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Application of digital image processing for seedling vigor estimation of primed tomato seed

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Abstract

Digital image processing was used to estimate the seedling vigor of tomato cv. Seeda-50 in lot A and lot B. Comparison was performed between seedling vigor of hydroprimed, osmoprimed seeds, and non-primed seeds (control). The results indicated that the osmoprimed seeds of the two lots had the highest germination, the shortest time to reach 50% germination, and the shortest mean germination time. However, priming had no effect on the growth and vigor of seedlings of lot A, except for leaf greenness and fresh weight of roots, whereas the seedlings of lot B from non-primed seeds showed better growth and vigor than seedlings from primed seeds. Images were taken by 48-megapixel digital camera using sunlight as a light source at a height of 30 cm above the seedling canopy. Digital image processing was performed with the MATLAB-based, image thresholding, and calculated pixels number per plant for all treatments. The correlation coefficients between number of pixels, seedling vigor index, and seed vigor were analyzed. The results show a correlation between the number of pixels and seedling vigor index. The regression equation was then conducted from lot A, where y was seedling vigor index and x was pixels per plant. The equation showed $y = 41.784 + 0.003x$, $R^2 = 0.678$. The equation was used to estimate seedling vigor index of lot B. The results indicated a highly significant relationship between seedling vigor index and seedling vigor index simulation, with a correlation coefficient ($r = 0.941$).

Keywords: Image analysis, Mean germination time, Seed priming, Seedling vigor index, Speed of germination

1. Introduction

Currently, tomato production is traditionally prepared by farmers as seed before transplanting to the fields in order to ensure even and steady growth of seeding vigor. Tomato seeds usually germinate slowly and unevenly due to dormant seeds, inadequate seed quality or unsuitable germination conditions. Moreover, transplanting could be harmful and promote unsafe growth and slow products [1]. Seed priming is a technique to improve seed germination and vigor, which contributes to high germination percentage, rapid germination, and uniform germination, including both supporting seedlings being able to persist and grow overall. In addition, vigorous seedlings were produced that were able to establish in and withstand harsh environments [2]. There were many methods such as hydropriming and osmopriming, with osmopriming performed by dissolved potassium nitrate (KNO_3). KNO_3 is used as a chemical agent to break dormancy [3,4] and is relatively cheap compared to other seed treatment agents and widely available in urban markets. There have been reports of primed tomato seeds with KNO_3 at different concentrations and durations, depending on the varieties and initial quality of seeds [5-7].

Transplanting tomato seedling occurs approximately 20-30 days after sowing or at the beginning of the 3-5 first true leaves. This is the period when a vigorous seedling with straight growth, healthy root system, and green leaf develops. There are many methods to measure the seedling vigor of tomato such as leaf greenness, leaf surface area, dry weight of shoot and root, shoot-root ratio, and seedling vigor index [8]. Some of these methods are harmful to seedlings (destructive methods), such as measurements used on leaf area and dry weight which require more time and labor. In addition, measuring the seedling vigor of a large number of seedlings is slow and increases seedling time. Therefore, an indirect method or non-destructive method to measure seedling vigor is required that must be fast and save costs. Currently, the technological development in the field of computer programming is so advanced that the calculations, especially for image processing, are simple and suitable. Digital images are processed or computed by a computer, so they are both qualitatively and quantitatively sophisticated. Using image processing would increase the accuracy of estimate procedure to seedling vigor. The result is a dramatically changed to translate a faster works, while there was a large amount of seedling depending on the size, figure, surface, and color of the objects [9-11]. These characteristics are recorded as pixels in a digital image and then specifically related to seedling vigor performance characteristics. In the mentioned activities, some limitations remain due to a lack of because accuracy because of some necessary influences such as seed characteristics and the image processing including calculation system. Silva and Cicero [12] utilized digital image processing for seedling vigor using a seed vigor imaging system (SVIS®) to estimate seedling vigor and seedling tomato cv. Santa Clara in 10 lots and obtained seedling length, seedling vigor index, and seedling growth with an efficient estimation of differences between a lot with high vigor and another lot with low vigor. In addition, Yamamoto et al. [13] reported node detection and estimation of internode length of tomato seedling by image analysis and machine learning. In this case, a computational simulation must be performed to more accurately determine the values derived from the images. The equation maintains the confidence capable of showing the relationships between various necessities and seedling vigor. Moreover, Beyaz et al. [14] determined the oxidation area and skin color change on damaged apples using an image analysis technique. It indicated that the impact tests response to impact brushing is dependent on flesh firmness and the impact height as well as the apple variety. Beyaz et al. [15] studied automatic methodologies based on computer vision to detect fly stings in infected olive fruit samples. The methodology to detect defective areas reached a success ratio of 93% and the best classification algorithms reached a success ratio close to 80%.

