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Análise de imagens de plântulas para avaliação do potencial fisiológico de sementes de tomate

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ABSTRACT - Computerized seedling image analysis are one of the most recently techniques to detect differences of vigor between seed lots. The aim of this study was verify the hability of computerized seedling image analysis by SVIS® to detect differences of vigor between tomato seed lots as information provided by traditionally vigor tests. Ten lots of tomato seeds, cultivar Santa Clara, were stored for 12 months in controlled environment at 20 ± 1 °C and 45-50% of relative humidity of the air. The moisture content of the seeds was monitored and the physiological potential tested at 0, 6 and 12 months after storage, with germination test, first count of germination, traditional accelerated ageing and with saturated salt solution, electrical conductivity, seedling emergence and with seed vigor imaging system (SVIS®). A completely randomized experimental design was used with four replications. The parameters obtained by the computerized seedling analysis (seedling length and indexes of vigor and seedling growth) with software SVIS® are efficient to detect differences between tomato seed lots of high and low vigor.

Key words: *Solanum lycopersicum* L.. SVIS. Vigor.

RESUMO - A análise computadorizada de imagens de plântulas é uma das técnicas mais recentes para detectar diferenças de vigor entre lotes de sementes. O objetivo deste trabalho foi verificar a possibilidade de utilização do sistema computadorizado de análise de imagens de plântulas SVIS® (Seed Vigor Imaging System) para detectar diferenças de vigor entre lotes de sementes de tomate em comparação as informações fornecidas por testes de vigor tradicionalmente utilizados. Dez lotes de sementes de tomate, cultivar Santa Clara, foram armazenados por 12 meses em ambiente controlado a 20 ± 1 °C e 45-50% de umidade relativa do ar. O teor de água das sementes foi monitorado e o potencial fisiológico avaliado aos 0, 6 e 12 meses após o armazenamento, com os testes de germinação, primeira contagem de germinação, envelhecimento acelerado tradicional e com solução saturada de sal, condutividade elétrica, emergência de plântulas e com o sistema de análise computadorizada de plântulas (SVIS®). O delineamento experimental utilizado foi inteiramente casualizado com 4 repetições. Os parâmetros obtidos na análise computadorizada de plântulas (comprimento de plântulas e índices de vigor e de crescimento de plântulas) com o software SVIS® são eficientes para diferenciar lotes de sementes de tomate de alto e de baixo vigor.

Palavras chave: *Solanum lycopersicum* L.. SVIS. Vigor.

INTRODUCTION

The use of high quality seeds is essential to obtaining high quality seedlings. Seed quality can be defined by four components: genetic, sanitary, physic and physiological, and the last one represents germination capacity and seed vigor. The seed quality evaluation is essential in quality control programs, which must employ efficient, low cost and replicable methods. This evaluation is usually made through germination test and can be complemented by vigor tests, especially because germination test, once it is performed under controlled conditions, might overestimate the seed potential.

Vigor tests have been used mainly to identify differences associated to the seed performance during storage or after sowing, trying to highlight more highly efficient lots for establishing the stand under broad variation of ambient conditions (MARCOS FILHO *et al.*, 2009). Among the vigor tests most commonly utilized, some are based on determinations of speed and uniformity of seedling growth or manual measurement of their parts. Tests of seedling length based on manual measurement present two limitations at least: results may vary from laboratory to laboratory due to the subject nature of the analysis and take excessive time to acquire results. By automating these tasks through the use of image processing computer programs, these two limitations can be addressed (HOFFMASTER *et al.*, 2003).

