

Computerized imaging analysis of seedlings for assessment of physiological potential of wheat seeds¹

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ABSTRACT – Nowadays, image analysis is one of the most modern tools in evaluating physiological potential of seeds. This study aimed at verifying the efficiency of the seedling imaging analysis to assess physiological potential of wheat seeds. The seeds of wheat, cultivars IAC 370 and IAC 380, each of which represented by five different lots, were stored during four months under natural environmental conditions of temperature (T) and relative humidity (RH), in municipality of Piracicaba, State of São Paulo, Brazil. For this, bimonthly assessments were performed to quantify moisture content and physiological potential of seeds by means of tests of: germination, first count, accelerated aging, electrical conductivity, seedling emergence, and computerized analysis of seedlings, using the Seed Vigor Imaging System (SVIS®). It has been concluded that the computerized analyses of seedling through growth indexes and vigor, using the SVIS®, is efficient to assess physiological potential of wheat seeds.

Index terms: *Triticum aestivum*, imaging analysis, vigor, SVIS®

Análise computadorizada de imagens de plântulas para avaliação do potencial fisiológico de sementes de trigo

RESUMO – Análise de imagens para avaliação do potencial fisiológico de sementes é uma das ferramentas mais modernas existentes atualmente. O objetivo deste estudo foi verificar a eficiência do sistema de análise computadorizada de plântulas para avaliação do potencial fisiológico de sementes de trigo. Sementes de trigo, das cultivares IAC 370 e IAC 380, cada uma delas representadas por cinco lotes diferentes, foram armazenadas durante quatro meses nas condições ambientais naturais de temperatura e umidade relativa do ar em Piracicaba, SP. Para isso, foram realizadas avaliações bimestrais para quantificar o teor de água e o potencial fisiológico das sementes, por meio dos testes de: germinação, primeira contagem de germinação, envelhecimento acelerado, condutividade elétrica, emergência de plântulas e análise computadorizada de plântulas, por meio do *Seed Vigor Imaging System* (SVIS®). Concluiu-se que a análise computadorizada de plântulas, por meio dos índices de crescimento e vigor é eficiente para a avaliação do potencial fisiológico de sementes de trigo.

Termos para indexação: *Triticum aestivum*, análise de imagens, vigor, SVIS.

Introduction

The assessment of physiological potential of seeds, which involves germination and vigor, is essential for an adequate management of seed lots during all steps of

productive process including processing, storage and commercialization. For this type of assessment, the germination test is commonly used. However, such test presents some limitations, once is carried out under ideal conditions, which may contribute for the super estimation

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of physiological potential of seeds from the lots assessed. Because of limitations of this test, it is necessary to assess vigor of seeds to provide complementary responses, this way allowing obtaining more consistent information.

For assessing vigor of wheat seeds, the tests of accelerated aging (Ohlson et al., 2010; Maia et al., 2007; Fanan et al., 2006; Lima et al., 2006) controlled deterioration (Modarresi and Van Damme, 2003) and growth of plants (Ohlson et al., 2010; Lima et al., 2006) are emphasized in the pertinent literature.

The standardization of methods for assessing seed vigor is of essential importance, for the analyses carried out in different laboratories are able to provide comparable and consistent results. Nevertheless, the standardization still represents a challenge to seed analysts, once seed vigor is reflex of a set of characteristics. Based in such fact, it becomes difficult the development of a single test, which would be able in providing coherent information concerning the possible behavior of seed lots in the field, after the sowing or during storage. The use of several vigor tests is based on premisses that seed vigor is a complex

response to different environmental conditions and that all seeds within a given lot do not respond in the same manner to these environments (Oakley et al., 2004).

The majority of vigor tests involve interpretative assessment by seed analysts, what brings at least two inconveniences: the results of seed vigor, achieved in distinct laboratories, may vary in consequence of subjective nature of these tests; many of such tests consume excessive time to produce results. Notwithstanding, these limitations may be overcome with the automatization of those tasks by using computerized imaging analysis (Hoffmaster et al, 2003).

In an attempt to automate assessment of seeds, Sako et al. (2001) have developed, at Ohio State University, the software SVIS® (Seed Vigour Imaging System). This system uses a scanner, mounted in inverted manner, in which images of seedling, usually three to four-days-old, are obtained and processed by the software, which also computes vigor index based on their growth and uniformity; parameters that are normally considered in assessment of seeds are in Figure 1.