Rapid and uniform germination and seedling establishment is essential to increase tomato yield and quality, which is of great economic importance in agriculture. The aim of this study was to investigate a method of digital image to process and estimate the seedling vigor of primed and non-primed tomato seeds for further seedling production.

2. Materials and methods

2.1 Seed material

Seeds of F1 hybrid tomato cv. Seeda-50 were collected from the Thai Seed & Agriculture Co., Ltd in two lots (lot A and lot B) with different initial quality, such as seed moisture content of 6.4% and 6.1%, germination 84.5% and 89.5%, and mean germination time 8.17 days and 7.55 days, respectively. Seeds were newly harvested and kept in an aluminum foil zip-lock bags at 20 ± 2°C and 40 ± 2% relative humidity (RH) before priming treatments.

2.2 Seed priming treatment

The experiment was conducted in the Seed Technology Laboratory and greenhouse in the Experimental Field 1, Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand between July, and September 2019. The experiment was laid out in a completely randomized design (CRD) with three treatments and four replicates: (1) non-primed seeds (control); (2) hydropriming; and (3) osmopriming. A total of 200 tomato seeds were placed in a 100 mL beaker containing 50 mL of either the solution of 0% (reverse osmosis [RO] water; hydropriming), 1% KNO_3 solution for 48 h in lot A and 2% KNO_3 solution for 48 h in lot B (osmopriming) under natural light at 30 ± 2°C. After each treatment, seeds were rinsed thoroughly with RO water and dried back in electrical desiccator cabinet at a humidity of 40% RH and temperature of 35°C until the seed moisture content decreased to 6%, then the seeds of both primed and non-primed seeds were tested in the greenhouse condition in four replicates with 50 seeds per replicate.

2.3 Germination

The two lots of primed and non-primed tomato seed were sown in peat moss in a plastic tray with 104 holes in four replicates with 50 seeds per replicate in the greenhouse condition between July and September 2019. The mean light intensity inside the greenhouse was 227 μmol/m^2/sec, the highest light intensity during the day at noon was 542 μmol/m^2/sec, the temperature during the day and night was 28°C and 25°C, and the mean relative humidity was 47.59%. First and final counts were recorded at 5 days and 14 days after sowing, respectively. The percentage of normal seedlings were recorded. Normal seedlings consisted of those seedlings with fully developed primary root, hypocotyl, and two cotyledons. The percentage of abnormal seedling, fresh ungerminated seed, and dead seed also were recorded and used to calculate the final count. Germination was calculated using Equation 1:

$$\text{Germination (\%)} = (\text{number of normal seedlings} / \text{total seeds}) \times 100 \quad (1)$$

2.4 Time to reach 50% germination (T_{50})

T_{50} was determined by counting the number of normal seedlings daily for 14 days. In the calculation, the number of days was used to reach the germination of half of the normal seedlings. T_{50} was calculated using Equation 2 [16]:

$$\text{T50} = \text{ti} + [[((\text{N} + 1) / 2 - \text{ni}) / (\text{nj} - \text{ni})] \times (\text{tj} - \text{ti})] \quad (2)$$

where N is the final number of germinated seeds and n_i and n_j are the total numbers of seeds germinated in adjacent counts at times t_i and t_j, respectively, for $n_i < (N + 1) / 2 < nj$.

