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Association between biometric characteristics of tomato seeds and seedling growth and development

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ABSTRACT

Background: The size and weight of tomato seeds depend on genetics and can be modified by environment and management. In some species, a strong relation has been described between physical aspects of the seeds and the quality of the corresponding seedlings, but this cannot be considered a general rule. The objective of this research was to identify any association between the biometric characteristics of tomato seeds and the growth and development of their seedlings.
Results: A total of 18 lots of hybrid tomato seeds were used (from indeterminate plants with round fruits), belonging to six varieties from two reproduction seasons. Each lot was evaluated for seed size and weight, and seed quality, in terms of the germination test (5 and 14 d after sowing). The number of normal roots emerged with a length above 2 mm was also evaluated at d 3, 4 and 5 after sowing. The length of the seedlings and their total and partial dry weight were measured 5 d after sowing. The results indicate that there was no association between seed size and weight and subsequent seedling emergence, and only weak correlations were found between the dry weight of the radicle and cotyledon and seed size.
Conclusion: There is little association between the physical characteristics of the seeds and the subsequent seedlings, making it impossible to propose the use of seed weight or size as a compliment to quality evaluation tests.

1. Introduction

The current definition of seed quality includes physical aspects such as size and weight [1] because there is often an association between size and quality in agricultural species [2]. However, this statement depends on the type of seed in question and cannot be used as a general rule for all different groups of vegetables that are sold by the global seed industry. Most information has been gathered in regard to monocots, but their particular nature, especially in terms of anatomy, composition and metabolism, can be very different to other seeds [3].

Though most information that is available is in regard to cereals and other agriculture seeds, it can be noted that for tomato, Nieuwhof et al. [4] found that heavier seeds produced heavier plants, while Khan et al. [5] demonstrated a high level of association between seed weight and seedling dry weight. Van der Merwe et al. [6] included biomass, particularly of the radicle, as an indicator of seed quality.

In the field of seed quality evaluation, there is agreement that standard germination does not provide sufficient information, and as such the parameter vigour has been considered [7]. Though this variable is complex, it has been defined by analysts [8] as the sum of seed properties that lead to the rapid production of uniform seedlings under a wide range of field conditions. Upon further analysis of this definition, it can be seen that it includes early growth and development stages, firstly in association with germination and later with emergence. This type of evaluation coincides with the current trends in growth tests and seedling evaluation [1]. This definition makes no association between the physical aspects of the seeds and the behaviour of the seedlings.

Several authors have evaluated the connection between seeds and seedling quality over short periods, without looking beyond 14 d after sowing [9,10]. For tomato, Akbudak and Bolkan [11] evaluated the quality of seedlings after 3 and 7 d while Khan et al. [5] conducted evaluations at d 5, and 10. One objective of the current analysis is to reduce analysis time. As such, in order to support some seedling evaluation methods, the analysis of digital images has been used to improve objectivity [12,13,14,15].

Other authors have evaluated seedling length, without making any connection with the physical characteristics of the seeds, *e.g.* Sako et al. [12] and Kikuti and Marcos-Filho [16] respectively implemented and used total, hypocotyl and radicle length as the basis for estimating

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a vigour index to facilitate comparison of lettuce seed quality (Seed Vigour Imaging System®). For tomato, it has been found that radicle length at d 3 and d 4 is significantly correlated with seed quality [11]. Van der Merwe et al. [6] also found that the root is a good estimation of the process of germination and the metabolic changes that occur. For bell peppers, Hacisalihoglu and White [17] found that the area of the radicle and the weight was strongly associated with germination. In flower seeds, Oakley et al. [14] used seedling length to categorise the quality of different seed lots.

Early evaluations comprise radicle protrusion, which though it is specific to each set of research conditions, for tomato it has been reported that it begins from 40 h after imbibition onwards [18]. Other key events also occur during this period, such as enzyme action [18], reserve protein mobilisation [19] and hormone participation [20]. It is, therefore, justifiable to use the radicle analytically because its early protrusion is associated with energy availability [21], and it grows more than the hypocotyl under conditions of stress [22]. Another important consideration is that abnormal seedlings germinate later [23].

