+ripening	37.0 a	6.5 b	1.0	0.5	3.5
50	32.0 ab	15.5 a	4.5	3.5	4.0
60	21.5 b	17.0 a	3.5	3.0	5.5
70	20.0 b	6.0 b	2.5	4.5	5.5

Means followed by different letters are significantly different ($P \leq 0.05$) by Fisher's least significant difference (LSD) test between the treatments within the same column ($n=4$ from 100 seeds per replicate).

that harvesting the seeds at 45+ripening, or at 60 and 70 DAP reduced the proportion of ungerminated seeds.

Discussion

Effect of season and fruit maturity on the physiological quality of seeds

Although providing the best conditions for crop growth and health is the foundation of seed quality, the climate during plant growth is a crucial factor for seed viability and vigor. The relatively high average temperature and humidity in the rainy season had a greater effect on expediting fruit maturity by ten days compared to those in winter (Fig. 3B, F). In agreement with Bakker (1991), planting tomatoes under high temperature (about 32°C) induced fruit maturation; however, it was not influenced by humidity. Demir et al. (2008) reported that low greenhouse temperatures in autumn (about 28°C in the day and 6°C at night) delayed seed maturity up to 115 days after anthesis (DAA), but the mature seeds could be harvested at 60 DAA in spring (about 35°C in the day and 18°C at night).

Effect of season on the physiological quality of seeds

Tomato seed quality (number, weight, dry matter, and size) is mainly dependent on the growing season and variety of tomato plants selected for seed production (Kanwar, 1990; Mendham et al., 1981). The climate in the rainy and winter seasons in this experiment did not affect the number of mature seeds per fruit, which may be due to the fact that the difference between the daily average temperature in both seasons was only 1.2°C (Fig. 3A, B, E, F). The highest weight of dried seeds was observed at 50 DAP, suggesting the physiological maturity of the seeds, which was ten days earlier than in winter (Fig. 3B, F). Based on the observation of the tomato fruit color being light red, the fruit could be applied for harvesting matured seeds. In the rainy season, all fruit stages had higher percentages of speckled and irregularly shaped seeds compared to the winter season (Table 1). The plant height of tomatoes in the rainy season was approximately 2-fold taller than that in winter

(data not shown). However, high light intensity in the winter season delayed tomato fruit maturity. This result was different from the report of Piringir and Heinze (1954). This suggests that light intensity plays an important factor in fruit ripening as well as seed maturation, but also that other crucial factors such as the different temperatures and RH in the day and at night affected our results. Khanal et al. (2013) demonstrated that a smaller difference in day/night temperature of $24^\circ\text{C}/17^\circ\text{C}$ accelerated fruit maturity, indicated by a greater percentage of dry matter, soluble solids and titratable acid of tomatoes compared to the fruit from day/night temperatures of $27^\circ\text{C}/14^\circ\text{C}$. This suggests that a larger difference in day/night temperature delayed the maturity of both the tomato fruit and the seeds.

Effect of fruit maturity on the physiological quality of seeds

Our results show that the highest seed number per fruit and the highest weight of dried seeds were in the fruits at 50 DAP in the rainy season, and at 60 DAP and the 45+ripening fruits in winter (Fig. 3A, B, E, F). The significant increase in these attributes in the 45+ripening fruits was mostly caused by nutrient translocation from the fruit to immature seeds (Demir and Samit, 2001; Petrov et al., 1981). The results were similar to the results from Dias et al. (2006) who reported that a short storage period of post-harvested tomato fruits improved physiological seed quality, especially if the fruits were harvested at the green-mature stage and then stored until the pericarp turned completely red. Development of immature seeds in berry fruits during post-harvest storage has also been reported for cucurbits and paprika chilli (Barbedo et al., 1999; Shamsheer Ahmed et al., 2008; Welbaum, 1999). The proportion of speckled seeds increased during the development regardless of whether the fruits were harvested at the green-mature stage (immature at 45 DAP) prior to ripening at ambient temperature or remained on the parent plant (Fig. 3G). The speckled appearance is a critical quality aspect for the seed industry because this

appearance suggests that the seed is infected. Commercially, this kind of seed is manually removed prior to sale. There was no incidence of microbial growth. The fruit maturity was not affected with irregularly shape seeds.