2.5 Mean germination time (MGT)

The number of normal seedlings was counted daily from the first days after testing until the day of the final count (14 days after sowing). The MGT was calculated using Equation 3 [17]:

$$\text{MGT} = \sum (\text{n T}) / \sum \text{n} \quad (3)$$

where n is the number of normal seedlings which germinated on day d and d is the number of days counted from the beginning of the test.

2.6 Seedling growth and vigor

Tomato seedlings at 28 days after sowing were randomized by four replicates with 10 plants per replicate. The following data was recorded: 1) plant height, by measuring from the base level of peat moss up to the shoot apex; 2) stem diameter, by measuring at the cotyledon node; 3) leaf number, by counting the true leaves at full surface with the leaf length and width around 1 cm; 4) leaf greenness, using the Soil Plant Analysis Development (SPAD)-502 Chlorophyll Meter, Konica Minolta; 5) fresh weight of shoot and root; 6) dry weight of shoot and root; 7) shoot-root ratio; and 8) seedling vigor index, calculated from Equation 4 [18]:

$$\text{Seedling vigor index} = \text{germination (\%)} \times \text{dry weight of seedling} \quad (4)$$

2.7 Digital image processing

Images were taken using a camera with a resolution of 48 megapixels (MP) at 72 dots per inch (dpi) using sun light as a light source at a height of 30 cm above the canopy of the seedling at 28 days after sowing, which was an appropriate time for transplanting the seedling. As the seedling was vigorous, it had a full leaf that was at a right angle to the stem so that the entire leaf could be seen during inspection.

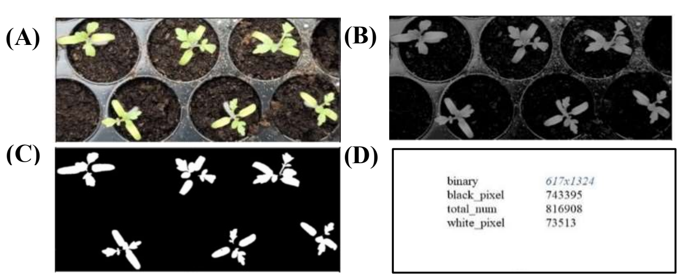

Figure 1 (A) Digital image processing steps during the pixel number calculation, (B) digital images were converted to gray scale, (C) converting the signal to a binary image, and (D) the pixel numbers on the leaf surface of tomato seedlings.

This study was developed using a matrix laboratory (MATLAB)-based image processing algorithm to calculate the pixel number per plant of all tomato seedling treatments (Figure 1A), from the sample group of treatment for digital images using 6 plants per mean of pixel. The seedlings of lot A and lot B under 24 experimental units were collected from a total of 144 plants. Digital images were converted to gray scale (Figure 1B) and image segmentation was performed by separating the front image from the threshold [19] by converting the signal to a binary image (Figure 1 C). Then, the pixel numbers on the leaf surface of tomato seedlings (Figure 1 D) and the pixel number per plant were calculated.

2.8 Statistical analysis

All data was analyzed using R program and were subjected to an analysis of variance according to the CRD used and the means were compared using Least Significant Difference (LSD). Equation analysis by estimating leaf area of tomato seedling to create simulation, where could be used to explain the relationship between seedling vigor and pixel number per plant in a comparison with efficiency of simulation. Based on the coefficient of determination (R^2), calculated and verification continued for the correct simulation as well. The simulation tests required to mimic the value of seedling vigor by image processing were compared to the seedling vigor index.