Matthews et al. [23] use periodical protruding radicle count to generate a value for seed vigour, information that cannot be obtained from the standard d 14. In addition, during germination and seedling emergence the accumulation of dry weight in the seedling increases [9]. Studying bell peppers, Demir et al. [24] also state that the lots seen to germinate early produce longer seedlings that are more uniform in comparison to those that germinate later.

The objective of the present research is to associate tomato seed size and weight with seedling growth and development, in order to propose their use as early quality estimates.

2. Materials and methods

2.1. Seed material

A total of 18 seed lots were used. They belong to six hybrid tomato varieties obtained by manual crossing and were studied over two seasons (2005–2006 and 2006–2007). The varieties denominated F, G, H and I were obtained in the first season while varieties J and K were from the second season. All seeds were produced in Chile, within the area located between latitudes 32°54′ and 34°21′ S and longitude 70°50′ and at an altitude of 120 m to 146 m above sea level.

All lots have different harvest dates for each variety. The genetic lines from which the seeds in question were derived were the property of private transnational companies. The multipliers were not made aware of the attributes or characteristics of the seeds, though they were known to be of indeterminate growth habits, simple racemes, and round multilocular fruit, and were grown under greenhouse conditions.

2.2. Seed characterisation

The physical characteristics of the seeds from each lot were determined using four repetitions of 100 seeds. The weight (SW) was recorded individually using an analytic scale. Seed size was characterised as seed length (SL), seed width (SWi) and seed area (SAr), and 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.

2.3. Germination test

The germination test was conducted by sowing four repetitions of 100 units each for each lot onto filter paper substrate saturated with distilled water. The procedure is based on ISTA standards [25]. The count was carried out on normal seedlings on two occasions: at 5 d after planting (G1) and at 14 d after planting (G).

2.4. Seedling emergence

The methodology is based on that described by Sako et al. [12] without calculating the vigour index developed by the author. Seeds from each lot were sown in four repetitions of 25 seeds on a double-layer of blue filter paper (Anchor Paper Co.) saturated with distilled water. The substrates were stored in transparent plastic boxes measuring $15 \times 23 \times 4$ cm. The boxes were placed in a germination chamber at 25°C $\pm$ 0.1, without light. The boxes were placed vertically at 85°C. Digital images were taken of each repetition on a daily basis using a Hewlett Packard model 4670 vertical scanner with a resolution of 200 dpi. The evaluations were conduction only with germinated seedlings with a radicle length of ≥2 mm. Counts were made of the number of seedlings germinated 3, 4 and 5 d after sowing (S3d, S4d and S5d respectively) and then were converted to be expressed as percentages. Germinated seedling length was measured 5 d after sowing and was broken down into radical length (RL), hypocotyl length (HL) and total length (TL). The dry weight of the germinated seedlings was taken 5 d after sowing and was broken down into radical dry weight (DWR), hypocotyl dry weight (DWH), cotyledon dry weight (DWC) and total dry weight (DWT). The dry weight was calculated by maintaining the seedling at a temperature of 70°C for 48 h.

2.5. Data extraction and digital image processing

The seed characterisation images were used to obtain data on the area, length and width of the seeds. The sprouted seedling images were used to obtain data on the lengths of the radicle and the hypocotyl. In both cases, this was done using the programme Sigma Scan Pro 5. Prior to this, all images were processed with calibration functions, intensity threshold, filter and number of objects.

2.6. Experimental design and data analysis

A fully randomised experimental design was used. The quantitative variables of the seed lots representing each variety were subjected to variance analysis and the means were compared using the Tukey or student's t-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 two variables was determined *via* Pearson correlations with a level of significance of 0.05.

The variables of seed size and weight and their correlation to the dry weight of the sprouted seedlings were analysed using multiple regressions with a level of significance of 0.05.

