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Effect of Seed Production Environment and Time of Harvest on Tomato (*Lycopersicon esculentum*) Seedling Growth*

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Abstract: This study was carried out to find out the effects of two growing seasons and harvest time on seedling growth in tomato. Seeds were harvested between 40, 45, 50, 55, 60, 70, 80 and 90 DAA (days after anthesis) in spring and 45, 50, 55, 60, 70, 80, 90, 100 and 115 DAA in autumn season from hybrid tomato plants grown under glasshouse. Maximnm seed weight occurred at 50 DAA in spring but went up to 80 DAA in autumn season. Maximum seedling emergence took place at 60 DAA in spring as 89% but 115 DAA as 90% in autumn. The highest seedling fresh and dry weights were also observed at the same harvests. As seeds matured the fewer seeds were germinated but unable to emerge in the compost. Advancement in seed maturation not only increased seedlings fresh and dry weight but also seedling uniformity ($R^2 = 0.75$, $R^2 = 0.69$) measured through coefficient of variation (CV). Fast emergence (lower mean time to emerge) resnlted in the higher seedling fresh weight ($R^2 = 0.80$, $R^2 = 0.84$). Occurrence of maximnm seedling quality in tomato was influenced by the growing environment. Low greenhouse temperatures in autumn delay occurrence of the maximum seedling growth up to 115 DAA. While, it can be obtained from seeds harvested at 60 DAA in spring.

Key words: *Lycopersicon esculentum*, tomato seedling growth, harvest time, growing season

INTRODUCTION

Physiological quality of seed lot is the resnlt of pre-storage factors, acquisition of the ability to produce vigorous seeds and post-storage factors (Powell *et al*., 1984). Mother plant environment affects seed quality through climate and growth conditions (Delouche, 1980).

Hybrid summer crop seeds are produced in two seasons, i.e., spring and autumn under glasshouse or plastic tunnels in the Mediterranean basin (Passam and Khah, 1992). Two serial seed production seasons provides more profitable use of glasshouse field and produce more seeds within the same year. The growing temperature in spring gradually increases during the seed filling period (April-June) towards summer, contrarily, in autumn; it declines as seed maturation progresses on the plant (October-December) towards winter. It has been reported in various crop seeds that temperature during the critical growth stage, i.e., the seed filling period, has affected the seed yield and quality (Kameswara Rao and Jackson, 1996; Spears *et al*., 1997; Craufurd *et al*., 2002; Thomas *et al*., 2003; Greven *et al*., 2004; Demir *et al*., 2004). When produced under warmer conditions, storage longevity of rice (Ellis *et al*., 1993) and watermelon (Demir *et al*., 2004) seeds were shown to be less than that from cooler environments. Warm climate also reduced the seed dry mass in both species. Similarly, soyabean and bean seeds produced at high temperatures during seed filling were smaller, wrinkled and poor quality (Siddique and Goodwin, 1980; Spears *et al*., 1997). Occurrence of low quality was more prominent particularly when high temperature was combined with high relative humidity which is called field weathering (TeKrony *et al*., 1980).

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Although not an environmental factor, harvest time is known to be a major factor responsible for physiological maturation level, size and vigour of seed during maturation (Delouche, 1980). The decision of when to harvest particularly under varying environmental conditions is therefore of importance to get maximnm seed quality. Various studies examining the influence of seed development on seed quality in tomato have shown that seeds extracted from fruits harvested 70-75 days after anthesis (Berry and Bewley, 1991; Demir and Ellis, 1992; Liu *et al*., 1996) or when fruits are firm red (Valdes and Gray, 1998; Demir and Samit, 2001; Ramirez-Rosales *et al*., 2004) had the maximum viability and vigour. Although growing environment was not considered in these studies, environment and seed maturation may co-interact and time of occurrence of maximnm quality may change. Moreover, changes in transplant quality, in relation to enviroument during seed development on the plant are valuable information for horticultural technology. Protected tomato cultivation by and large was done by using high value hybrid seeds. Therefore, high emergence percentage, uniform and developed transplant production has utmost importance (Cantliffe, 1994).

