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Is there Association between the Biometric Variables of Tomato Seeds and their Physiological Quality?

Patricia Penaloza Aspe*

Pontifical Catholic University of Valparaíso, chile

Research Article

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***For Correspondence**

Patricia Penaloza Aspe, Pontifical Catholic University of Valparaiso, chile. Tel: +56 32 2274501

E-mail: patricia.penaloza@pucv.cl

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ABSTRACT

Tomato seeds are chosen by weight and size due to the positive association between these properties and the physiological quality of the seeds, as shown by many studies using large seeds and cereals. Nevertheless, for horticultural production, information on this subject is scarce and contradictory. This is the reason for the present research, whose objective is to determine the effect of the weight and size of tomato seeds (*Solanum lycopersicum L.*) on the physiological quality of the plant expressed as germination and vigor.

A total of 30 blocks of nine hybrid tomato seed varieties were used. The seeds came from two different reproduction seasons. In all cases, the plants were of indeterminate growth habits and produced round fruits. The seeds were characterized by weight and size, and their quality was evaluated through tests of germination and vigor using accelerated ageing tests (stress) and usable transplant tests (seedling growth and evaluation). A fully randomized design was used; blocks from each season were compared using variance analysis, and means were compared using the Tukey or t-student test. The association between the two variables was determined using Pearson correlations. A level of significance of 0.05 was applied.

The seed blocks for each variety presented significant differences for the size variables but not for weight. It was not possible to discriminate between block quality via the germination tests and usable transplant tests, although the accelerated ageing stress test did show statistical differences between blocks for most of the varieties in question. There was only weak association between the physiological quality of the seeds evaluated using germination and vigor tests and the physical characteristics of said seeds.

INTRODUCTION

Seed quality includes aspects of health, genetics, physiology [1] and currently appearance [2, 3]. The level required depends on the use or destination of the seeds in question [4, 5]. Physiology is evaluated through germination and then complemented with vigor [6]. The latter is a broad and complex term, as it associates seed properties with their capacity to rapidly produce uniform seedlings under field conditions [6-9].

Commercial seed selections are carried out by separating them by size and weight, criteria that are widely used for agricultural crops [3]. According to Boligon et al. [10], the general opinion is that large seeds are better than smaller ones in terms of viability and germination rate. The latter statement has also been shown in a large number of plant species that grow in natural environments [11].

Research concerning cereals found direct association between larger values for seed size and weight and higher germination and vigor [12]. However, Peltonen-Sainio et al. [13] found no connection between physical seed characteristics and germination. It has also been found that smaller maize seeds showed higher vigor, and that the composition of the grain was more significant than its size [3]. Thus, complete concordance does not arise between seed size and weight for different farmed species and their effect on physiological quality.

Concerning vegetable seeds, information on weight and size and their association with physiological quality is scarce and contradictory [9, 14,15]. Some studies have found significant differences in weight between seeds of different tomato cultivars. In particular Ganeva [16] studied hybrid tomato seeds, finding a marked trend of seeds that were heavier than those from the original genetic lines used in the hybrids. Nieuwhof et al. [17] found that seed weight was significantly correlated with plant dry matter generated ten days after planting. Khan et al. [18] found that tomato seed weight is weakly and negatively correlated with germination. Whittington and Fierlinger [19] found that germination was directly related to tomato seed size, highlighting that the smaller seeds germinated early. Orsi and Tanksley [20] proved that the embryo-endosperm relationship influences seedling quality, independent of seed size.

The characteristics of tomato seed weight and size are related to the maternal genotype [17, 18, 20-22]. Therefore, when two plants are purposefully crossed, as in the case of manual hybrids, it is expected that differences generated by the characteristics of the maternal line will exist between varieties in terms of seed size or weight [16].

Several molecular studies posit that the relationship between seed size and weight and physiological quality depends more on genetic and metabolic aspects that occur during the seed's development [3], and as a result there are particular characteristics between different vegetables that must be considered in commercial quality criteria [23].