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

3. Results and discussion

3.1. Seedling emergence

The emerged plants and their growth and development characteristics are presented as an alternative for evaluating quality through new vigour tests, as they satisfy the needs established by several authors for quick low cost analysis methods that are non-destructive and easy to implement within the seed industry [26].

As can be seen in Table 1, the emerged seedling showed significant differences between lots in three of the six varieties evaluated in the study; these were varieties H, I and J. This was the case for the different d after sowing, though it was more frequent on d 4 (S4d) and 5 (S5d). These results partially complement the information from the germination test (G), which is insufficiently sensitive to distinguish the quality of lots according to several authors [7,23]. Akbudak and Bolkan [11], identified the importance of seedlings at d 3 or 4 for differentiating the quality of tomato seed lots. In addition, the values from d 4 (S4d) onwards were higher than those obtained in

Table 1
First count germination (G1), germination (G), seedling emergence on d 3 after sowing (S3d), seedling emergence on d 4 after sowing (S4d), and seedling emergence on d 5 after sowing (S5d) of the 18 tomato seed lots from six hybrid varieties.

Variety	Lot	G1	G	S3d	S4d	S5d
			(%)		(%)	
F	1	12b	84a	62a	93a	95a
	2	41a	85a	56a	94a	96a
	3	42a	86a	67a	92a	95a
CV (%)		38.37	10.18	8.20	11.72	11.18
G	1	39ab	73a	75a	94a	97a
	2	52a	69a	56b	97a	98a
	3	36 b	83a	55b	93a	93a
CV (%)		14.76	11.87	13.73	10.85	10.14
H	1	53a	69a	55a	84b	88b
	2	47a	69a	62a	94a	98a
CV (%)		9.86	2.42	8.24	9.50	13.71
I	1	59a	87a	58a	94a	97a
	2	52a	72b	64a	82b	87b
CV (%)		6.74	11.92	6.99	10.09	12.52
J	1	31d	35c	28d	40c	55c
	2	62b	64b	53b	68b	76b
	3	81a	85a	73a	83a	90a
	4	78a	80a	45c	58b	71b
	5	48c	48c	55b	63b	71b
CV (%)		24.20	23.77	20.25	17.35	13.96
K	1	51a	53a	54a	63a	75a
	2	54a	54a	61a	70a	83a
	3	60a	60a	59a	69a	81a
CV (%)		6.92	6.63	5.91	5.57	5.55

Measurements followed by the same letter presented no statistical differences with a probability of 5%, Tukey Test. Varieties with two lots analysed using the student's t-test. Measurements followed by the same letter showed no statistical differences with a probability of 5%. CV: coefficient of variation.

Table 2
RL, HL, TL, DWR, DWH, DWC and DWT of the 18 tomato seed lots from six hybrid varieties.