Effect of season on the physiological quality of seedlings

The germination percentage of the seeds from fruits growing in the rainy season was higher than that in winter at all maturity stages (Table 1). The highest germination percentage and dried seed weight were at 50 DAP in the rainy season and at 60 DAP and the 45+ripening in winter, which indicated a delay in the physiological maturity of the seeds in winter. The low germination of the seeds in winter correlated with the high percentage of ungerminated seeds and abnormal seedlings at all maturity stages, especially in the seeds harvested at 45 DAP (Fig. 4E). Seed dormancy could be induced by temperature integration during seed maturation (Guterman, 2000). In this tomato F_1 hybrid line in the winter, a larger difference between day and night temperatures and also a lower temperature at night (low accumulated temperature) induced seed dormancy. This was also found in both *Arabidopsis* and tomatoes (Bewley and Black, 1994; Munir et al., 2001). A low temperature (15°C) increased the ABA content 2-fold during *Arabidopsis* seed development and resulted in higher seed dormancy compared to those grown at 22°C (Kendall et al., 2011). The vigor of seeds, expressed as GI, from fruits growing in the rainy season was higher than that in winter at all maturity stages (Table 1). This indicated that uniformity of seedlings in the rainy season could be obtained. Seed maturation under high temperatures showed higher seed germinability compared to those under low temperature, as observed in *Amaranthus retroflexus* and *Aegilops ovate* (Guterman, 2000). However, the more accumulated days of high temperature (32°C/28°C), the lower the germinability of soy bean and *Chenopodium album* seeds was observed (Karssen, 1970; Keigley and Mullen, 1986). More abnormal seedlings and ungerminated seeds (dormant seeds) were affected by the larger difference in temperature and RH between day and night and lower temperature in the night in winter, which in turn resulted in a lower GI for the F_1 hybrid seeds compared to the rainy season (Fig. 4C, D, G, H). Occurrence of maximum seedling quality in tomatoes was influenced by the growing environment in spring (about 35°C and 18°C) compared to autumn (28°C and 6°C) (Demir et al., 2008).

Effect of fruit maturity on the physiological quality of seedlings

The high germination capability of the 45+ripening seeds (Fig. 4E) had a positive relationship with the increases in mature seed number (Fig. 3E) and weight per

fruit (Fig. 3F). It is well-known that seeds as a sink accumulate food from the fruit (source) to fulfill physiological development during post-harvest ripening of berry fruits, including tomatoes (Dubreucq et al., 2010). This means that good seed quality of these parental lines can be achieved at 45 DAP in the mother plant with subsequent ripening at ambient temperature. The 45+ripening seeds showed statistically high GI values (Fig. 4F). This confirms that increases in seed vigor, germination, and seed weight can occur during post-harvest ripening of a green-mature stage tomato fruit (45 DAP in this cultivar) under ambient conditions. Therefore, harvesting tomatoes at 45 DAP and subsequently ripening at ambient temperature for about ten days resulted in seed quality that was similar to that harvested at 60 and 70 DAP. The percentage of abnormal seedlings in winter was relatively high and such seeds were classified as seeds of poor quality (Fig. 4G). Generally, the F_1 hybrid seeds of these parental lines (female line: 25%–45%) had high amounts of abnormal seedlings, especially those with seed coats that were less than 50% removed from the cotyledons. The high proportion of ungerminated seeds in the 45 and 50 DAP (Fig. 4H) was caused by under-development of the embryo. This was consistent with a low germination percentage (Fig. 4E), small number of mature seeds per fruit (Fig. 3E), and low weight of dried seeds per fruit (Fig. 3F).