Recently, for seed vigor evaluation based on seedling growth, Sako et al. (2001) developed at Ohio State University/USA an automatized system for lettuce seed vigor evaluation, named Seed Vigor Imaging System (SVIS®); for that, scanned seedlings, which parts were identified and marked by a specific software, were worked on. After image processing on a computer, the following data were simultaneously obtained: the length of the primary root, of the hypocotyl and of the whole plant, besides the ratio root/hypocotyl and parameters based on speed and uniformity of growth. This kind of analysis has been making possible the determination of seed vigor of other species such as wheat (SILVA *et al.*, 2012a), sweet corn (ALVARENGA *et al.*, 2012), cucumber (CHIQUITO *et al.*, 2012), sunn hemp (SILVA *et al.*, 2012b), soybean (MARCOS FILHO *et al.*, 2009), melon (MARCOS FILHO *et al.*, 2006), evaluation of the effect of priming on sweet corn seeds (GOMES JUNIOR *et al.*, 2009) and the evaluation of the effect of fungicide treatments in the castor bean seed quality (KOBORI *et al.*, 2010) and peanut (MARCHI *et al.*, 2011). The method consists on the caption of multiple digital images, by scanning, of seedling, which is next processed on a computer, generating values which, collectively, represent the physiological potential of the seeds. Data include seedling lengths and vigor and growth indexes (SAKO *et al.*, 2001). After image processing, the software automatically generates values which refer to the rate of vigor (values from 0 to 1000, in direct proportion to the vigor) and of growth (also from 100 to 1000).

Nowadays, there are not researches related to the use of this technique for tomato seed quality evaluations, therefore this paper had the aim to verify the hability of computerized seedling image analysis by SVIS® to detect differences of vigor between tomato seed lots as information provided by traditionally vigor tests.

MATERIAL AND METHODS

The research was performed at the Laboratories of Seed and Image Analysis of the Crop Production Department of the Agriculture College "Luiz de Queiroz" at Sao Paulo University, located in Piracicaba, SP, Brazil. Ten tomato seed lots, cultivar Santa Clara, treated with Thiram were stored in aluminium packages, hermetically sealed, in a controlled environment room at 20 ± 1 °C and 45-50% of relative humidity of the air, after verification of seed moisture content. Physiological seed potential was evaluated through tests described as follows.

Moisture content: the oven method was adopted, at 105 ± 3 ºC, for 24 hours with two samples of 1.0 g seeds per each lot and the results were registered in percentages (wet basis) (BRASIL, 2009). **Germination and first counting of germination:** four replications of 50 seeds for each lot were distributed over two blotting paper sheets, moisturized with an amount of water equivalent to 2.5 times the dry mass of the subtract, inside plastic boxes (11.0 x 11.0 x 3.5 cm) and exposed to 20-30 °C with 8 hours of light and 16 hours of darkness. The evaluations were performed at 5 and 14 days after sowing (BRASIL, 2009). The results were registered in percentages of normal seedling per each lot. **Traditional accelerated ageing:** a layer of seeds was distributed over a metal screen attached to a plastic box (11.0 x 11.0 x 3.5 cm) containing 40 ml of distilled water on the bottom. Each box was covered and exposed to 41 °C for 48 hours in an incubator BOD; next, it was determined the seed moisture content and a germination test (four replications) was performed with evaluations after five days (MARCOS FILHO, 1999). **Saturared salt accelerated ageing**: made similarly to the traditional procedure, however with the use of 40 ml of saturated NaCl solution instead of water (JIANHUA; McDONALD, 1996). **Electric conductivity:** four replications of 25 seeds

for each lot were weighted and, following to that, immersed in 75 ml of distilled water, where they were left for 24 hours at 25 °C (ISTA, 1995). The reading of electric conductivity of the solution was made on a conductivemeter branded DIGIMED, model DM-31. The values of the readings were divided by the mass of the seeds and the results were expressed in $\mu S\ cm^{-1}\ g^{-1}$ per seed. **Seedling emergence**: four replications of 50 seeds per lot were sowed on trays containing proper commercial subtract for production of oleraceous seedlings (Plantmax®). At 14 days after the sowing the immersed seedlings were counted (RODO, 1998).