One way of measuring the seed quality is by the use of a methodology proposed by ISTA (International Seed Testing Association) and AOSA (Association of Official Seed Analysts). With regard to the physiological quality of seeds, the germination test is the most commonly accepted [24]. Corbineau [4] states that with high quality seeds, full, rapid and simultaneous germination occurs to produce normal, vigorous plants with low sensitivity to external factors.

Barros et al. [25] and Santorum et al. [26] posit that the results of the germination test overestimate the physiological potential of the seed, as it is conducted under optimal conditions. It is therefore necessary to conduct tests of other attributes to complete the seed quality evaluation. Seed vigor then becomes a concept that completes this need for information and describes other characteristics associated to seed performance in the field and in storage [8]. According to Milošević et al. [2], current vigor tests include the physical aspects of the seeds, determining characteristics such as size or mass. There are other three types of vigor test: some related to stress or deterioration, others biochemical, and the more modern are related to growth and seedling evaluation. Stress tests are based on the fact that field conditions are usually less than optimal for the species, and therefore, in suboptimal or stressing conditions seeds with high levels of vigor have a greater potential to emerge and thrive [7]. Out of such tests, accelerated ageing is one of the most commonly used to estimate the vigor of vegetable seeds. Considering tomato, Panobianco and Marcos-Filho [27] found that it was the best test to identify levels of seed quality. Later, Barros et al. [25] and Korkmaz et al. [28] found similar results.

Growth tests and seedling evaluation measure development and growth [8]. Regarding evaluation time, Burg et al. [29] focus on seedlings 10 to 14 days after planting. These tests have only one method suggested by ISTA or AOSA for vegetable species, and as such researchers commonly conduct the tests considering that the factors that affect plant growth must be controlled or described. Thus, Leskovar [30] warns that this test depends on environmental factors and crop characteristics. In olericultural species, this type of test is used to complement the information obtained from germination. Burg et al. [29] proposed the term usable transplants as a way to evaluate seed quality. This type of test is of greater interest to seed users [2].

The present research aims to determine the effect of tomato seed weight and size on physiological quality and the association between these variables.

MATERIALS AND METHODS

Seed material and location

Nine varieties of hybrid tomato seeds were used, with the number of blocks per variety varying from two to four. Varieties were crossed manually during two seasons (2004-2005 and 2005-2006), both in summer. The genetic lines whose seeds were collected for the study are the property of transnational companies and the description of their particular attributes or characteristics were not divulged to the multipliers. However, they were plants of indeterminate growth habits, simple roots, and round multilocular fruit. All seeds were produced in a single part of Chile, located between latitudes 32° 54' and 34° 21' S and longitudes 70° 50' and 71° 16' W, and at an altitude of 120 m to 146 m above sea level. This area present Mediterranean climate (warm temperate and dry season in summer).

Seed characterisation

The physical characteristics of the seeds from each block were determined using four repetitions of 100 seeds. The weight was recorded individually using an analytic scale with a sensitivity of 0.0001 g. Seed size (area, length, width) was obtained from digital images acquired with a Hewlett Packard model Precision Scan Pro 3.02 flatbed scanner, with a resolution of 300 dpi; the images were stored in jpeg format.

Image processing

The images were used to obtain data on the area, length and width of the seeds using the program Sigma Scan Pro 5. Prior to this, all images were processed with calibration functions, intensity threshold, filter and number of objects.

Germination test

The germination test was conducted by planting four repetitions of 100 units each for each block on filter paper substrate saturated with distilled water in a germination chamber with control temperature (25°C ± 0.5°C). The count was carried out on normal seedlings on two occasions: at 5 days after planting and at 14 days after planting. The procedure is based on ISTA standards [24, 31].