Effect of storage at 0°C on the physiological quality of seedlings

The primary purpose of seed storage is to preserve storage economic crops from one season to another. In many kinds of seeds, short term storage at low temperature (below 0°C) did not significantly affect physiological changes or quality attributes. The percentage of germination and GI of F_1 hybrid tomato seeds after storage at 0°C for four months showed the same patterns as fresh seeds (non-stored seeds) (Fig. 4E, I). This indicated that germination of the tomato seeds harvested at 45+ripening was as high as that at 60 and 70 DAP. This confirms that high quality seeds could be obtained from fruits harvested at 45+ripening. The GI of stored seeds is consistent with the initial GI values (Fig. 4F, J). This indicates that storability of the seeds from 45+ripening and 60 DAP fruits would be the longest. Based on our results, it would be an advantage for farmers and seed companies to harvest tomato fruits earlier (fifteen days in this study) at the light green stage and then store them until ripening at ambient temperature ($28 \pm 2^\circ\text{C}$). Additionally, this would be a protection against insects, pests, and diseases hazard which can occur during fruit ripening on the mother plant. Storage for four months significantly reduced the number of ungerminated seeds. We found about 98%–100% viability of non-stored seeds (data not shown). This was apparently associated with an increase in the proportions of

deformed and imbalanced seedlings (abnormal seedlings) (Table 2), meaning that the ungerminated seeds were able to establish either deformed or imbalanced (radicle emergence and short hypocotyls) seedlings. In other words, the 45 and 50 DAP treatments of fresh seeds growing in winter had a high dormancy level (Fig. 4H, L). It can be concluded that these F_1 hybrid tomato seeds from early-harvested fruits without postharvest ripening or the ones which were immediately processed, were dormant, and would be released after four months of storage. In contrast, fresh and stored seeds of 45+ripening fruits had almost the same proportions of ungerminated seeds meaning that harvesting the seeds at 45+ripening reduced the proportion of ungerminated seeds or reduced the level of seed dormancy. Our results reveal that post-harvest fruit ripening of the early-harvested tomato fruits (45+ripening) improved seed qualities and reduced the degree of dormancy. Dias et al. (2006) reported that seeds from fruits harvested at an immature and completely red stage had a similar physiological quality to immature fruits ripened during short post-harvest storage. In the significantly reduced amount of ungerminated seeds in our study, this suggests that the dormancy decreased with longer storage. This response is well understood (Copeland and McDonald, 1999). Recent research showed that increasing the level of reactive oxygen species during seed storage contributes to a reduction in dormancy (El-Maarouf-Bouteau and Bailly, 2008; Graeber et al., 2012). However, these parental lines may not be suitable for the production of high quality seeds in the winter because of the significantly high proportions of undesirable seedlings, namely seed coats with less than 50% removed from the cotyledons.

Conclusions

Higher light intensity, a larger range of night and day, temperatures, and RH in winter delayed fruit and seed physiological maturity by ten days (60 DAP in winter). The physiological qualities of the seeds and seedlings were apparently influenced by climate during plant growth. The physical appearance of seeds, particularly speckled and irregularly shaped seeds, was induced by a rainy climate. The proportion of speckled seeds increased with the increase in seed maturity while the proportion of irregularly shaped seeds and weak seedlings was not influenced by maturity. The climate in the rainy season caused lower seed dormancy, resulting in an improvement in germination and seedling quality. The seeds harvested at 45 DAP and subsequently ripened at ambient temperature (45+ripening) displayed physiological qualities similar to that of fruits that ripened on the mother plant (60 DAP). Storing the seeds from the winter season at 0°C for four months improved germination or caused a decrease in seed dormancy by reducing the proportion of fresh ungerminated seeds in the immature fruits (45 and 50

DAP), as indicated by germination, GI, and increased percentage of abnormal seedlings in all treatments, except for 70 DAP.

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