Computerized seedling image analysis by software SVIS®: preliminary tests were at first performed aiming to check the most adequate method for obtaining seedlings for this analysis. Several conditions were tested to obtain seedlings as at 3, 4 and 5 days at 20-30 °C or 25 °C, in rolls of paper towel or paper in plastic box (11.0 x 11.0 x 3.5 cm), with light or darkness. The best condition for obtaining tomato seedlings, which was used in this research, was to submit four replications of 25 seeds for each lot at 20-30 °C in a germination chamber during 4 days in the absence of light in paper towel rolls moisturized with distilled water 2.5 times the mass of the paper.

Next, the seedlings were transferred from the paper towel to a black colored paper sheet being the seedling images obtained through scanning by a machine model HP Scanjet 2410, assembled upside down within an aluminum box operated by the software Photosmart, at a 100 dpi resolution. Next, the images were analyzed using the software SVIS® (Seed Vigor Imaging System), installed in a Pentium IV computer operated by Windows XP Professional system. In this analysis, the axis root/hypocotyl of each seedling was marked red, using the software itself. After the analysis and evaluation of each seedling, the software SVIS® provided the values for the rate of vigor (VI) and of growth (GI) and seedling length (SL). The data of seedlings length were provided by the software in pixels and were converted to cm, being a pixel considered as equivalent to 0.0254 cm.

The experimental design was completely randomized with four replications. Three evaluation periods were made, at 0, 6 and 12 months after storage. The data were submitted to analysis of variation and comparison of means through the Tukey test at 5% significance. Besides, the type of distribution of percentual data was verified and the Cochran test was applied aiming to check the uniformity of the variance. The data which did not present normal distribution and variance uniformity were changed into arc sen $(x/100)^{1/2}$.

RESULTS AND DISCUSSION

The data of initial characterization of tomato seed lots can be observed in Table 1. The seed moisture content for the 10 lots varied from 6.2 to 7.7% in the first period; it is desirable, considering that a huge difference between the lots regarding to moisture content, could influence results of vigor, which has not occurred in this research in all periods (Tables 1 and 2).

The percentage of tomato seed germination was similar to all the lots in the first period and over 85% in most of the lots (Table 1); it is important to highlight that lots with a distinct physiological potential may present similar germination, which can be granted to the lack of sensitivity of this test as to identify small differences between lots, considering that the loss of germination capacity is one of the last stages in the process of seeds deterioration (DELOUCHE; BASKIN, 1973). Besides, the germination test is performed in controlled environment, possibly overestimating the potential of the seeds performance.

Despite germination results, vigor tests revealed differences between the lots. However, the lots classification based in vigor levels was not always the same; in general, lots 4 and 9 had a worse performance and lot 3 a better performance in the first period (Table 1). It must be highlighted that the seed vigor is not a single measurable property, like standard germination, but a concept describing several characteristics associated with one or more aspects of seed lot performance (TEKRONY, 2003); therefore, reunion of these information can make the forecast of seeds behavior more efficient.

The computerized seedling analysis with SVIS® was effective to differed tomato seed lots with less or more vigor in the first period of evaluation, based on the three parameters utilized. The best physiological potential of lots 3, 6 and 7 as compared to lot 9 was observed from the vigor index, the same way for lots 3, 6, 7 and 8 as compared to 5 from the growth index and for lot 3 as compared to 9 from seedling length (Table 1). An example of seed imaging analysis by SVIS is presented in Figure 1, in which are highlight the vigor index (555), uniformity of seedling development (998), and total seedling length (indicated by white arrow- 222 pixels).

In general, considering all three parameters, lots 3, 6 and 7 were superior to lot 9. Comparing these results to the other vigor tests, it's possible to observe coherence, because in the set of tests most commonly used lots 6 and 7 had higher physiological potential compared to others and lot 9 also had low performance in these evaluations.

