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Assessment of Vigor Characteristics of Processing Tomato Cultivars by Using Various Vigor Tests

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Abstract: Experiments were conducted in order to assess the effectiveness of accelerated ageing, controlled deterioration, electrical conductivity and tetrazolium tests to determine the vigor differences among the seed lots of processing tomato cultivars. For this purpose, C-37 open pollinated and Shasta and Brigade hybrid cultivars were chosen as experimental materials. It was found that accelerated ageing and controlled deterioration tests gave the most satisfactory results in determining the vigor differences among the seed lots. Although the results obtained from electrical conductivity and tetrazolium tests were in accordance with the results of other tests employed in this research, it is necessary to increase the number of replications and number of seeds used in each replication in order to obtain satisfactory results.

Key words: Seed vigor, vigor test, accelerated ageing, controlled deterioration, electrical conductivity

INTRODUCTION

The importance of seed quality in realizing the full potential of a cultivar is well known. Seed quality includes seed vigor and viability, which are two interrelated characters. The standard germination test, which is considered to be the universal test for seed quality, evaluates the maximum potential of a particular seed lot under favorable conditions[1]. Since the standard germination test is conducted under ideal conditions, it does not necessarily reflect the potential performance of that seed lot under a wide range of field conditions. High standard germination does not always result in rapid and uniform emergence or vigorous stand under actual planting conditions[2]. On the other hand, seed vigor denotes the ability of a seed to germinate rapidly and produce a normal seedling under a wide range of conditions[3]. AOSA[4] defines seed vigor as properties that determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions. Perry[5] suggested that a vigor test would provide a better indication of seed performance in the field than the standard germination test.

High seed vigor can improve the rate and uniformity of germination and rate of early seedling growth, especially as it translates to plant growth under less than optimum conditions[6]. Therefore, high-vigor seeds should be used in all instances to ensure good stand establishment under field conditions. At times, it is necessary to store seeds for a period of several months to a year or more. However, no storage procedure guarantees that seeds will remain viable forever. Seeds eventually lose vigor and then viability with time[7]. Storage leads to considerable loss in viability and vigor of the seeds, which in turn can affect yield of a crop by decreasing germination percentage resulting in a sub-optimal population of plants per unit area and lack of vigor in the surviving plants[8].

There is not one universal vigor test for all seeds. A wide array of testing methods has been used to characterize seed vigor. According to Ellis[9] seed vigor can be assessed in laboratory and various procedures can be used to detect high and low vigor seed lots. Several examples of seed vigor tests include the cold test[10] accelerated ageing test electrical conductivity test controlled deterioration test seedling vigor classification test[11] and tetrazolium test[12].

Tomato is one of the most important vegetable crops in Turkey. Production of processing tomatoes exceeded 1.6 million tons in 2002, constituting approximately 20-25% of the total tomato production[13]. Tomato seeds have a high commercial value and the availability of satisfactory vigor tests for tomato seeds is desirable. It is important to determine vigor characteristics of open-pollinated and hybrid tomato cultivars used in processing tomato production in order to make decisions regarding which

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seeds lots to market or to buy. The results of vigor tests can also be used to decide whether to keep a particular seed lot in storage for a longer period of time. Since seed vigor is a concept describing several characteristics associated with seed performance and not a single measurable character, there is not an adopted standard procedure to measure vigor, but rather a number of options. Therefore, the objective of this study was to compare the performance of various seed vigor tests in determining the vigor differences among the seed lots of processing tomato cultivars.

MATERIALS AND METHODS

This study was carried out at Department of Horticulture, Aegean University, Izmir, Turkey. Seeds of Shasta and Brigade F_1 and open-pollinated C-37 processing tomato (*Lycopersicon esculentum* Mill.) cultivars stored for various durations were used as experimental materials. Two seed lots of Shasta and four seed lots of Brigade and C-37 were subjected to the vigor tests (Table 1). Accelerated ageing, controlled deterioration, electrical conductivity and tetrazolium test methods were conducted to determine the vigor differences among the seed lots of processing tomato cultivars.