Accelerated aging test

The stress vigor test was carried out by distributing a layer of seeds (600 units) over a plastic structure (10.3 cm x 10.3 cm x 3 cm) covered with a 60 mesh grille. This structure, with the samples on the surface, was inserted into a plastic box (11.0 cm x 11.0 cm x 3.5 cm) with 40 ml of deionized water in the bottom. These boxes were placed inside a water-jacketed incubation chamber, where they remained for a period of 72 hours at 41°C ± 0.1 and 100% relative humidity. The percentage humidity of the seeds was also determined (ISTA, 1995). They were then planted and evaluated as described for the germination test [24, 31].

Usable transplant test

The test was carried out in accordance with the proposal of Burg et al. [29]. Four repetitions of 30 seeds were sowed for each block. Polyethylene trays with 40 ml containers were used, the substrate was a mixture of peat, perlite, dolomitic limestone and an initial nutrient mix (Sunshine Mix 4). The seeds were watered daily, though no fertilization or cleaning tasks were performed. The seedlings were grown under greenhouse conditions, with an average daily temperature of 18°C.

Experimental design and data analysis

A fully randomized experimental design was used. The quantitative variables of the seed blocks representing each variety were subject to variance analysis and the means were compared using the Tukey or t-student test, with a level of significance of 0.05. Values expressed in percentages were transformed using the arcsine function $\sqrt{x}/100$.

The association between the two variables was determined via Pearson correlations with a level of significance of 0.05.

Minitab 16, by Minitab Inc. was used for the statistical analysis.

RESULTS AND DISCUSSION

Physical characterization of the seeds

In both seasons, the tomato seed blocks for each variety were found to be only fractionally different in terms of seed weight **(Tables 1 and 2).** These results indicate the determinant effect of the maternal genotype on this variable, as stated by Nieuwhof et al. [17] and Khan et al. [18]. The slight differences were attributed to the fact that in this study the seed blocks came from plants crossed for the purpose of obtaining hybrid seeds [16], in which the low fruit yield and the appropriate cultivation conditions were not limiting factors for the weight of the seeds.

Table 1. Seed weight (SW), seed area (SAr), seed length (SL), seed width (SWi), first count germination (G1), germination (G), abnormal seedlings (Ab), dead seeds (De) for 20 tomato seed blocks from five hybrid varieties (100 seeds used per replicate). Quillota (Chile), PUCV, 2004 - 2005.

Variety	Block	SW	SAr	SL	SWi	G1	G	Ab	De
		(mg)	(mm2)	(mm)	(mm)	(%)			
A	1	3.5a	8.0a	3.9a	2.8a	82a	88a	5a	7a
	2	3.8 a	7.5a	3.7b	2.7a	84a	85a	10a	5a
	3	3.9a	7.8a	3.7b	2.7a	84a	90a	6a	4a
	4	3.6a	7.4a	3.7b	2.7a	84a	87a	7a	6a
CV (%)		7	6.4	5.5	2.4	4.6	4.5	17.7	26.7
B	1	3.2a	5.7a	3.1a	2.4a	88ab	93b	1a	6a
	2	3.3a	4.9b	2.9a	2.2b	96a	100a	0a	0b
	3	3.1a	5.4ab	3.0a	2.4a	80b	97a	1a	2b
	4	3.1a	5.6a	3.0a	2.4a	88ab	95ab	1a	4a

CV (%)		5.9	7.9	4.1	4.2	8.1	3.2	153.2	121.7
C	1	3.2ab	6.2b	3.2b	2.5b	86a	91a	4ab	5a
	2	3.5a	8.0a	3.9a	2.8a	84a	89a	2b	9a
	3	3.0b	5.7b	3.1b	2.4b	72b	79b	10ab	11a
	4	3.0b	6.0b	3.2b	2.5b	80a	86ab	11a	3a
CV (%)		10.6	15.1	9.8	6.7	8.2	6.9	36.1	39.9
D	1	3.5a	7.4a	3.4a	2.9a	90a	90a	6a	4a
	2	3.1a	6.9a	3.4a	2.8ab	84a	87a	8a	5a
	3	3.3a	6.8a	3.3ab	2.8a	68b	78a	14a	8a
	4	3.0a	6.1b	3.1b	2.6b	82a	90a	5a	5a
CV (%)		6.4	8.8	4.2	4.5	12.3	8	40.6	50.2
E	1	3.9a	6.7b	3.2b	2.7b	70ab	77a	14a	9ab
	2	3.9a	8.4a	3.7a	3.0a	70ab	73a	12a	15ab
	3	4.1a	8.4a	3.2a	3.0a	64b	75a	9a	16a
	4	4.0a	8.2a	3.7a	2.9a	76a	84a	9a	7b
CV (%)		5.2	11.3	6.9	6.2	6	7.3	19.2	21.1