Variety	Lot	RL	HL	TL	DWR	DWH	DWC	DWT
		(mm)	(mm)	(mm)	(mg)	(mg)	(mg)	(mg)
F	1	39.90a	9.00a	48.90a	0.46a	0.58a	1.71a	2.75a
	2	27.34b	8.25a	36.13b	0.41a	0.53a	1.56a	2.50ab
	3	29.20b	6.92a	35.59b	0.32b	0.41a	1.55a	2.29b
CV (%)		22.06	21.26	18.80	16.00	29.86	6.92	10.36
G	1	33.53a	8.50a	42.03a	0.28b	0.42a	1.25a	1.96a
	2	33.63a	9.75a	43.38a	0.32a	0.46a	0.98b	1.76a
	3	34.48a	8.50a	42.98a	0.30ab	0.47a	1.09ab	1.86a
CV (%)		4.06	16.19	5.80	7.82	29.02	12.62	8.27
H	1	34.78a	10.50a	45.28a	1.10a	0.27a	0.39a	1.76a
	2	36.65a	10.75a	47.40a	1.18a	0.29a	0.41a	1.88a
CV (%)		7.02	9.98	7.03	7.98	10.52	5.81	6.21
I	1	38.38a	11.00a	49.38a	1.46a	0.31a	0.46a	2.23a
	2	36.70a	10.25a	46.95a	1.44a	0.33a	0.38a	2.15a
CV (%)		6.96	12.26	7.02	12.53	11.05	36.95	10.95
J	1	29.87a	8.93a	38.80a	0.28a	0.38a	1.41a	2.07a
	2	33.63a	7.10a	40.73a	0.29a	0.40a	1.27a	1.96a
	3	30.25a	7.52a	37.77a	0.31a	0.36a	1.49a	2.17a
	4	32.13a	9.38a	42.50a	0.30a	0.17b	1.39a	1.86a
	5	34.50a	7.72a	42.22a	0.31a	0.34a	1.00a	1.65a
CV (%)		9.58	16.32	8.38	11.91	29.95	29.22	21.03
K	1	34.28a	8.49a	42.77a	0.33b	0.46a	1.29a	2.10a
	2	37.33a	8.38a	45.70a	0.37a	0.43a	1.20a	2.00a
	3	34.95a	7.50a	42.45a	0.36a	0.47a	1.06b	1.88b
CV (%)		8.58	13.62	6.56	1.83	4.78	9.57	5.19

Measurements followed by the same letter presented no statistical differences with a probability of 5%, Tukey Test. Varieties with two lots analysed using the student's t-test. Measurements followed by the same letter showed no statistical differences with a probability of 5%. CV: coefficient of variation.

the germination test (G) with the exception of lot 4 of variety J. Seedling emergence increased by differing percentages depending on the time between sowing and observation. This coincides with Demir et al. [24], who studied bell peppers, finding association between early germination and longer and more homogenous seedlings.

With regard to the capacity to discern quality, it can be said that his test partially differentiated between the lots from d 3 onwards, which is fully in line with Akbudak and Bolkan [11], in terms of the early evaluation time, although in this case we did not see the high sensitivity reported by these authors. However, the current research is a contribution to the small number of examples for this species, which was recently studied by Ferreira et al. [15].

3.2. Association between biometric characteristics of the seeds and seedling emergence

No significant correlations were found between the physical variables of the seeds and seedling emergence at the three evaluation times (S3d, S4d and S5d). Very little correlation was also seen between the seed and the resulting seedling (Table 3). This conclusion is contrary to what usually happens with cereal species [3,12] and with some large seeds, such as legumes [7,10]. However, Khan *et al.* [5], coinciding with the results of this research, found very low correlation between seed weight and early seedling germination for tomato seeds. These results may be due not only to the fact that the size and weight characteristics of the seeds include seedling development, but also that the composition, metabolism and genetics also affect the germination and emergence process [3], along with the presence of any abnormalities [23].

3.3. Seedling length

It can be seen in Table 2 that only the lots of variety F showed significant differences in RL and TL, while no differences were seen for all other lots. Sako et al. [12] studied the early utility of this variable for discerning seed quality, based on the notion that radicle and seedling growth (length) and the growth rate are direct components in defining seed vigour [8]. Thus, as with Oakley et al. [14], several different seed lots have been successfully ranked, particularly in lettuce and impatiens. Researchers such as Van der Merwe et al. [6] and Akbudak and Bolkan [11] have found the same in tomato. The existence of differences with regard to the present research shows that working with seeds with relatively low germination percentages (<87%, Table 1), the presence of seedling abnormalities can affect root growth [23] or lead to low energy availability [21]. It can also be noted that these researchers include the variable of seedling length in several more complex formulae, which they use to build their vigour indices, sometimes comprising standard deviation and others [12], with the germination percentage [11], though this form of analysis was not used in the present research. With regard to the percentage distribution of seedling length, it can be noted that RL comprised more than 75% of the total in all lots (data not published).