Accelerated Ageing test (AA): It is a vigor test, where seeds are stressed with high temperature and relative humidity for a period of time prior to the germination test. High quality seeds tolerate such stress conditions and retain their capabilities to produce normal seedlings. This test was conducted by ageing seeds using the method suggested by Ramamoorthy[14]. The tomato seeds (10 g from each seed lot) in cheesse-cloth bags were placed on a wire mesh tray in a desiccator containing 500 ml of distilled water at the bottom. The seeds were aged by closing the desiccator and placing them in an ageing chamber at 40±0.5°C for 10 days. Upon completion of the ageing period, the seeds were removed from the desiccator and taken to germination and emergence tests. Germination tests were carried out in darkness in a temperature-controlled incubator held at 23±0.5°C[15]. Seeds were placed on two layers of filter paper (Whatman No.1) moistened with deionized water in covered 10-cm petri dishes. Each dish contained 50 seeds, with ten replicate dishes per treatment. Radicle protrusion to 1 mm was scored as germinated and germinated seeds were removed from the petri dishes. Aged seeds were also sown in plastic pots (8x10 cm) filled with peat-lite and emergence test was conducted at 13±0.5°C. Seed germination and emergence were recorded at 24 h intervals until the numbers stabilized (10 days for germination and 21 days for emergence). Final germination percentage (FGP), final emergence percentage (FEP) and, emergence rate as expressed by days to 50% of FEP (E_{50}) values were calculated for each seed lot and the results were subjected to analysis of variance (ANOVA).

Controlled Deterioration test (CD): The controlled deterioration test is very similar in principal to the accelerated ageing test except the seeds are equilibrated to certain level of moisture and are held at this moisture level constantly during the deterioration period. Controlled deterioration test was conducted according to Bradford and Argerich[16]. After determining their initial water contents by oven method, water contents of seeds were raised to 13.5% by adding necessary amount of water. The seeds were placed in sealed cryogenic bottles and incubated at 4°C for 24 h for every seed to reach equal water content. After incubation period seeds were placed in a water-bath at 50°C for 6 days. Following incubation, the seeds were subjected to germination and emergence tests and statistical analysis as described above.

Electrical Conductivity test (EC): Another experiment was designed to determine the electric conductivity of seed leakage following the method suggested by Matthews and Powell[17]. This test measures the integrity of the cell membrane. Low vigor seeds have leaky membrane structure and release electrolytes into the soak-water. The level of electrolytes is reflected in high electric conductivity of the soak-water. In this experiment, untreated seeds and seeds subjected to accelerated ageing and controlled deterioration tests were used as experimental units. Ten replicates of 50 seeds were weighed and incubated at 20°C for 6 h in 200 mL flasks containing 50 mL of deionized water. Following incubation, soak water was separated from the seeds using cheesecloth and its electric conductivity was measured with a digital conductive meter (Model: WPA CDM 400).

The EC values measured were expressed as μsiemens/cm ($\mu s\ cm^{-1}$) after using the following formula for each replication of a seed lot:

EC = (Conductivity in soak water-conductivity in deionized water)/ weight of 50 seeds

Tetrazolium test (TZ): The most widely known biochemical test is tetrazolium staining. This test is used to evaluate seed vigor in numerous agricultural seeds[4,12]. The intensity of the staining reaction is used as a basis of seed vigor. In this test conducted according to Grabe[18]

Table 1: Tomato cultivars and seed lots used in the study

Cultivar	Seed lot	Storage duration (years)	Company
Brigade F_1	1	1	Asgrow
Brigade F_1	2	1	Asgrow
Brigade F_1	3	3	Asgrow
Brigade F_1	4	3	Asgrow
Shasta F_1	1	1	Champbell
Shasta F_1	2	3	Champbell
C-37	1	3	Akfa
C-37	2	3	Akfa
C-37	3	4	Akfa
C-37	4	4	Akfa

0.1% tetrazolium chloride solution was prepared and its pH was adjusted to 7.0. Tomato seeds from each seed lot were incubated for 24 h in water at room temperature to remove seed coats easily. The embryos were taken out by removing the seed coats under binocular microscope. The embryos (10 embryos in 10 replicates from each seed lot) were incubated for 5 h in tetrazolium chloride solution at 35°C. Following incubation, the embryos were rinsed with deionized water. The TZ test was also conducted for the seeds obtained from the AA and CD tests. The data obtained from the TZ test were subjected to ANOVA.

The scale developed by Perry[19] to interpret the result of TZ tests is given below. The embryos were grouped in eight categories according to their staining intensities and locations. Seeds belonging to the categories 1-3 were considered as high vigor seeds while seeds belonging to the categories 4-8 were considered as low vigor seeds:

- Embryo stained completely.
- Minor unstained areas on cotyledons.
- Except the radicle tip entire embryo stained.
- More than one third of cotyledons unstained.
- More than tip of radicle unstained.
- Unstained area on cotyledons extends into region where radicle and cotyledons are attached.
- Majority of embryo unstained.
- Embryo completely unstained.

RESULTS AND DISCUSSION

According to Pollock and Ross[20] vigor tests are conducted to reveal differences in the field performance of seeds that have the same genetic origin. Vigor tests are performed to find out vigor differences among the seed lots of the same cultivar that may have resulted from environmental, storage and growing conditions. Therefore, in this study, the results of different vigor tests conducted on each tomato cultivar were compared separately for each cultivar tested.