The objective of this study was then to evaluate effects of temperature during the seed filling period, on capacity of seedling growth in serially harvested hybrid tomato seeds in two consecutive growing seasons (spring and autumn) under glasshouse.

MATERIALS AND METHODS

Plant Husbandry and Seed Harvest

Plants were grown between February and July 2005 in spring and between August 2005 and February 2006 in autumn season. Seeds of male and female parent lines of hybrid tomato cultivar (*Lycopersicon esculentum* Mill.), Safir (Anamas Seed Company/Antalya/Turkey) were sown in seedling trays on 15 February and 21 August 2005 in spring and autumn season, respectively. Seedlings were transplanted to the glasshouse on 13 March and 12 September. Male and female lines were planted in the same glasshouse with spacing of 80 cm between and 40 cm within rows. Ratio of male to female plants was arranged as 1:4 and male parent plants were isolated by using insect-proof plastic nets. Ammonium sulphate (15 kg/1000 m^2) and potassinm nitrate (15 kg/1000 m^2) were applied at transplanting and again at the seed filling phase (15 days after anthesis) via watering. Drip irrigation was applied and approximately 4 L of water was given every day in the warm period (May-July, August-September) and once in two days in the cool period (March-April, October-December). Plants started to flower on 12 April in spring and on 8 October, in autumn season. Maximum and minimum temperatures were measured daily and shown in Fig. 1 for each growing season. Pollens were collected from fully open flowers of male parent and transferred by hand pollination to stigmas of those flowers in which petals just turned to yellowish green in the first three trusses of the female parent. Pollinated flowers were isolated with transparent paper bags to prevent foreign pollens until fruit set. At each growing season at least 300 fruits were tagged out of simultaneously pollinated 550-600 flowers. Fruit harvests were arranged according to tagging day (fruit set) and were 40, 45, 50, 55, 60, 70, 80 and 90 DAA (days after anthesis) in spring; 45, 50, 55, 60, 65, 70, 80, 90, 100 and 115 DAA in autumn season. Seeds were removed from the harvest fruit by hand. Seeds were fermented at 25°C for 24 h by adding the same amount of water to the slurry (1:1 v/v), then they were washed in tap water and dried on mesh trays in the dark for two days at 25°C at which time the seed moisture content was <10%. Seed moisture content was determined by high temperature oven method (130°C, 1 h) (ISTA, 1996) after drying. Changes in fruit colour by the harvest time were determined in both seasons. Seed dry weight determination was carried out by drying four replicates of 20 seeds per harvest at 130°C for 1 h and weighing after cooling in a desiccator with silica gel. Mean dry mass of individual seeds was then determined.