3. Results and discussion

3.1 Gemination

The germination percentage of tomato seeds of lot A and lot B after priming treatments and non-primed seeds is shown in Table 1. The results show that the seedlings of lot A and lot B after osmopriming had the highest germination of 98.00% and 98.50%, respectively without any statistical difference from hydroprimed seeds, which had germination at 95.00% and 97.50%, respectively. From the results, non-primed seeds had the lowest germination at 94.50% and 89.50%, respectively. The results indicate that osmoprimed seeds of lot A increased germination from 94.50% (control) to 98.00% or by 3.50%, as compared to non-primed seeds. However, hydroprimed seeds of lot A showed that there was not any effective statistic on germination compared with non-primed seeds, whereas hydroprimed and osmoprimed seeds of lot B increased germination from 89.50% (control) to 97.50-98.50% or by 8.00-9.00% when compared with non-primed seeds.

Therefore, germination of osmoprimed tomato seeds in lot A and osmoprimed or hydroprimed seeds in lot B could be increased. This might be due to the different initial quality of seed lot A and lot B responding to the different seed priming. Moreover, the seeds of lot A had a rather high germination at 94.50% even after hydropriming, the germination was not affected on germination, but osmoprimed seeds using KNO_3 resulted in enhanced germination. KNO_3 is a chemical agent that is used to break seed dormancy [4]. KNO_3 is distinct into K^+ and NO_3^-while NO_3^- is an electron acceptor for respiration in the pentose phosphate pathway, replacing oxygen in NADPH in the respiration process and leading to seed germination [20]. Similarly, Siri et al. [21] reported that tomato F1 hybrid seeds with high initial quality as seed process by hydropriming or osmopriming, however, when using some types of chemical materials. This might be affected by slightly enhanced seed germination, in agreement with Mirabi and Hasanabadi [7], who reported seed priming tomato cv. ZD610 with KNO_3 had the highest germination (100%) in a greenhouse condition when compared with non-primed seeds (only 70% germination).

Table 1 Germination, time to reach 50% germination (T_{50}), and mean germination time (MGT) of primed tomato seeds in lot A and lot B at 14 days after sowing.

Treatment	Germination (%)		T_{50} (days)		MGT (days)	
	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B
Non-primed seeds (control)	94.50^b	89.50^b	4.81^a	4.65^a	5.45^a	5.32^a
Hydropriming	95.00^{ab}	97.50^a	3.80^b	3.66^b	4.65^b	4.42^b
Osmopriming	98.00^a	98.50^a	3.55^b	3.53^c	4.16^c	4.13^c
F test	*	*	*	*	*	*
CV (%)	2.17	3.45	6.34	1.19	6.03	2.86

*Significantly different at $p \leq 0.05$.
Mean values in the same column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

3.2 Time to reach 50% germination

Tomato seed in lot A treated with osmopriming and hydropriming had the highest speed of 50% germination and was not statistically different from hydropriming (3.55 days and 3.80 days, respectively), while non-primed seeds had the lowest speed of 50% germination (4.81 days) (Table 1). It was shown that seeds in lot A primed by hydropriming and osmopriming resulted in 50% faster germination than non-primed seeds. The highest germination rate of 50% was achieved in lot B by osmopriming at 3.53 days, whereas the germination rate was slower for hydroprimed seeds (3.66 days) and non-primed seeds (4.65 days), respectively. It was shown that seed priming in lot B by osmopriming resulted in 50% germination faster than hydropriming and non-primed seeds. Thus, seed priming resulted in faster germination because the primed seeds could trigger the metabolic process (phase 2) or lag phase, in which water is not fully absorbed so that the seeds required osmosis for amino acids, proteins and a greater amount of sugars in the seeds [22]. Therefore, when the primed seeds start to germinate, the seed can absorb more water and germinate faster in phase 2, especially faster in phase 3 than non-primed seeds [2].