Shasta F_1 cultivar: The germination percentages of different seed lots of this cultivar were not significantly different. The germination percentages of control seeds of seed lot 1 and seed lot 2 were 96 and 95%, respectively (Table 2). The results of TZ test, emergence percentage and emergence rate of seedlings showed that there were no significant differences between two seed lots of this cultivar indicating that both seed lots had high vigor. However, EC test results demonstrated that the seed lot 1 had higher electrical conductivity than seed lot 2. Low conductivity indicates low electrolyte leakage from the seed and thus high vigor where as high conductivity accordingly indicates low vigor[21]. According to this principle, we can conclude that seed lot 2 higher vigor than the seed lot. This result conflicts the germination, emergence and TZ test results and may have resulted from experimental errors as indicated by Matthews and Powell[17].

With respect to AA and CD tests, both seed lots had similar germination and emergence percentages and emergence rates indicating that they had same vigor levels.

Brigade F_1 cultivar: The results of germination tests conducted on control seeds of different lots showed that FGP of all seed lots were similar except that seed lot 2 had slightly lower FGP than the others (Table 3). Therefore, it is incorrect to say that all seed lots have similar vigor levels. However, when the FEP of all seed lots at 13°C considered, it became apparent that seed lot 4 had higher FEP followed by seed lot 3 and seed lots 1 and 2. Similar results were obtained from the EC test in which seed lot 4 had the highest vigor with the lowest EC reading while seed lot 1 had the lowest vigor level with the highest EC level according to the principle developed by Agrawal[21]. These results confirm those of Tomer and Maqurie[22] who reported that sdandard germination tests were ineffective in determining the vigor differences among the seed lots and that their results did not correlate well with field performance under stressfull conditions.

The results of germination and emergence tests conducted on the seed lots aged by AA and CD tests also showed that there were significant vigor differences among the four seed lots of this cultivar even though standard germination test conducted on control seeds showed that no significant differences occurred within seed lots of the cultivar. For example, AA test detected the seed lot 4 as the most vigorous seed lot with highest of FGP of 92% and FEP of 52% and the seed lot 1 as the least vigorous seed lot with an FGP of 28% and FEP off 6% (Table 3). These finding are in agreement with those of Karuna and Aswathaiah[23] and Basra *et al.*[24] who reported that AA test was a useful method to detect the vigor defferences among the seed lots of carrot and sugarbeat and cotton, respectively.

Table 2: Final Germination Percentage (FGP), Final Emergence Percentage (FEP) and Emergence rate (E_{50}) of seeds of two lots of Shasta F_1 tomato cultivar subjected to various vigor tests

	Seed lots	FGP (23°C)	FEP (13°C)	E_{50} (days)[z]	EC (µs/cm)	TZ (scale 1-8)[x]
Control	1	96.0	78.0	17.0	700.6a[y]	2.1
	2	95.0	79.0	16.5	670.3b	2.0
	$LSD_{0.05}$	-	-	-	4.9	-
AA test	1	81.0	14.0	22.4	742.2a	5.3
	2	82.0	14.0	20.3	687.8b	5.0
	$LSD_{0.05}$	-	-	-	9.8	-
CD test	1	85.0	21.0	24.5	733.1a	4.2
	2	87.5	28.0	22.2	709.7b	3.3
	$LSD_{0.05}$	-	-	-	14.8	-

Table 3: Final Germination Percentage (FGP), Final Emergence Percentage (FEP) and Emergence rate (E_{50}) of seeds of four lots of Brigade F_1 tomato cultivar subjected to various vigor tests

	Seed lots	FGP (23°C)	FEP (13°C)	E_{50} (days)[z]	EC (µs/cm)	TZ (scale 1-8)[x]
Control	1	98.5a[y]	49.0c	19.1b	772.6a	4.2
	2	94.5b	51.0c	18.7b	748.4b	4.4
	3	99.0a	62.0b	16.9a	702.1c	3.2
	4	98.5a	77.0a	16.0a	644.5d	2.4
	$LSD_{0.05}$	2.1	8.7	1.6	6.3	-
AA test	1	28.0d	6.0b	24.9c	896.8a	6.0
	2	59.0c	6.5b	24.8c	853.8b	6.1
	3	66.0b	11.5b	23.4b	798.7c	5.2
	4	92.0a	52.0a	21.1a	673.9d	3.4
	$LSD_{0.05}$	4.9	8.7	1.4	17.0	-
CD test	1	43.5c	11.0c	23.5c	824.3a	5.6
	2	75.0b	13.0bc	23.8c	774.9b	5.3
	3	94.5a	20.0 b	22.2b	739.7c	5.3
	4	96.5a	43.0a	20.9a	698.1d	5.0
	$LSD_{0.05}$	4.8	8.2	1.5	7.8	-