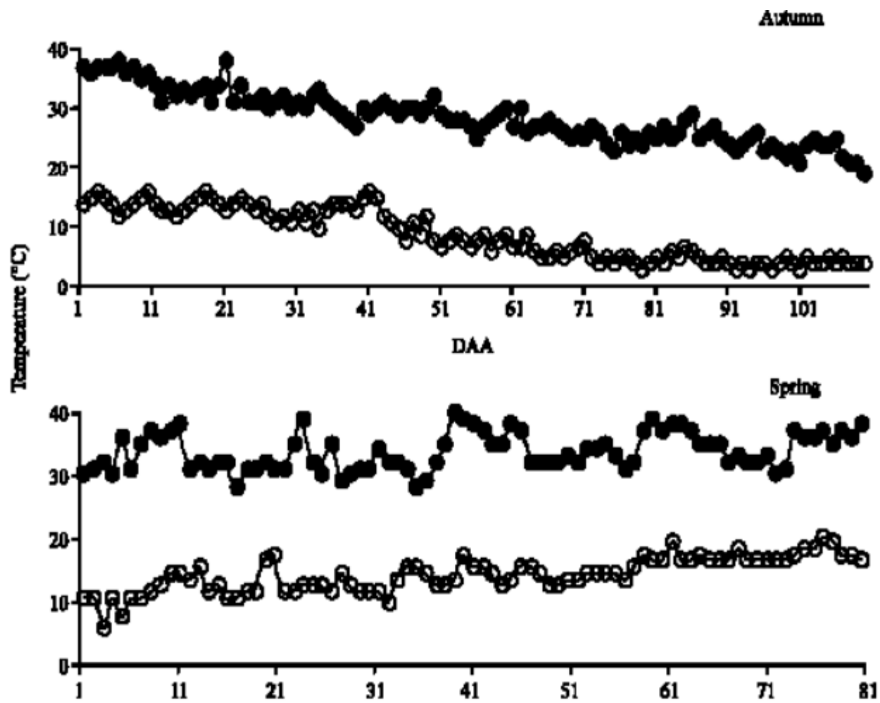

Fig. 1: Daily maximum (●) and minimum (○) temperatures recorded during seed maturation in spring and autumn growing season

Seedling Growth Test

The seedling growth test was conducted in a glasshouse at Amanas Seeds Company, Antalya/Turkey.

Seeds during that period were stored at 5°C and 8-9% moisture content in air tight glass jars in the dark. Seeds, four replications of 100 seeds/harvest, were sown 2 cm deep in compost (Plantaflor-Humus Verkaufs GmBH, Germany) in seedling modules. Seed sowings were done on 18 July 2005 and 12 February 2006 in spring and autumn seasons. Seedlings were grown in a temperature controlled greenhouse for 25 days. Daily temperature in the greenhouse changed between 18 and 23°C throughout the experimental period. Regular irrigation was carried out during the emergence test. Seedlings 25 days after sowing were counted and cleaned and above ground seedling fresh and dry weights were determined. Seedling dry weight was determined by drying at 80°C for 24 h and both values were expressed as mg plant^{-1}. Following, seedlings were removed from seedling modules, compost was searched and germinated but non-emerged seed percentages were determined in each harvest.

Using the daily counts, the mean emergence (MET) was calculated for each lot using the formula cited by Ellis and Roberts (1980):

$$MET = \sum nt/\sum n$$

Where:

n = No. of seeds newly germinated at time t

t = Days from sowing

$\sum n$ = Final emergence

Means of seed weight and fresh and dry seedling weight in each harvest were compared by Duncan's multiple range tests by using the SPSS package program at the 5% level. Angular transformation for percentages was carried out before analyses. Regression analyses were conducted between seedling fresh weight and mean emergence time and its coefficient of variation (uniformity) of seedling fresh weight and harvest times in two different seasons.

RESULTS

Seed dry weight gradually increased from 3.1 mg at 40 DAA to 4.2 mg at 50 DAA in spring and from 1.6 mg at 45 DAA to 4.2 mg at 80 DAA in autumn season and thereafter seed weight remained stable. Maximum seed weight was attained at 50 DAA in spring and at 80 DAA in autumn when fruit colour is pink. Fruit colour changed from green to soft red during maturation (Fig. 2). Seeds harvested between 45 and 70 DAA in spring season were significantly ($p<0.05$) heavier than those of autumn.

Maximum seedling emergence in modules was attained at 60 DAA and 90 DAA, being 89% and 91% in spring and autumn season respectively (Fig. 3). This value was significantly higher than all other harvests in spring ($p<0.05$) but was significantly higher than earlier but not later ($p>0.05$) harvests in autumn season. Although it is too low, seeds harvested 45 DAA and onwards started to emerge in spring but no emergence was observed until 70 DAA in autumn season.