Table1 - Moisture seed content (MC), germination (G), first count germination (FCG), traditional accelerated ageing (AA), saturated salt accelerated ageing (AASS), seedling emergence (SE), electrical conductivity (EC), vigor index (VI), seedling growth index (SGI) and seedling length (SL) of ten tomato seed lots, cultivar Santa Clara, first and second analyzed periods

Lot	MC	G*	FCG*	AA	AASS*	SE	EC	VI	SGI	SL
				-------(%)-------			$\mu S\ cm^{-1}\ g^{-1}$			
					First period					
1	6,2	89 a[1]	77 ab	46 ab	61 abc	80 b	241 d	599 ab	521 ab	4,0 ab
2	6,3	88 a	60 ab	51 ab	72 ab	84 ab	229 bcd	598 ab	531 ab	3,9 ab
3	6,3	90 a	68 ab	62 a	56 bc	92 a	235 cd	645 a	572 a	4,7 a
4	6,5	91 a	59 b	35 b	68 ab	90 ab	213 abcd	608 ab	525 ab	4,2 ab
5	6,4	84 a	71 ab	52 ab	65 ab	89 ab	216 abcd	572 b	479 b	3,5 ab
6	7,2	87 a	65 ab	58 a	62 abc	84 ab	192 a	640 a	586 a	4,2 ab
7	7,7	89 a	77 ab	59 a	76 a	86 ab	218 abcd	642 a	566 a	4,4 ab
8	7,6	91 a	83 a	53 ab	76 ab	83 ab	204 abc	604 ab	594 a	4,4 ab
9	7,0	86 a	59 b	54 ab	44 c	81 ab	193 ab	554 b	507 ab	2,8 b
10	7,5	89 a	73 ab	65 a	74 ab	83 ab	240 cd	588 ab	517 ab	4,2 ab
CV (%)	-	5.7	13.8	17.8	11.6	6.1	6.9	4.4	6.9	18.2
					Second period					
Lot	MC	G*	FCG*	AA	AASS*	SE	EC	VI	SGI	SL
				-------(%)-------			$\mu S\ cm^{-1}\ g^{-1}$			
1	7,7	77 ab[1]	40 b	51 ab	66 abc	87 bcd	194 e	478 ab	401 ab	2,0 bc
2	7,7	74 ab	44 b	54 ab	73 abc	91 bc	164 d	451 ab	329 bcd	2,0 bc
3	7,5	73 ab	57 ab	55 ab	75 abc	89 bcd	155 cd	481 ab	377 abc	2,1 bc
4	7,3	77 ab	47 b	60 ab	74 abc	90 bc	140 cd	422 b	299 d	1,5 c
5	7,1	77 ab	48 ab	48 b	70 abc	96 a	135 bc	453 ab	323 cd	1,9 bc
6	7,3	68 b	57 ab	55 ab	54 c	83 de	105 a	494 ab	420 a	2,0 bc
7	7,3	76 ab	63 ab	48 b	75 abc	90 bc	110 ab	484 ab	380 abc	2,5 ab
8	7,2	80 ab	58 ab	49 b	81 a	92 ab	107 ab	469 ab	371 abcd	2,3 abc
9	6,5	83 a	73 a	48 b	57 bc	79 e	274 f	516 a	425 a	2,9 a
10	6,5	84 a	73 a	68 a	63 abc	85 cde	290 f	522 a	418 a	3,0 a
CV(%)	-	9.3	19.2	13.9	12.0	3.4	6.9	6.8	8.5	14.6

*Data transformed at arc sin $(x/100)^{1/2}$. [1] Means comparison within each column by the Tukey's test ($p<0.05$)

As well as computerized seedling analysis, most of vigor tests used only showed differences between lots of higher and smaller potential, not allowing separation of lots of medium vigor. It is important to emphasize that there was not any significant difference in germination speed, evaluated in the first counting of the test, therefore possibly owing to that most lots have not differed regarding SVIS® indexes and seedling length, because these parameters are calculated based on speed and uniformity of seedlings growth.

Afterwards, in the second and third period, the germination test showed differences of potential between the lots; in the second period, lots 9 and 10 were better than lot 6 (Table 1), and in the third period, lot 4 was greater than lots 1, 3 and 9 (Table 2). It must be emphasized that in the third period, although the germination percentage of most lots have reached minimal standards for trading in Brazil, the vigor tests showed low physiological potential of these seeds.