Table 4: Final Germination Percentage (FGP), Final Emergence Percentage (FEP) and Emergence rate (E_{50}) of seeds of four lots of C-37 tomato cultivar subjected to various vigor tests

	Seed lots	FGP (23°C)	FEP (13°C)	E_{50} (days)[z]	EC (µs/cm)	TZ (scale 1-8)[x]
Control	1	86.0	26.1c[y]	20.5b	839.6a	5.0
	2	89.0	38.0b	20.7b	746.8b	4.7
	3	89.5	43.0ab	20.2ab	719.8c	4.6
	4	92.0	46.0a	19.2a	697.1d	3.9
	$LSD_{0.05}$	-	6.1	1.3	14.9	-
AA test	1	50.0b	4.0b	24.6b	930.2a	6.4
	2	48.5b	4.0b	24.3b	833.1b	6.1
	3	58.5a	12.5a	22.2a	818.9b	5.7
	4	59.0a	15.0a	22.5a	773.6c	5.5
	$LSD_{0.05}$	3.5	4.4	1.7	23.3	-
CD test	1	51.0c	4.0c	25.1 b	852.0a	6.1
	2	62.0b	5.0c	24.7 b	781.1b	6.4
	3	68.5a	8.0b	24.5 b	763.3c	5.7
	4	70.0a	18.0a	22.6 a	749.9c	5.4
	$LSD_{0.05}$	3.5	2.4	1.5	16.3	-

[z]Days to 50% of final emergence percentage [y]Mean comparison within each column (Fisher's LSD test at p=0.05) [x]1= lowest vigor and 8=highest vigor

Moreover, these findings were also confirmed by the results of CD test in which seed lot 4 had the highest FEP (43%) and the fastest emergence rate (E_{50}=20.9 days) while the seed lots 1 and 2 had the lowest FEP and E_{50} values. The accuracy of the test confirmed previous results by Larsen *et al.*[25] who reported that germination tests even after CD test was ineffective in detecting the vigor differences among the seed lots and that a stress factor such as low temperature emergence test should be used instead. Tests such as emergence test at low temperatures can presumably reflect a seed lot's potential performance or vigor better than the standard germination test.

The results of TZ test, which was conducted on the control seeds and the seeds obtained from AA and CD tests, indicated that there were statistically no differences among seed lots even though seed lot 4 had the highest staining rating with the lowest TZ scale values (Table 3).

C-37 cultivar: In the experiments conducted with the seed lots of C-37 cultivar, similar results were observed with the cultivar Brigade (Table 4). The standard germination percentages of four seed lots were not significantly different from each other. However, the results of emergence tests conducted on the seed lots at 13°C showed that there were significant differences among the seed lots of this cultivar. Results of the emergence test provided information that the seed lot 4 had the highest FEP while the seed lot 1 had lowest FEP, which is a clear indication of vigor differences among the seed lots (Table 4). These findings were also confirmed by the result of the EC test in which seed lot 1 had the highest EC value and seed lot 4 had the lowest EC value.

When seeds were subjected to the AA and CD tests, findings were similar to those of control seeds (Table 4). Seed lots 3 and 4 had higher FGP, FEP and E_{50} values compared to seed lots 1 and 2 indicating that these seed lots were more vigorous than the seed lots 1 and 2. These results are in agreement with those of Tesnier *et al.*[26] who reported that controlled deterioration test could be used to reveal genetic differences in arabidobsis seed quality. The results of EC test conducted on controlled deteriorated seeds confirmed AA test results. It is quite normal that seed lots 3 and 4 had higher vigor than the seed lots 1 and 2 considering that they were physiologically one year younger than the seed lots 1 and 2. However, standard germination test was ineffective to detect the vigor differences among seed lots (Table 4). The results of TZ test conducted with the seeds obtained from AA and CD tests also indicated that although there were statistically no differences among seed lots, lots 3 and 4 had higher TZ scale values, which is an indication of higher vigor (Table 4).

The results of this study showed that it was possible to determine the vigor differences among the seed lots of a cultivar using different vigor tests. The results of the vigor tests showed that two seed lots of hybrid tomato cultivar Shasta were similar considering the vigor levels; the seed lot 4 of Brigade had highest vigor while the seed lot 1 had lowest vigor and quality. This study also indicated that the seed lots 3 and 4 of the cultivar C-37 had higher vigor than the seed lots 1 and 2. Among the vigor tests conducted, accelerated ageing and controlled deterioration tests gave the most reliable and consistent results. The results of electrical conductivity test were inconsistent and gave varying results compared to other tests. Therefore, the number of replications must be increased in order to obtain consistent and reliable results from the EC test. Tetrazolium test provided results parallel to the AA and CD tests; however, it is not as accurate as the other tests since it is a subjective test and requires a trained eye. Therefore, more replications with more seeds should be used when conducting this test.

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