Maximum seedling fresh and dry weight occurred at 60 DAA as 844 mg and 68.3 mg plant^{-1} in spring season. Both criteria in this season showed gradual increase by the maturity. Having reached the maximum level, seedling fresh weight remained the same until penultimate harvest but seedling dry weight continuously decreased (Table 1). In autumn season, both maximum seedling fresh and dry weights were obtained from seeds harvested at 115 DAA as 739 mg plant^{-1} and 54.5 mg plant^{-1}, respectively. Both increased gradually by the maturity starting with 80 DAA. As seeds matured the percentage of seeds germinated but unable to emerge in the compost was reduced; this was in the lowest at 60-70 DAA in spring and 100-110 DAA in autumn season as 2-3%. It was much higher in less mature lots, 21%, at 45 DAA and 70 DAA, respectively (Table 1).

Maximum hypocotyl lengths were observed at 60 DAA in spring and 115 DAA in autumn, as 33.1 mm and 32.6 mm, respectively. These harvests produced significantly ($p<0.05$) longer hypocotyls than those of other harvests in both growing seasons (Table 1).

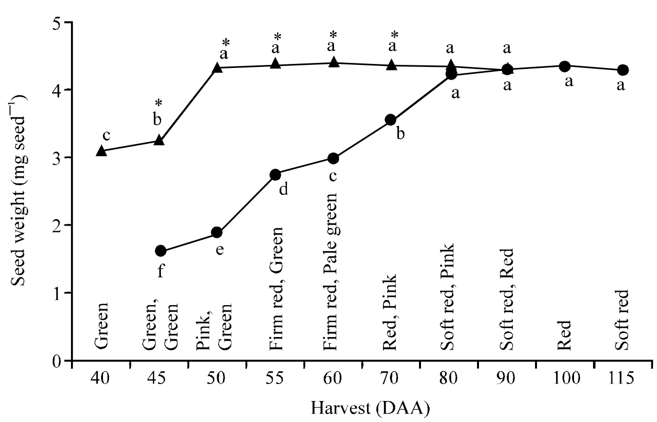

Fig. 2: Changes in seed weight and tomato (*Lycopersicon esculentum*) fruit colour (upper row for autumn, lower row for spring season) during seed development in spring (▲) and autumn (●) season. Means with the same letters within the same season are not significantly different ($p = 0.05$). Means with asterisks are significantly different ($p = 0.05$) harvests between two seasons

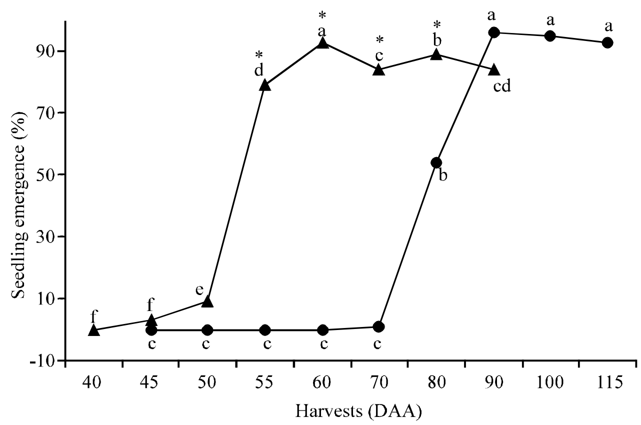

Fig. 3: Seedling emergence in relation to time of harvest and seed development in spring (▲) and autumn (●) grown tomato (*Lycopersicon esculentum*) seeds, letters are to compare harvests in the same season, asterisks are the time of harvests in different seasons. Means with different letters and asterisk are significantly (p = 0.05) different