Values labeled with the same letter show no statistical differences with a 5% probability Tukey Test.

Table 2. Seed weight (SW), seed area (SAr), seed length (SL), seed width (SWi), first count germination (G1), germination (G), abnormal seedlings (Ab), dead seeds (De) for 10 tomato seed blocks from four hybrid varieties (100 seeds used per replicate). Quillota (Chile), PUCV, 2005 - 2006.

Variety	Block	SW	SAr	SL	SWi	G1	G	Ab	De
		(mg)	(mm2)	(mm)	(mm)	(%)			
F	1	4.3a	8.4a	4.0a	3.0a	12b	84a	3a	13a
	2	4.3a	8.2ab	3.6b	2.9a	41a	85a	3a	12a
	3	4.2a	8.2ab	3.6ab	2.9a	42a	86a	3a	11a
CV (%)		1.8	4.1	2.6	1.9	38.4	10.2	54.5	29.7
G	1	3.1a	7.4b	3.6b	2.7b	39ab	73a	5a	22a
	2	3.2a	7.5b	3.6ab	2.7b	52a	69a	11a	20a
	3	2.9a	7.9a	3.7a	2.8a	36b	83a	6a	11a
CV (%)		6.9	5.7	2.9	3.9	14.8	11.9	70.9	26.8
H	1	4.3a	8.7a	3.8b	2.9a	53a	69a	28a	3a
	2	3.8b	8.8a	3.9a	2.9a	47a	69a	30a	1a
CV (%)		6.2	3.6	2.1	2.1	9.9	2.4	5.5	51.5
I	1	3.7a	8.8a	3.9a	3.0a	59a	87a	13a	0b
	2	3.7a	8.1b	3.6b	2.9a	52a	72b	19a	9a
CV (%)		1.6	5.3	3.9	1.5	6.7	11.9	21.7	91.4

Values labeled with the same letter show no statistical differences with a 5% probability. Tukey Test.
Varieties with two blocks analyzed using the t-Student test. Values labeled with the same letter show no statistical differences with a 5% probability.

Regarding seed size (area, length and width), the results from both seasons indicate significant differences between blocks **(Tables 1 and 2).** This is explained by the development of the flower and of the seed. In the former case, the size of the ovule prior to fertilization determines the final size of the seed. During subsequent development, the size is determined 10 to 15 days after pollination [20], and as such it is probable that having branches pollinated on the same plant in a short timeframe leads to competition that affects the size and not the weight of the seeds, as it is during this period that the size curve shows a higher gradient than the weight curve [20].

Association between physical characteristics of the seeds

The association between the characteristics of size and weight of the tomato seeds evaluated in season 1 is presented in **(Figure 1 A, B and C).** There were significant and positive associations between almost all variables, though the correlation coefficients are low for both seasons (data for season 2 not shown).

These results are in agreement with Khan et al. [18], who found association between tomato seed size and weight, though the coefficients of correlation found by these authors are lower that than those of the present research. This may be because Khan et al. [18] crossed different Solanum species.