Table 2 - Moisture seed content (MC), germination (G), first count germination (FCG), traditional accelerated ageing (AA), saturated salt accelerated ageing (AASS), seedling emergence (SE), electrical conductivity (EC), vigor index (VI), seedling growth index (SGI) and seedling length (SL) of ten tomato seed lots, cultivar Santa Clara, third analyzed period

Lot	MC	G*	FCG*	AA	AASS*	SE	EC	VI	SGI	SL
			----------------------------(%)--------------------------------				$\mu S\ cm^{-1}\ g^{-1}$			
1	7,1	66 bc1	55 ab	47 abc	58 abc	91 a	132 a	413 c	261 d	2,2 c
2	7,0	64 abc	64 ab	24 e	44 cde	88 ab	120 a	444 abc	303 abcd	2,5 abc
3	6,9	61 bc	61 ab	30 de	30 e	84 bc	119 a	426 bc	299 bcd	2,1 c
4	6,8	89 a	64 ab	40 bcde	58 abc	79 c	120 a	413 c	254 d	2,2 c
5	6,4	82 ab	60 ab	46 abcd	53 abc	80 c	123 a	429 bc	279 cd	2,3 bc
6	6,8	80 ab	76 a	61 ab	63 ab	83 bc	98 a	470 abc	348 abc	2,7 abc
7	6,7	82 ab	70 a	59 ab	66 a	82 bc	100 a	490 a	373 ab	3,0 ab
8	6,5	86 ab	72 a	64 a	63 ab	84 bc	92 a	477 ab	359 ab	2,8 abc
9	6,1	56 c	43 b	29 cde	34 de	83 bc	325 b	484 ab	348 abc	3,1 a
10	6,0	81 ab	65 ab	31 cde	48 bcd	87 ab	334 b	482 ab	377 a	2,8 abc
Cv (%)	-	9.3	10.9	13.0	13.6	2.8	11.4	5.3	9.8	12.9

*Data transformed at arc sin $(x/100)^{1/2}$. [1] Means comparison within each column by the Tukey's test ($p < 0.05$)