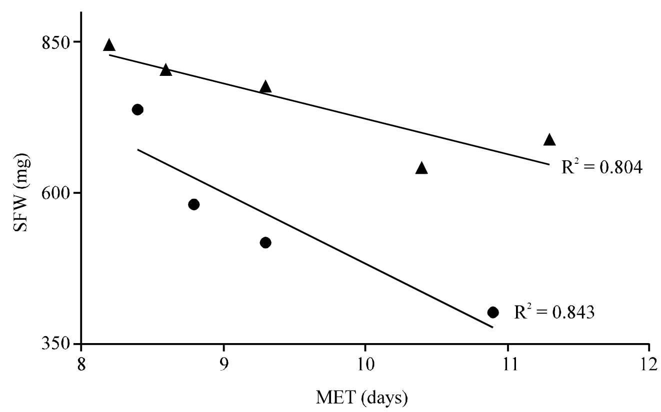

Fig. 4: Relationship between mean seedling emergence time (day) of spring (▲) and autumn (●) grown tomato (*Lycopersicon esculentum*) seeds and seedling fresh weight (mg plant^{-1})

Table 1: Changes in seedling fresh weight, dry weight, hypocotyls length and germinated but unable to emerged seed percentages in spring and autumn grown and serially harvested tomato (*Lycopersicon esculentum*) seeds. The means with different letters in the same column are significantly different (p = 0.05)

	Spring				Autumn			
Harvests (DAA)	SFW (mg)	SDW (mg)	HL (mm)	GBU (%)	SFW (mg)	SDW (mg)	HL (mm)	GBU (%)
45+	124^{e}	25.0^{e}	28.3^{e}	21	0^{d}	0^{d}	0	0
50	333^{d}	34.8^{d}	29.1^{d}	16	0^{d}	0^{d}	0	0
55	641^{c}	54.3^{c}	31.2^{b}	7	0^{d}	0^{d}	0	0
60	844^{a}	68.3^{a}	33.1^{a}	3	0^{d}	0^{d}	0	18
70	775^{ab}	61.3^{b}	31.3^{b}	3	0^{d}	0^{d}	0	21
80	803^{a}	60.5^{b}	30.7^{c}	7	403^{c}	36.5^{c}	28.1^{d}	11
90	688^{bc}	53.0^{c}	30.1^{c}	8	518^{b}	44.5^{b}	30.8^{c}	6
100	*	*	*	*	582^{b}	46.3^{b}	31.1^{b}	3
115	*	*	*	*	739^{a}	54.5^{a}	32.6^{a}	2

*: No harvest was done, DAA: Days after anthesis, SFW: Seedling fresh weight, SDW: Seedling dry weight, GBU: Germinated but unable to emerge, HL: Hypocotyl length, +: 40 DAA in spring was not shown since no seedling emerged

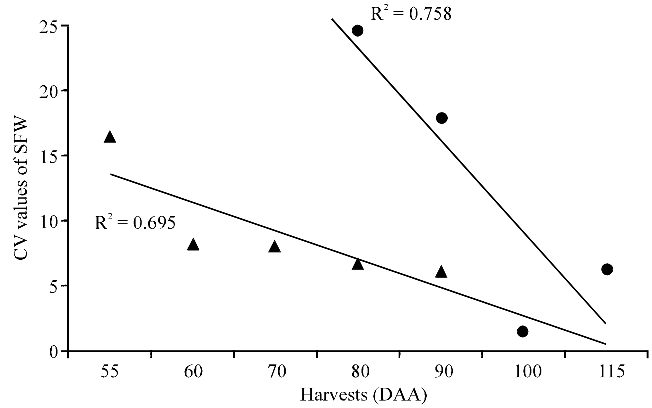

Fig. 5: Relationship between time of harvest and coefficient of variation (CV) of seedling fresh weight in spring ( ) and autumn (●) grown tomato (*Lycopersicon esculentum*) seeds

The mean emergence time and above ground seedling fresh weight is highly significant ($p<0.05$) in seed lots of both growing seasons; the slower the emergence, the lower the mean seedling fresh weight (Fig. 4).

As seed maturity progressed, seed lots showed less variation in seedling size as indicated by the significant positive regression of coefficient values between CV of seedling size and harvests in both growing seasons (Fig. 5). However, seeds harvested during spring season had much lower CV values than those of autumn, which indicates that they produce more uniform seedlings. Although spring grown seeds emerged faster and produced more uniform seedlings than those of autumn, the lower CV value was related to earlier emergence in seed lots of both seasons.