Germination percentage

For both seasons, there were significant differences between blocks in the first germination count **(Tables 1 and 2).** However, as stated by Barros et al. [25] the first count, which is used to estimate vigor, shows low sensitivity when detecting small differences between tomato blocks. Nevertheless, the total germination percentage present few differences between the blocks **(Tables 1 and 2).** According to Matthews et al. [5], this is due to the limitation of this test in overestimating potential, as it is conducted under optimal conditions. The varieties with highest and lowest germination percentage remained so for the first count and for total germination. The lack of discrimination of this test is also observed in the categories of abnormal seedlings and dead seeds **(Tables 1 and 2).** It can be noted that the highest variation coefficients are seen for dead seeds and abnormal seedlings.

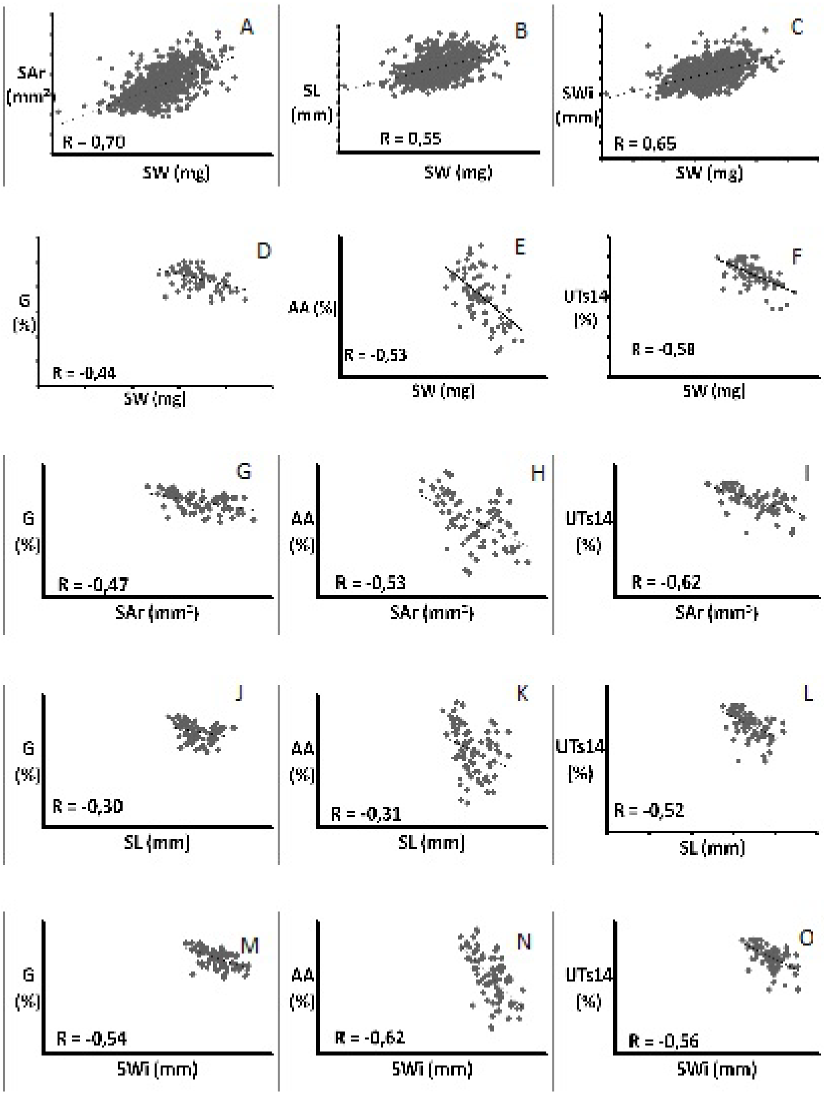

Figure 1. Correlations between biometric variables and physiological quality in tomato seeds. Seed weight (SW), seed area (SAr), seed length (SL), seed width (SWi), germination (G), accelerated ageing (AA), usable transplants on day 14 after planting (UTs14) for 20 tomato seed blocks from five hybrid varieties. Quillota (Chile), PUCV, 2004 - 2005.