Figure 1. Example of imaging analysis of wheat seedlings by the software SVIS®.

The system allows performing the imaging analysis of seedling in a form relatively fast (generally less than 60 sec. for assessing the 50 plants usually contained in each roll of paper towel), representing significant time-saving, as compared to the majority of routine tests for the seed quality assessments (Marcos-Filho et al., 2009). After the image processing, the software automatically generates numeric values, referent to the vigor index (between 0 and 1,000, directly proportional to vigor) and to development

uniformity (also from 0 to 1,000). Thus, the most vigorous seedlings would be those with the longest length and best uniformity between each other. Besides for these parameters a value for growth is obtained, representing the total length for the seedlings analyzed by the software (Marcos-Filho et al., 2009).

Some research studies have indicated success in using this system in the seed vigor assessment of soybean [*Glycine max* (L.) Merrill (Hoffmaster et al., 2003; Marcos-Filho et

al., 2009), corn (*Zea mays* L.) (Otoni and McDonald, 2005), melon (*Cucumis melo* L.) (Marcos-Filho et al., 2006) and castor bean (*Ricinus communis* L.) (Kobori et al., 2010); as well as for assessment of physiological conditioning of sweet corn seeds (Gomes Junior et al., 2009) and mutamba (*Guazuma ulmifolia* Lam.) (Brancalion et al., 2010).

Therefore, the aim of this study was verifying the efficiency of the Seed Vigour Imaging System (SVIS®) in assessing physiological potential of wheat seeds.

Material and Methods

The study was carried out in the Seed Analysis Laboratory and Imaging Analysis Laboratory of the Department of Plant Production, Campus of Superior School of Agriculture “Luiz de Queiroz”, University of São Paulo, municipality of Piracicaba, São Paulo state, Brazil. Wheat seed of cultivars IAC 370 and IAC 380, each of which represented by five different seed lots, presenting difference of physiological potential, were used in the experiments. These seed were stored into permeable packages (Kraft paper bags) under natural environmental conditions of temperature and RH in the laboratory.

Seed samples of each lot were assessed at beginning of study (before storage) and after two and four months of storage, by using the tests following described

Seed moisture content: the oven method was used at 105 °C, during 24 h, with two replications of 4 g of seeds each, per each seed lot, according to Rules for Seed Testing (Brasil, 2009).

Germination and first count of germination: this test was conducted with four replications of 50 seeds each, for each seed lot and cultivar, distributed in three sheets of paper towel, moistened with distilled water in a volume equivalent to 2.5 times the mass of dry substrate, made into rolls, and then kept in a seed germinator, at 20 °C. Assessments were performed at the fourth day (first count) and seventh day after the sowing, according to criteria established by Rules for Seed Testing (Brasil, 2009) and results were expressed in percentage of normal seedlings that emerged in each seed lot.

Accelerated aging: for this test, a single layer of seeds was evenly distributed on a metallic screen, attached to the upper portion of a plastic box, measuring 11 cm x 11 cm x 3 cm (gerbox), containing, at bottom, 40 mL of distilled water. The gerbox were then lidded and maintained into a BOD type chamber, at 43 °C, during 48 h (Lima et al., 2006). After such period, the seeds were placed to germinate, using the procedure already described for the germination test.

Electrical conductivity: was performed with four

replications of 50 seeds each, per lot of seeds, previously weighed in an analytical balance with 0.01 g precision. The seed were then immersed into 75 mL distilled water, at 25 °C, during 16 h (Lima et al., 2006) and the electrical conductivity reading was performed with the aid of a conductivimeter (brand DIGIMED, model DM-31). The values of readings were divided by the mass of seeds and the results were expressed in $\mu\text{S} \cdot \text{cm}^{-1} \cdot \text{g}^{-1}$ of seeds.

Seedling emergence: for such test, were used four replications, of 50 seeds each, per seed lot and cultivar. For this, the seed were evenly distributed on the surface of a 5 cm layer of sterile sand, into plastic trays measuring 32 cm x 28 cm x 10 cm. After the sowing, the covering of seeds was performed with another sand layer of 2 cm thick. Substrate moisturizing has been achieved with distilled water, corresponding to 60% of retention capacity. The trays containing the seeds were kept under laboratory environment, without environmental control (T and RH) during 14 days; and the emergence percentage was determined by percentage seedling that emerged in each sample of seed and cultivar.