3.3 Mean germination time

Seed priming in tomatoes of lot A and lot B by osmopriming resulted in a mean germination time of 4.16 days and 4.13 days, respectively, and more days with priming seeds by hydropriming (4.65 days and 4.42 days, respectively) and non-primed seeds (5.45 days and 5.32 days, respectively) (Table 1). It was shown that osmoprimed seeds were found to germinate faster in mean germination time than hydroprimed and non-primed seeds. Since primed seed was refilled with water and germinated rapidly, the physical and biochemical processes inside the seed were most important for germination [23]. Moreover, the KNO_3 solution had related with nitrate reductase associated with the processing of osmotic protein in seeds to contribute to better and faster germination [24]. In addition, Mirabi and Hasanabadi [7] reported tomato seed of cv. ZD 610 primed with 5% KNO_3 solution for 48 h, which resulted in the seed having the fastest mean germination time in the greenhouse, with a germination time of 5.63 days compared to non-primed seeds (14.00 days), similar to Srepho [25] who reported that hydroprimed and osmoprimed seeds of tomato for 9 hours and incubation periods of 9 hours had the fastest mean germination time in greenhouse. Similarly, Farooq et al. [5] reported that four varieties of osmoprimed tomato seeds with KNO_3 solution (-1.25 MPa or 2.5%) for 24 hours had the highest and fastest germination. The results of this study revealed that seed priming treatments improved the germination percentage and speed of germination or seed vigor, such as T_{50} and MGT of both tomato lots under greenhouse conditions.

3.4 Seedling growth and vigor at 28 days after sowing

Seedlings from the primed tomato seed of lot A by hydropriming and osmopriming did not statistically affect plant height, stem diameter, leaf number, fresh weight and dry weight of shoot, and dry weight of root when compared with seedlings from non-primed seeds, except for leaf greenness as shown in Tables 2-4. Seedlings of lot A from osmoprimed seeds had the highest leaf greenness but there was no statistical difference when compared with seedlings from hydroprimed seeds with 15.72 and 15.35 SPAD unit, respectively, while seedlings from non-primed seeds had the lowest leaf greenness of 12.23 SPAD unit (Table 3). This is consistent with Shafiq et al. [26] which reported that cotton plants from KNO_3 primed seeds had higher values for chlorophyll *a* and *b* content that elevated total chlorophyll content. These results can be explained on the basis of a positive influence of nitrogen and potassium of plant physio-biochemical functioning. In contrast, seedlings from non-primed seeds of lot B had the highest plant, stem diameter, leaf number, shoot and root fresh weight, shoot and root dry weight, and seedling vigor index (Tables 2-4). However, our findings are contradictory with previous reports, such as Mirabi and Hasanabadi [7] who reported that primed tomato seed cv. ZD 610 using KNO_3 had the highest seedling and fresh weight of shoot and root. Ziaf et al. [27] reported primed pepper seed had potential for vigorous seedlings. In addition, Amjad et al. [28] reported that pepper seed primed with KNO_3 solution had the highest seedling height, fresh weight, and vigor.

Table 2 Plant height, stem diameter, and leaf number of tomato seedling in lot A and lot B at 28 days after sowing.

Treatment	Plant height (cm)		Stem diameter (mm)		Leaf number	
	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B
Non-primed seeds (control)	5.43	6.36^{a}	0.97	1.14^{a}	2.38	2.53^{a}
Hydropriming	5.20	4.75^{b}	0.93	0.86^{b}	2.43	2.05^{b}
Osmopriming	5.48	4.82^{b}	1.02	0.84^{b}	2.33	2.00^{b}
F test	ns	*	ns	*	ns	*
CV (%)	11.31	11.32	13.98	12.31	13.19	8.84

ns not significant.
*Significantly different at $p \leq 0.05$.
Mean values in the same column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

Table 3 Leaf greenness and fresh weight of shoot and root of tomato seedlings in lot A and lot B at 28 days after sowing.