Association between physical characteristics of the seeds and germination

Association was found for only one of the seasons **(Figure 1 D, G, J and M).** Seed weight was correlated significantly, though weakly and negatively, with the germination percentage, for the first count (data not reported) and for total germination; this result coincides with Khan et al. [18] for tomato. Regarding this association, several authors have found contradictory results. For Brassica oleracea there was significant and positive correlation, mainly between seed weight and germination [9], while for cereals, seed weight was only slightly correlated with germination [13]. Some authors have argued that higher seed weight influences germination capacity because the seeds are well formed and have more reserves [32]. This cannot be concluded from the present research which, as with Komba et al. [15], did not find correlation between seed size and quality. Thus, the physical, compositional and functional differences between seeds of different types, such as monocots and dicots, may indicate that the association between the seeds' physical characteristics and germination occurs in a singular manner [23]. This may be partly because in some research, such as the present, the relationship between seed size and weight and physiological quality differs from what is commonly described for cereals and legumes.

Accelerated ageing

After application of the stress test, germination in the blocks was reduced **(Tables 3 and 4).** This is in agreement with the delayed emergence of shoots described by Matthews et al. [33], which is attributed to enzyme failure and a reduction in carbohydrates [34]. Though the loss of quality is estimated at 23% for tomato [34], in the present research it is associated with initial seed quality, especially in season 1, thus ratifying the notion that the initial condition of the seeds influences their response to the vigor test [7]. Several authors state that this test achieves better results in tomato than other tests [25, 28], mainly by identifying differences in vigor between blocks. With regard to abnormal seedlings and dead seeds, it was seen that in blocks with initially high germination percentages, the fraction of abnormal seedlings increased after the accelerated ageing test, while the blocks with lower initial quality attained higher values for dead seeds, similar to the results described by Matthews et al. [5]. The highest coefficients of variation were also seen for the fraction of abnormal seedlings and dead seeds.

Table 3. Germination with accelerated ageing (AA), abnormal seedlings with accelerated ageing (AAAb), dead seeds with accelerated ageing (AADe), usable transplants on day 7 after planting (UTs7), usable transplants on day 14 after planting (UTs14), usable transplants on day 21 after planting (UTs21) for 20 tomato seed blocks from five hybrid varieties. Quillota (Chile), PUCV, 2004 - 2005.

Variety	Block	AA	AAAb	AADe	UTs7	UTs14	UTs21
			(%)			(%)	
A	1	76a	10b	14c	70a	81a	88a
	2	64ab	18ab	18bc	70a	80a	84a
	3	58bc	14ab	28ab	79a	81a	85a
	4	46c	22a	32a	74a	83a	86a
CV (%)		23.8	50.6	43	30.8	7.9	8.6
B	1	61b	11a	28a	60ab	100a	99a
	2	83a	9a	8b	78a	98a	99a
	3	78a	19a	3bc	39b	93a	95a
	4	89a	10a	1c	46b	98a	99a
CV (%)		15.3	49.1	106.7	24.9	9.2	7.6
C	1	52b	30a	18a	58a	85a	87a
	2	60ab	30a	10a	58a	83a	86a
	3	75a	11b	14a	48a	88a	88a
	4	61ab	28ab	11a	52a	89a	93a
CV (%)		12.6	27.3	34	16.1	10.9	9.4
D	1	57a	28a	16a	48a	90a	95a
	2	51a	32a	17a	45a	81a	88a
	3	57a	21a	22a	33a	88a	94a
	4	56a	27a	17a	35a	90a	92a
CV (%)		10.4	19.1	15.5	24.5	8	8.7
E	1	27a	25ab	49a	68a	73a	78a
	2	35a	24ab	41ab	58a	69a	72a
	3	35a	22b	43ab	57a	78a	83a
	4	35a	36a	29b	75a	80a	82a
CV (%)		14.1	16.9	14.8	12.7	10.8	10

Values labeled with the same letter show no statistical differences with a 5% probability. Tukey Test.