Figure 1 - Digital images of 4 day-old tomato seedlings, cv. Santa Clara, from seed lot 2, and assessed by the software SVIS®. In highlight: vigor index (555), uniformity of seedling development (998), and total seedling length (indicated by white arrow-222 pixels)
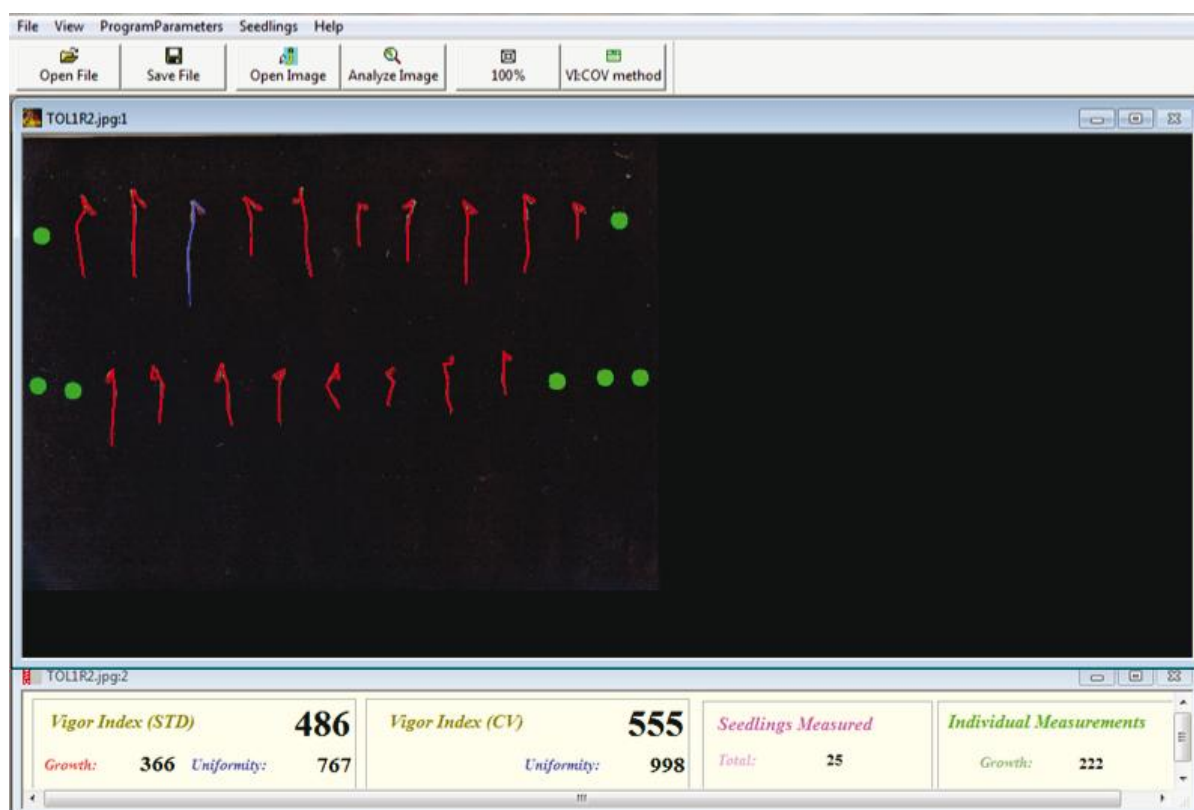
Similarly to the initial characterization of the lots, the information obtained in the vigor tests did not rank the lots the same way. Information provided by different tests may be apparently paradoxical but, although unusual, in specific cases some samples of seeds considered more vigorous in a test may be seen as less vigorous in other tests (CHIQUITO et al., 2012). Therefore, the seed lots performance in the set of evaluations must be taken into consideration in order to identify the seed vigor for each lot.

Among the obtained results, it is interesting to note that in the first count germination besides separation of lots in vigor levels at all times of analysis, was observed differences between the lots that were not identified in the final count germination, as for example for lot 4, which in all periods of evaluation had a high percentage of normal seedlings, however their speed of germination was smaller than in other lots (Tables 1 and 2). According to Marcos Filho (1999) one of the problems of the utilization of the germination test is that this one does not allow the differentiation of lots regarding germination speed, which can be identified at first counting.

The traditional accelerated ageing provided different results in each period of evaluation, regarding vigor of the lots, as in general it was possible to identify the lots of higher vigor (2, 8 and 10), however, the lots of intermediate behavior and less vigor alternated between periods; although the lots are bunch equally, it is well known that the seeds within a lot and even parts of a same seed deteriorate at different speed (MARCOS FILHO, 2005).

For most of the lots, traditional accelerated ageing provided more stressful conditions for the seeds, as compared to the alternative method (saturated salt solution) which diminished germinate capacity of the seeds (Tables 1 and 2).

The seedling emergence did not allow the identification of differences between lots as occurred at the first germination counting and accelerated ageing in the second period (Table 1); however, it was possible to verify higher vigor in lot 3 as compared to lot 1 in the first period (Table 1), in lot 5 as compared to most of the lots in the second period and in lot 1 as compared to most of the lots in the third period (Table 2).

It is valid to emphasize that in the seedling emergence test only the aerial part of the seedling is checked, differently from germination test, in which, although there was high percentage of immersed seedlings, they presented problems in their roots, which was the main kind of abnormality seen on germination, and they were not capable of producing useful seedlings for transplant. It is important to remind that this evaluation was made 14 days after sowing and usually the production of tomato seedlings involves at least 30 days.

Besides the already mentioned vigor tests, the computerized seedling analysis by SVIS® was efficient to differ the tomato seed lots in all times of analysis (Tables 1 and 2).