DISCUSSION

Findings of this study showed that temperature during seed development affected seed dry matter accumulation, seedling emergence and growth of hybrid tomato seeds. Seeds grown in the spring (March- July) reached to maximum dry mass earlier than those grown in autumn (September-January). Maximum seed quality in relation to seedling growth was obtained from seeds harvested at 60 DAA in spring and 115 DAA in autumn season.

Following fertilization of the ovule, development of seed until harvest involves embryo growth (Phase I, increase in size, weight and number of cells) and accumulation of reserves (Phase II, i.e., proteins, oils, starch) seeds do not generally have vascular connection with phloem, so that substances (i.e., proteins, fats, starch) should move apoplastically unloading from phloem (Naylor, 2003). When weather conditions, were lower than optimum, specifically low temperature in our case, the rate of biomass accumulation impaired and reduced seed weight. In autumn season due to the cool and low temperature period and the lower rate of photosynthesis, tomato seeds took 30 days longer to get the maximum dry weight compared to those of spring season, although they gained equal weight finally (Fig. 2).

Temperature during seed filling may affect seed weight and vigour in various crops (Tashiro and Wardlaw, 1990; Jansen, 1995). Egli *et al.* (1985) reported that reductions in seed weight result primarily from a reduced seed fill duration rather than any reduction in seed growth rate. Results of our work confirmed this conclusion that the time of the seed filling period is completed by 50 DAA in spring season, while it extends to 80 DAA in autumn season. Changes in temperatures not ouly slow down the accumulation of dry mass but also reduce total seed quality measured by storage longevity in rice (Ellis *et al*., 1993); watermelon (Demir *et al*., 2004), seed harvest index in peanut (Craufurd *et al*., 2002) and in normal seedlings in soyabean (Spears *et al*., 1997). The basic reason of slow seed filling in protected cultivation in the autumn growing season is that plants are subjected to gradually reducing cooler environment as maturation progress. The minimum temperature in autumn is lower than 10°C at 50 DAA and afterwards. However, temperature reduced such level in only a few cases in spring (Fig. 1). Hybrid tomato seed production was done under cover due to the easy application of isolation methods and production control. Results indicated that autumn growing conditions seed maturation extends over 115 DAA which is almost doubled compared to that of spring (Table 1, Fig. 2).

Tomato fruit colour is evaluated as an improved measure of seed quality development (Valdes and Gray, 1998; Demir and Samit, 2001; Ramirez-Rosales *et al*., 2004) along with days after anthesis (Berry and Bewley, 1991; Demir and Ellis, 1992; Liu *et al*., 1996). In most of the studies seeds extracted from red tomato fruits possessed maximum quality measured in various tests, compared to those extracted from earlier stages of fruit maturity. More recently, Ramirez-Rosales *et al*. (2004) found in high lycopene tomato cultivar that seed quality was higher at the mature green breaker and pink breaker compared to red mature and overripe stages. In present research, seeds produced highest emergence, seedling fresh and dry weights obtained from firm and red fruit colour in spring while soft and red stage in autumn (Fig. 2, 3, Table 1). This shows that fruit development is influenced by environmental conditions and the identification of specific physiological stages of seed development based on fruit colour may not be universal in all growing and environmental conditions. Agreeing with that conclusion, seeds within the late soft-red stage started to deteriorate, shown by germination after a saturated salt solution accelerated aging in high lycopene (Ramirez-Rosales *et al*., 2004) and by mean germination time, normal seedling percentage and storage longevity in some other cultivars (Valdes and Gray, 1998; Demir and Samit, 2001). Contrarily, seed quality remained at maximum level in the late seed development period in soft red fruits i.e., 90 DAA; it was postulated that this was due to occurrence of the repair mechanism under very high seed moisture within the fruit (Demir and Ellis, 1992). It can be postulated that this may depend on co-interaction of cultivar and environmental conditions, which was confirmed by our results. Seedling fresh and dry weight, hypocotyl length and mean emergence time declined gradually having reached to a maximum level at 60 DAA in spring season, whereas in autumn all seed quality criteria continuously increased until 115 DAA (Table 1). One cause of reduction in seedling growth can be the supra-optimal temperatures in the glasshouse in late seed maturation stage (June-July, daily maximum temperatures varies between 32 and 39°C) in spring season (Fig. 1). This is in agreement with the conclusions of various studies (Tashiro and Wardlaw, 1990; Ellis *et al*., 1993; Jansen, 1995; Spears *et al*., 1997).