Treatment	Leaf greenness (SPAD unit)		Fresh weight (mg/plant)			
			Shoot		Root	
	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B
Non-primed seeds (control)	12.23^{b}	11.77	14.01	17.84^{a}	6.61^{ab}	7.80^{a}
Hydropriming	15.35^{a}	14.36	12.17	9.79^{b}	6.01^{b}	4.36^{b}
Osmopriming	15.72^{a}	12.38	14.64	10.18^{b}	9.06^{a}	5.26^{b}
F test	*	ns	ns	*	*	*
CV (%)	12.19	30.96	24.43	22.65	25.23	22.22

ns not significant.
*Significantly different at $p \leq 0.05$.
Mean values in the same column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

Table 4 Dry weight of shoot and root, shoot-root ratio, and seedling vigor index of tomato seedlings in lot A and lot B at 28 days after sowing.

Treatment	Dry weight (mg/plant)				Shoot-root ratio		Seedling vigor index	
	Shoot		Root					
	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B	Lot A	Lot B
Non-primed seeds (control)	0.89	1.31^{a}	0.41	0.62^{a}	2.17	2.26	123.09	174.48^{a}
Hydropriming	0.93	0.75^{b}	0.38	0.32^{b}	2.52	2.34	124.03	104.10^{b}
Osmopriming	1.00	0.75^{b}	0.38	0.34^{b}	2.86	2.31	135.49	107.00^{b}
F test	ns	*	ns	*	ns	ns	ns	*
CV (%)	22.85	27.17	28.40	30.54	28.56	28.76	23.12	24.37

ns: not significant.
*Significantly different at $p \leq 0.05$.
Mean values in the same column followed by the same letter are not significantly different at $p \leq 0.05$ by LSD.

3.5 Digital image analysis of seedling vigor

Analysis of the correlation coefficients of each study factor using data from the A and lot B tomatoes (Table 5) revealed that the number of pixels per plant with seedling vigor index had only one value with a correlation coefficient of 0.904**. Since dry weight of seedling was related to leaf size [29], resulted to relation on pixel number per plant, it was considered that possibly image processing data (pixel number per plant) should be provided to estimate seedling vigor index, while pixel number per plant and seedling vigor index had no relationship with seed vigor factors. Since seedling dry weight is related to leaf size [29], it was found to be correlated with pixel number per plant, so it is considered that possibly image processing data should be provided to estimate seedling vigor index, while image processing data and seedling vigor index have no relationship with seed vigor factors.

It was found that the positive factors composted of germination were unfavorably related to the mean germination time and time to reach 50% germination with correlation coefficients of -0.653** and -0.628**, respectively. Whenever the seed showed high germination, it resulted in an increase in mean germination time and time to reach 50% germination. The data shows that the seeds had vigorous and rapid germination, while the seeds had a mean germination time and were related with time to reach 50% germination with the correlation coefficient of 0.978**. Moreover, leaf greenness was not found to be related to other factors.

Table 5 Correlation coefficient between pixel, seedling vigor index, and seed vigor.

Factor	Pixel	Seedling vigor index	Leaf greenness	Germination	MGT	T_{50}
Pixel	1.000	0.904**	-0.088 ns	-0.259 ns	0.268 ns	0.276 ns
Seedling vigor index	0.904**	1.000	-0.076 ns	-0.078 ns	0.139 ns	0.166 ns
Leaf greenness	-0.088 ns	-0.076 ns	1.000	-0.001 ns	-0.193 ns	-0.283 ns
Germination	-0.259 ns	-0.078 ns	-0.001 ns	1.000	-0.653**	-0.628**
MGT	0.268 ns	0.139 ns	-0.193 ns	-0.653**	1.000	0.978**
T_{50}	0.276 ns	0.166 ns	-0.283 ns	-0.628**	0.978**	1.000

** = significantly different at $p \leq 0.01$.
ns = not significant.

The results of the correlation coefficient analysis, which included all the tomato data from lot A, considered the relationship between the number of pixels per plant and seedling vigor index through the required equation, where y was the seedling vigor index and x was the number of pixels per plant. The equation resulted in $y = 41.784 + 0.003X$, $R^2 = 0.678$, as shown in Figure 2. Then, the equation was applied to the seedling vigor of lot B by simulation and compared with the seedling vigor index in the greenhouse. It was found that there was a highly significant relationship and correlation coefficient ($r = 0.941$), as shown in Figure 3. This shows that the ability to obtain better results in digital image processing for seedling vigor could be used to estimate seedling vigor without harming the seedlings. However, there were some limitations with this estimation program when the seedling had a full leaf and a one-side stem. Future research should be conducted to develop equation angles with stems, including the use of digital images from different angles captured for collective estimates.