The vigor index revealed higher potential in lots 9 and 10 as compared to lot 4 in the second time (Table 1) and lot 7 as compared to lot 1, 3, 4 and 5 in the third period (Table 2). Similarly to this research, the use of the vigor index was efficient in the evaluation of the physiological potential of cucumber seeds (CHIQUITO et al., 2012), crotalaria (SILVA et al., 2012) and melon (MARCOS FILHO et al., 2006).

Another parameter provided by SVIS®, the growth index, showed a higher vigor in lots 6, 9 and 10 as compared to lots 2, 4 and 5 in the second period (Table 1) and in lot 10, as compared to lots 1, 3, 4 and 5 in the third period (Table 2). The use of growth index was also efficient for the vigor evaluation of Impatiens wallerana seeds (OAKLEY et al., 2004) in a system of seedlings images analysis similar to SVIS®. According to the same authors, the evaluation of the seedlings growth after germination can be considered an interesting vigor test. There is a tendency that seedling growth decreases as seed deterioration advances, a process to which seeds are subject from physiological maturity on; it is known that more vigorous seed lots must deteriorate slower than the less vigorous ones. For seeds of maize it was observed that the small rate of growth of deteriorated seeds is related to the decreasing of the expansion and cellular division growth areas (SVEINSDOTTIR et al., 2009).

Another information obtained through seedling image analysis with SVIS® was the seedlings length, which highlighted a better performance of lots 9 and 10 as compared to most of the lots in the second period (Table 1) and lot 9 as compared to lots 1, 3, 4 and 5 in the third period (Table 2). The seedling length evaluation has been considered efficient to detect differences in the physiological potential of seeds for several species (NAKAGAWA, 1999). Simultaneously, besides this sensitivity, this results can show relation with the seedlings emergence on the field (VANZOLINI et al., 2007); this kind of information is highly desirable in seed analysis, because it might make possible the selection of lots with higher probability of a good performance for the establishment of plants, an essential stage in agricultural production systems.

Similarly to the rest of the vigor tests, the seedlings images analysis by SVIS® allowed identification of lots with more or less vigor. The identification of lots with low vigor may contribute for the improvement of the efficiency of agricultural production systems, making possible the correct choice of the sowing materials. It is important to highlight that once less vigorous seeds may take more time to establish plants, this might benefit pathogens attack, which ones usually are already present in the soil and this might lead to seedlings damping-off or even seed death. As a consequence, plant stand decreases may occurs, which will directly reflect on production, besides facilitating establishment of weeds, which will compete for nutrients and other essential resources for the development of the plants.

According to Rodo and Marcos Filho (2003), the use of high vigor seeds can be justified to assure the adequate establishment of the stand of plants in different environmental conditions. Besides, the identification of low vigor lots might allow discharge of them and thus reduce costs for seed processing and storage.

Another important aspect which must be mentioned, is that the use of SVIS® is not restricted only to direct comparison of the vigor of different lots through the analysis of seedlings growth, but involves the possibility of its introduction as a complement of other tests (SEKHARAM *et al*., 2005).

Companies which produce seeds have the need of developing specific tests which can guarantee the adequate analysis to identify lots with a higher level of quality for trading and efforts must be applied in the future aiming the use of this kind of technologic information, which can be included as a key to global precision and sustainability agriculture, and to the standardized data transfer for seeds analysts for decision making (DELL'ÁQUILA, 2009).

The possibility of using a seeds quality evaluation system which provides accurate, objective and quick information is essential for any seed quality control system and must be a priority of researches. The use of SVIS®, besides the advantages previously mentioned, provide images archiving for later analysis and it is a low cost system, equivalent to germination test (GOMES JUNIOR *et al*., 2009).

This technology has been commonly used by labs of certifying entities of seeds and production companies in the United States of America, particularly in the State of Ohio, being considered a promising procedure for a safe evaluation of seed vigor for several cultivated species (MARCOS FILHO *et al*., 2009).

CONCLUSION

The parameters obtained in the computerized seedlings analysis with the software SVIS® are efficient to differ lots of tomato seeds of low and high vigor.

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