Table 4. Germination with accelerated ageing (AA), abnormal seedlings with accelerated ageing (AAAb), dead seeds with accelerated ageing (AADe), usable transplants on day 7 after planting (UTs7), usable transplants on day 14 after planting (UTs14), usable transplants on day 21 after planting (UTs21) for 10 tomato seed blocks from four hybrid varieties. Quillota (Chile), PUCV, 2005 - 2006.

Variety	Block	AA	AAAb	AADe	UTs7	UTs14	UTs21
			(%)			(%)	
F	1	70a	20a	10b	18a	38a	48a
	2	64a	29a	7b	20a	30a	40a
	3	40b	28a	32a	20a	38a	53a
CV (%)		18.2	27.6	58.4	61.7	36.3	33.9
G	1	50b	29a	23a	35a	60a	70a
	2	45b	29a	27a	20a	48a	58a
	3	72a	9b	20a	28a	60a	73a
CV (%)		16.1	34.6	18.9	29.7	22.6	22.3
H	1	65a	29a	6a	8a	43a	50a
	2	61a	33a	6a	13a	38a	45a
CV (%)		4.6	8.5	40	86	11.39	13.9
I	1	80a	20a	0b	5a	63b	70a
	2	58b	22a	20a	8a	88a	90a
CV (%)		15.3	12.6	83.4	186.7	23.9	21.1

Values labeled with the same letter show no statistical differences with a 5% probability. Tukey Test.

Association between physical characteristics of the seeds and accelerated ageing

As with the germination test, the correlation between physical attributes and the germination capacity response accelerated aging are negative **(Figure 1E, H, K and N).** It is proposed that it is the constituent parts of the seed and not the quantity that influence the response, and thus since the composition of tomato seeds is mainly lipid and protein, this would directly affect germination and the respective enzymes [34] independent of the size or weight. In season 2, significant correlations were only found between the length and width of the seeds and the stress response, where this association was quite weak (data not shown).

Usable transplants

In both seasons there was little significant difference between blocks **(Tables 3 and 4)**, and those that did occur were seen in the first 14 days after planting. Though this test has a very low capacity for discerning quality, it is of important commercial interest for vegetable seeds [2]. In comparison with the accelerated ageing test discussed above, it shows lower sensitivity for differentiating between the quality of the blocks, a result which is in agreement with Panobianco and Marcos-Filho [27].

It can be noted that this test encompasses several aspects involved in the ISTA definition of vigor [8], including plant stage (germination and emergence) and uniformity characteristics.

The emergence values attained on day 7 after planting **(Tables 3 and 4)** differ from those of the first count of the germination test for both seasons **(Tables 1 and 2).** It is therefore necessary to consider the influence of initial block quality, since, as is noted by Boligon et al. [10], when quality is low it does not correlate with plant emergence; this was corroborated in season 2. The plants that have emerged by day 14 and day 21 after planting are in the process of developing actual leaves [30]. In season 1, from day 14 onwards, the emergence percentages recorded are higher than those observed in the germination test, while in season 2, the emergence percentages remain lower than those of the germination test for several seed blocks. This observation coincides with Burg et al. [29], who state that the test in question is more demanding than the germination test for normal plant qualification.

The coefficients of variation decreased with the increasing development of the plants, showing that as the plant grows the differences detected in the first stages tend to decrease. This coincides with the findings of other authors, who recommend evaluating plant vigor at 10 and 14 days after planting [29]. Even when studying non-cultivated species, Moles and Westoby [11] also corroborated that the association between seed mass and seedling survival only occurs during the first weeks.

Association between physical characteristics of the seeds and usable transplants

The plants emerged at day 14 after planting were negatively correlated with seed size and weight in the first season **(Figure 1 F, I, L and O).** Although, Nieuwhof et al. [17] found strong association between seed weight and the weight of the resulting plants, this association held only up to day 10 after planting and then decreased. The plants with 14 or more days of development were possibly generating photosynthesis, which may be why they were inversely dependent on the original seed (season 1) or why there was no association at all (season 2, data not shown).

Finally, we conclude that in this research there was weak and slight negative association between the tomato seed size and weight variables and their physiological quality.

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