Emergence speed index (ESI): this index was determined by the daily record of the number of seedlings that have emerged until the fourteenth day after the sowing; the data so obtained were then used to compute the ESP through the equation proposed by Maguire (1962).

Computerized imaging analysis of seedling: for this assessment, four replications of 25 seed each, for each seed lot and cultivar, were placed to germinate in two rows, located in the upper surface third of paper towel sheets, at 20 °C, during 3 days. After germination, the imaging of the seedlings were digitalized, with the aid of a scanner (brand HP, model Scanjet 2004), inversely mounted inside an aluminum box (60 cm x 50 cm x 12 cm) run by a software Photosmart, with 100 dpi resolution. After this procedure, the seedlings were transferred from the paper towels to a black sheet of pasteboard, measuring 30 cm x 22 cm (corresponding to the actual size of area scanned), placed on an internal platform of the metallic box. The scanned images were analyzed by the software Seed Vigor Imaging System (SVIS®). The maximum length established for 3-days-old seedlings has been 10.2 cm and this value was then used as referential and registered by user in the software. On computing vigor index, the variations of percentage growth and uniformity of seedlings were assessed aiming at verifying the best combination for obtaining consistent results, where the 70:30 ratio (70% for the growth: 30% for the uniformity) has been selected.

Statistical procedures: a completely randomized experimental design, with four replications, was used to

perform the experiment. The ANOVA was computed, and treatment means were compared by the Tukey test, at 5% probability. The type of distribution of percentage data was verified and the test of Cochran was then applied to verify if variables were presenting homogeneity. Data transformation was not needed.

Results and Discussion

Based on data obtained in all assessments performed in the first period of evaluation (before storage), statistically significant differences of physiological potential among seed lots, for both the cultivars, were detected by the ANOVA, except in the electrical conductivity test (Table 1). Emergence

speed index (ESI) and emergence percentage of seedlings (SE), for seed lots 6 and 7 have been low, what could be an indicative of the low physiological potential of these lots. However, it is also possible to observe that the performance of such lots, in the remaining vigor tests, has been adequate; in considering that germination, after the accelerated aging test, has been similar to results obtained for the best seed lots (5 and 10), what was indicated by this variable (Table 1). Another possible cause for these results would be the presence of pathogens in the seeds. Nevertheless, symptoms of contamination by microorganisms have not been observed in any moment during assessments, and besides, the substrate used had been sterilized before the test, what would impair proliferation of pathogenic agents.

Table 1. Moisture content (MC), germination (G), first count of germination (FC), accelerated aging (AA), seedling emergence (SE), emergence speed index (ESI) and electrical conductivity (EC) of wheat seeds, cv. IAC 370 and cv. IAC 380, performed before storage.

Seed lot	MC	G	FC	AA	SE	ESI	EC ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$)
			(%)				
cv. IAC 370							
1	11.0	95 A ¹	87 A	87 A	96 A	7.1 A	29 A
2	11.4	94 A	91 A	88 A	84 B	6.0 B	29 A
3	11.7	93 AB	92 A	86 A	94 AB	7.0 A	28 A
4	11.4	92 AB	89 A	72 B	90 AB	6.3 AB	28 A
5	11.1	86 B	79 B	80 AB	89 AB	6.5 AB	30 A
CV(%)	-	3.9	3.2	7.6	6.3	6.3	6.7
cv. IAC 380							
6	11.3	92 AB	82 ABC	69 AB	20 B	2.7 B	48 A
7	11.7	93 A	88 AB	68 AB	20 B	2.3 B	41 A
8	12.1	96 A	88 AB	67 AB	65 A	5.0 A	40 A
9	11.4	90 AB	78 BC	59 B	78 A	5.5 A	43 A
10	11.5	86 B	73 C	71 A	90 A	6.0 A	43 A
CV(%)	-	3.2	5.8	9.9	16.0	12.8	10.1

¹Means followed by the same letter in the column, for each cultivar, do not statistically differ between each other by the Tukey test, at 5% probability.

Through the computerized imaging analysis of seedlings, by using the software SVIS[®], it was possible to detect statistically significant differences in vigor among seed lots in the first assessment period (Table 2). The seedling growth index (GI) of lot 1 was been higher, in relation to lot 3 (cv. IAC 370) and of lot 10, compared to lots 7 and 8 (cv. IAC 380). The vigor index emphasized physiological potential of seed lot 1, when compared to lots 3 and 4 (cv. IAC 370) and of lot 10 in relation to lots 6 and 7 (cv. IAC 380). For these parameters, the high physiological

potential of lot 1 was evidenced in the percentage and speed of emergence of seedling; in the same manner that the lot 10 was evidenced in the accelerated aging test.