3.4. Association between biometric characteristics of the seeds and seedling length

For all the physical characteristics of seed size and weight, there were no significant correlations with seedling length as can be seen in Table 3. The results of this research differ from those described by Bertholdsson and Brantestam [27], who found a positive correlation between length and dry weight of the radicle and seed weight in cereals. These results show that seedling development also depends on the species and on other aspects, such as complex metabolic systems and their composition, including new elements for the study of seed quality [28].

3.5. Seedling dry weight

It can be seen in Table 2 that the lots of varieties F, G and K showed significant differences for DWR, while the DWH showed no differences in relation to the dry matter that it generates, with the exception of one

Table 3
Correlations between seed and seedling attributes. SW, SL, SWi, SAr, seedling emergence on d 3 after sowing (S3d), seedling emergence on d 4 after sowing (S4d), seedling emergence on d 5 after sowing (S5d), RL, HL, TL, DWR, DWH, DWC and DWT of the 18 tomato seed lots from six hybrid varieties.

	SW	SL	SWi	SAr	S3d	S4d	S5d	RL	HL	TL	DWR	DWH	DWC
SL	0.411												
	0.000												
SWi	0.744	0.423											
	0.000	0.000											
SAr	0.572	0.865	0.563										
	0.000	0.000	0.000										
S3d	-0.057	0.057	-0.165 0.165	-0.097 0.416									
	0.636	0.632											
S4d	0.094	0.146	0.067	0.069	0.624								
	0.413	0.221	0.573	0.564	0.000								
S5d	0.060	0.139	-0.001	0.040	0.67	0.962							
	0.619	0.245	0.994	0.736	0.000	0.000							
RL	-0.220	0.206	-0.052	0.135	0.180	0.138	0.125						
	0.063	0.083	0.664	0.257	0.130	0.247	0.296						
HL	0.037	0.204	0.152	0.233	-0.065	0.213	0.215	0.308					
	0.756	0.086	0.202	0.049	0.590	0.072	0.069	0.009					
TL	-0.173	0.242	0.008	0.193	0.130	0.189	0.178	0.947	0.598				
	0.147	0.040	0.948	0.104	0.277	0.113	0.135	0.000	0.000				
DWR	0.237	0.549	0.354	0.523	0.144	0.316	0.307	0.371	0.571	0.506			
	0.045	0.000	0.002	0.000	0.229	0.007	0.009	0.001	0.000	0.000			
DWH	-0.048	-0.155	-0.193	-0.172	0.162	0.248	0.208	0.082	-0.245	-0.014	-0.003		
	0.688	0.192	0.105	0.148	0.174	0.036	0.080	0.494	0.038	0.906	0.004		
DWC	0.099	-0.350	-0.093	-0.337	-0.043	-0.194	-0.180	-0.330	-0.486	-0.442	-0.753	0.379	
	0.408	0.003	0.436	0.004	0.719	0.102	0.130	0.005	0.000	0.000	0.000	0.001	
DWT	0.396	0.136	0.230	0.117	0.172	0.209	0.203	0.036	-0.049	0.014	0.078	0.463	0.560
	0.001	0.253	0.052	0.328	0.149	0.079	0.087	0.761	0.683	0.907	0.513	0.000	0.000

The first number in each cell indicates the Pearson coefficient of correlation. The second number in each cell indicates the *p*-value with an α-level of 0.05.

lot of variety J. For the cotyledons, the dry weight (DWC) gave some differences between lots of varieties G and K. The DWT showed differences between lots of varieties F and K. These results coincide to some degree with those of Van der Merwe et al. [6], who included biomass, particularly of the radicle, as a quality indicator for tomato seeds. In the present research, the dry weight of the radicle was able to differentiation of the lots for only half the varieties in question.

With regard to the distribution of dry weight in the seedlings (data not published), the highest percentage, close to 60%, was seen in the cotyledons, with the exception of varieties H and I. This is followed by the hypocotyl, and finally the radicle holds less than 20%, with the aforementioned exception.