Maximum seed weight (mass maturity) occurred at 50 and 80 DAA in spring and autumn season growing, respectively. However, maximum seed quality improved after mass maturity. This changed between 10 and 35 days according to the quality test (Fig. 2, 3, Table 1). Thus, the first part of the hypothesis of Harrington (1972) that seed quality is maximal at the end of the seed filling phase (i.e., at mass maturity) can be rejected on the basis of the current results. This agrees with results of a number of different studies in tomato (Kwon and Bradford, 1987, Demir and Ellis, 1992, Berry and Bewley, 1991; Demir and Samit, 2001). However, the second part of the hypothesis, that seed quality declines thereafter, is supported by the results of spring season but not those of autumn (Table 1). That conclusion indicates that total accuracy of both parts of the mentioned hypothesis may depend on environmental conditions during the seed-filling phase as well as the cultivar. Results based on hybrid cultivar in the present work unlike open-pollinated ones in previous studies.

Recently, gradually increasing trend in the vegetable industry is to grow transplants in modules. In tomato, along with other crops, transplants have been used for various aims: To improve stands; to reduce seed usage; especially of high value hybrids; to shorten the time from planting to harvest and to get better seedlings for grafting (Cantliffe, 1994). Earliness and uniformity are important characteristics in transplant production. Use of well-developed and strong transplants is the prerequisite of uniform and fast plant growth in glasshouse crop production. Results of the present study indicate the ability of tomato seeds to produce a good transplant is based on the environment that it was grown. Longer mean emergence time of autumn season grown seeds was closely related to lower seedling fresh weight, while faster emerged spring grown seeds had larger seedlings (Fig. 4). More mature seeds produced larger seedlings in both growing seasons. This relationship between emergence time or germination time and seedling size was also reported by Ellis and Roberts (1980) in barley, wheat, onion and cabbage; by Gray (1984) in carrot; by Demir and Ellis (1993) in marrow and Matthews and Khajeh Hosseini (2006) in maize. Spring growing not only reduced mean emergence time but also increased uniformity in seedling size. CV values of seedling fresh weight were well correlated with harvest time. Figure 5 shows that spring growing and maturity assures better seedling production and uniformity.

Halmer and Bewley (1984) expressed the view that crop emergence losses are overwhelmingly due to a failure of seedlings to grow under the soil surface rather than to germination failure. Our resnlts indicated that the failure of emergence is related to seed maturation level. As seeds matured fewer seeds which germinated in the compost failed to emerge to the surface, showing that maturation increases the seedling vigour of the tomato seeds. The number of germinated but unable to emerged seed percentages were inversely related to hypocotyl length. Those lots that had longer hypocotyls also had lower percentages of germinated but non-emerged seed (Table 1). This conclusion was in agreement with previous findings that maturation enhances seed and seedling vigour of tomato in optimum and adverse conditions (Kwon and Bradford, 1987; Valdes and Gray, 1998; Demir and Samit, 2001).

In conclusion, autumn season growing delays the maximum maturation stage of tomato seeds in turn seedling emergence and size in tomato seeds up to 115 DAA. However, in spring maximnm seedling growth and quality can be obtained from seeds harvested at 60 DAA. This showed that the time of occurrence of maximum seed quality, affecting seedling growth in this work, may depend on the growing enviroument.

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