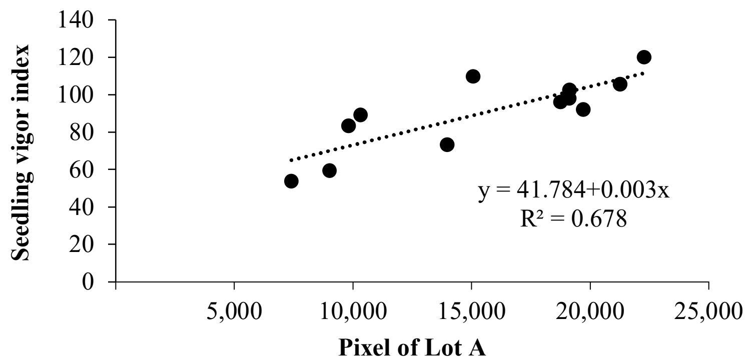

Figure 2 Relationship between the number of pixels and seedling vigor index of lot A.

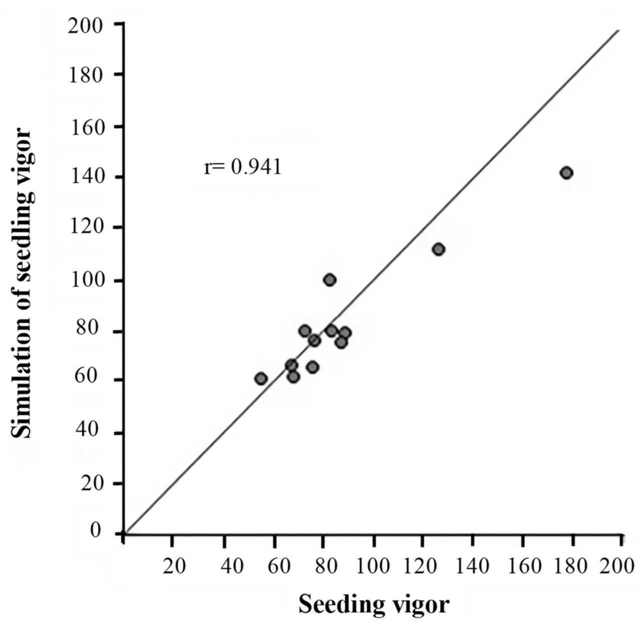

Figure 3 Relationship between seedling vigor index and seedling vigor index simulation of lot B.

4. Conclusion

Osmoprimed tomato seed of the two lots had the highest germination, the shortest time to reach 50% germination, and the shortest mean germination time. However, priming had no effect on the growth and vigor of seedlings of lot A, except for leaf greenness and fresh weight of roots, whereas the seedlings of lot B from non-primed seeds showed better growth and vigor than seedlings from primed seeds. Moreover, the correlation between the number of pixels and seedling vigor index. The regression equation was conducted from lot A, where y is the seedling vigor index and x is the number of pixels per plant. The equation showed $y = 41.784 + 0.003x$, $R^2 = 0.678$. The equation was used to estimate the seedling vigor index of lot B. The results show a highly significant relationship between seedling vigor index and seedling vigor index simulation, with a correlation coefficient ($r = 0.941$). Image processing based on the pixel number proved to be an efficient procedure characterization in the evaluation of tomato seedling vigor. The feasibility of using an outdoor natural light machine vision system for seedling vigor estimation and develop robot seedling vigor classification in the future.

5. Acknowledgements

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