3.6. Association between biometric characteristics of the seeds and seedling dry weight

As can be seen in Table 3, there is very little correlation between the total of the biometric characteristics of the seeds and the physical aspects of the resulting seedlings. Fig. 1 includes only the statistically significant correlations between the physical attributes of the seeds and the resulting seedlings. Only total dry weight of the seedlings was lightly associated with seed weight (Fig. 1f). The dry weight of the radicles showed a weak positive association with the seed size characteristics (Fig. 1a, Fig. 1b and Fig. 1c). The dry weight of the hypocotyl was not correlated with their respective seeds (Table 3). The latter result is contrary to those of Khan et al. [5], who found positive association between seed weight and radicle and hypocotyl weight for tomato seeds. The dry weight of the cotyledon was negatively correlated with seed area and seed length (Fig. 1d and Fig. 1e). These results can be explained by the work of Orsi and Tanksley [29], who found that the relation between the components of the seed (germ and endosperm) and its composition influences its behaviour.

Regarding the relation between the size and weight attributes of the seeds and the dry weight of the seedlings they produce, multiple regression showed that this association is low (Table 4). Thus, the highest coefficient was obtained for the model that represents how the DWR depends on the SL, SWi and SAr of the seeds. The DWC and the total DWT also present a relatively low R^2, though only the DWT showed association with the SW. These results demonstrate that there are other factors in the seeds that also determine quality, as seen in the definition of vigour [8]. Many authors have recently associated quality more with seed composition [3].

3.7. Association between seedling emergence and dry weight

The DWR was the only part of the seedlings to show positive correlation with seedling emergence on d 4 (S4d) and d 5 (S5d) after sowing (Fig. 1g, Fig. 1h). The DWR was also associated with the length of each of the parts and the total length of the seedlings (Fig. 1i, Fig. 1j, Fig. 1k). Bertholdsson and Brantestam [27] found a positive association between the weight and length of the radicle and seed quality in cereals. The DWH showed no association with seedling characteristics, while the dry weight of the cotyledon (DWC) was inversely related to total TL (Fig. 1l). The latter observation coincides with Bertholdsson and Brantestam [27].

3.8. Association between seedling emergence per observation day

There was association between seedling emergence on the 3 d of observation (Fig. 1m, Fig. 1n, and Fig. 1o), most notably on d 4 (S4d) and d 5 (S5d), with correlation coefficients above 0.90. As described previously by several researchers, the main germination events take place from 48 to 96 h after sowing [18,19,20], and as such mainly undamaged plants are to be expected [23]. Akbudak and Bolkan [11] found that the germination percentages at d 3 and 4 were more efficient for predicting seedling emergence, thus allowing the proposal of an early methodology for evaluating tomato seed quality.

4. Conclusions

Finally, it should be noted that the present research found little association between the physical characteristics of the seeds and the subsequent seedlings, making it impossible to propose the use of seed weight or size as a complement to quality evaluation tests.