The sorting of seed lots has not occurred in a identical form for all assessments; once vigor tests are based on different principles and for such reason this analysis has to consider the set of information and cannot be based in the analysis of results in an isolated form. It is important to stress that for the initial quality of seeds small differences may increase difficulties in sorting seed lots by vigor levels.

Table 2. Values of growth index (GI), growth uniformity index (UI), and vigor index (VI) of wheat seedlings, cv. IAC 370 and cv. IAC 380, obtained after 0 (control), 2 and 4 months of storage.

Seed lot	GI	UI	VI
First assessment period – cv. IAC 370			
1	742 A*	890 A	786 A
2	581 AB	876 A	669 AB
3	451 B	879 A	579 B
4	558 AB	875 A	654 B
5	626 AB	892 A	704 AB
CV(%)	14.3	1.6	8.9
First assessment period – cv. IAC 380			
6	511 AB	889 A	624 AB
7	478 B	876 A	597 B
8	399 B	860 A	530 B
9	514 AB	893 A	627 AB
10	637 A	886 A	711 A
CV(%)	10.3	1.6	6.2
Second assessment period – cv. IAC 370			
1	597 A	896 A	687 A
2	549 A	895 A	653 A
3	605 A	869 A	684 A
4	446 B	869 A	572 B
5	533 AB	882 A	635 AB
CV(%)	8.2	2.2	5.1
Second assessment period – cv. IAC 380			
6	421 A	824 A	541 A
7	384 AB	833 A	519 AB
8	319 AB	849 A	481 AB
9	301 B	796 A	449 B
10	385 AB	847 A	523 AB
CV (%)	14.8	4.0	7.2
Third assessment period – cv. IAC 370			
1	317 A	875 A	484 A
2	284 AB	871 A	459 AB
3	272 B	849 A	445 B
4	250 B	850 A	430 B
5	250 B	869 A	436 B
CV (%)	7,3	2.6	4.0
Third assessment period – cv. IAC 380			
6	281 A	861 A	455 A
7	255 AB	849 A	433 AB
8	220 BC	814 A	397 B
9	169 C	802 A	358 B
10	250 AB	812 A	418 AB
CV (%)	10.3	4.7	4.2

*Means followed by the same letter in the column, for each period and cultivar, do not statistically differ between each other by the Tukey test, at 5% probability.

In the second assessment period (two months after the storage) and among assessments used, only the electrical conductivity test, for the seeds of cv. IAC 380, and the test of accelerated aging, for both cultivars, have indicated statistically significant differences among seed lots (Table 3). It has to be emphasized the best physiological potential of lot 1, when compared to lot 4, for cv. IAC 370, lot 6 in relation to lot 9, for cv. IAC 380, in the test of accelerated aging and also for lot 10 when compared to lot 6, in the electrical conductivity test.

The computerized imaging analysis of seedlings, by the vigor and growth indexes, was able to detect the worst performance for the seed lot 4 (cv. IAC 370), when compared to lots 1, 2, and 3 of the same cultivar and lot 9 compared to lot 6, for cv. IAC 380 (Table 2). These results were coherent with results found in the test of accelerated aging. Similar to what was found within this study, in results achieved by Chiquito et al. (2012) for seed of cucumber (*Cucumis sativus* L.), the vigor index obtained with the imaging analysis of seedlings with the software SVIS® were efficient in detecting the lot with the worst physiological potential. The same authors have stressed that generally the objective of tests for assessing physiological potential is the distinction of seed lots with high performance among lots presenting low probability in providing the adequate establishment of seedling in the field after sowing, or identifying differences of storability. Similarly, in the results herein obtained, the GI obtained by the software SVIS® was efficient in assessing physiological potential of soybean seeds, being this the most sensitive parameter in assessing vigor through SVIS® (Marcos-Filho et al., 2009). The assessment of seedling growth is important, since their low physiological performance may result in difficulties for the establishment of plants in the field and occasionally cause gaps in the stand of plants (Soltani et al., 2006).

The test of electrical conductivity, although able of statistically differentiating the seed lots of cv. IAC 380, has provided results incoherent with results obtained in remaining assessments. Possibly, ions quantification leached from the wheat seeds is not adequate for vigor identification in seeds of this species. Since wheat seeds are composed of approximately 75% of carbohydrates, which in their majority are in the form of starch. Such carbohydrates have their interference reduced in the imbibition process, once they possess low electrical charge and low affinity with the water molecules; thus interfering in the electrical conductivity detected by conductivimeter.