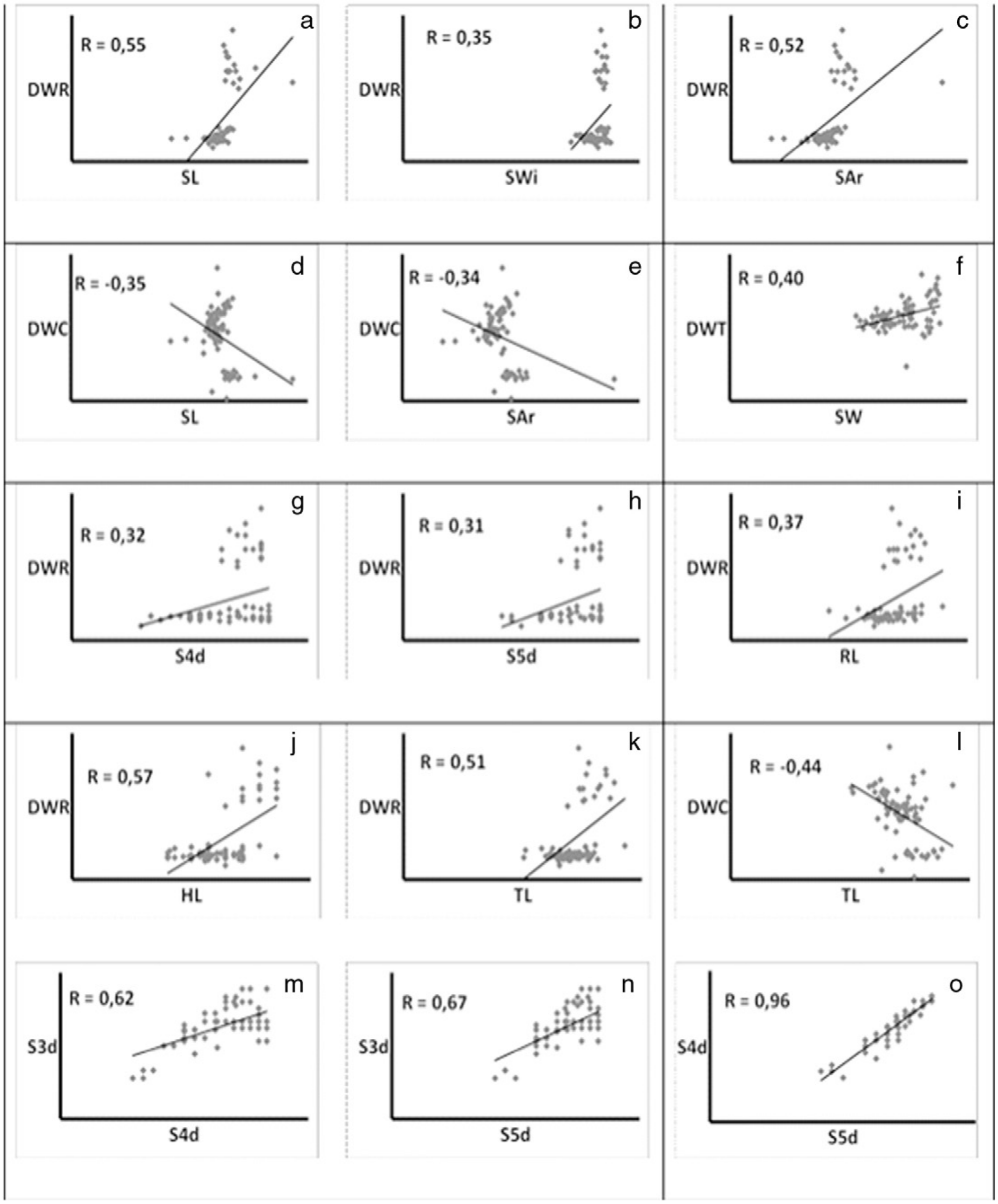

Fig. 1. Correlations between biometric characteristics of the seeds and seedling emergence. SW, SL, SWi, SAr, seedling emergence on d 3 after sowing (S3d), seedling emergence on d 4 after sowing (S4d), seedling emergence on d 5 after sowing (S5d), RL, HL, TL, DWR, DWH, DWC and DWT of the 18 tomato seed lots from six hybrid varieties.

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Table 4
Linear multiple regression model and correlation coefficient (R^2) between biometric characteristics of the seeds (SL, SWi, SAr, SW) and the dry weight of emerged seedlings (DWR, DWC, DWT) of the 18 tomato seed lots from six hybrid varieties.

Y	Significance of the equation ($p < 0,05$)	R^2
DWR	y = -2.984 + 0.47 · SL - 0.72 · SWi + 0.031 SAr	0.30
DWC	y = 2.921 − 0.35 · SL - 0.088 · Sar	0.20
DWT	y = 1.007 + 0.21 · SL + 0.386 · SW	0.14

Conflict of interest

No conflict interest.

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