In the third assessment period (after four months storage) the first count of germination and the test of

accelerated aging were sufficiently sensitive for detecting differences among seed lots of both cultivars, what was opposite to remaining assessments used (Table 4). Possibly,

during seedling emergence test, environmental conditions were very suitable for germination, thus not allowing identification of differences on vigor.

Table 3. Moisture content (MC), germination (G), first count of germination (FC), accelerate aging (AA), seedling emergence (SE), emergence speed index (ESI) and electrical conductivity (EC) of wheat seeds, cv. IAC 370 and cv. IAC 380, obtained after two months of storage.

Seed lot	MC	G	FC	AA	SE	ESI	EC ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$)
(%)							
cv. IAC 370							
1	12.0	95 A ¹	91 A	65 A	91 A	4.0 A	33 A
2	11.3	96 A	92 A	59 AB	89 A	3.8 A	31 A
3	12.0	94 A	92 A	57 AB	93 A	4.1 A	35 A
4	12.0	92 A	89 A	47 B	91 A	3.8 A	30 A
5	11.0	92 A	89 A	53 AB	90 A	4.0 A	34 A
CV(%)	-	5.0	5.0	12.2	3.4	4.3	7.1
cv. IAC 380							
6	11.0	95 A	85 A	48 A	87 A	3.4 A	56 C
7	11.0	83 C	83 A	47 AB	88 A	3.6 A	49 AB
8	12.0	89 B	83 A	41 AB	88 A	3.5 A	51 BC
9	11.0	90 B	81 A	34 B	87 A	3.4 A	45 AB
10	12.0	88 B	84 A	44 AB	91 A	3.8 A	37 A
CV(%)	-	2.6	4.9	14.4	4.3	5.8	10.0

¹Means followed by the same letter in the column, for each cultivar, do not statistically differ between each other by the Tukey test, at 5% probability.

Table 4. Moisture content (MC), germination (G), first count of germination (FC), accelerate aging (AA), seedling emergence (SE), emergence speed index (ESI) and electrical conductivity (EC) of wheat seeds, cv. IAC 370 and cv. IAC 380, obtained after four months of storage.

Seed lot	MC	G	FC	AA	SE	ESI	EC ($\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$)
(%)							
cv. IAC 370							
1	12.3	95 A ¹	93 AB	68 A	94 A	5.5 A	35 A
2	12.3	95 A	93 AB	71 A	89 A	5.2 A	35 A
3	12.0	97 A	97 A	51 B	88 A	5.1 A	36 A
4	12.0	92 A	89 B	40 B	86 A	5.0 A	31 A
5	11.7	95 A	93 AB	47 B	85 A	5.0 A	34 A
CV(%)	-	3.0	3.5	13.8	6.7	6.7	6.7
cv. IAC 380							
6	12.4	88 A ¹	78 B	45 AB	80 A	4.6 A	56 A
7	12.3	94 A	92 A	55 A	81 A	4.6 A	50 A
8	12.4	89 A	83 B	42 AB	83 A	4.8 A	48 A
9	12.1	88 A	79 B	37 B	76 A	4.3 A	51 A
10	12.2	90 A	83 B	41 AB	84 A	4.9 A	60 A
CV(%)	-	4.6	4.5	16.3	8.4	8.2	11.9

¹Means followed by the same letter in the column, for each cultivar, do not statistically differ between each other by the Tukey test, at 5% probability.

Through computerized imaging analysis of seedlings, by using the software SVIS[®], it was possible to identify statistically significant differences of physiological potential among seed lots (Table 2). The growth and vigor indexes showed the highest physiological potential of seeds of lot 1, in the first and third assessment periods for the cv. IAC 370, and of lot 6 in the second and third assessment periods, for cv. IAC 380. These results agree in part with results obtained in the test of accelerated aging, as for example: in the second assessment period, the identification of superiority in physiological potential of lot 1 in relation to lot 4 (IAC 370), of the lot 6 in relation to lot 9 (IAC 380), and on third assessment period, of seeds of the lots 1 and 2, when compared to the remaining seed lots for the cv. IAC 370 (Tables 3 and 4).

The identification of differences on seed vigor, obtained by means of computerized image analysis of seedlings, by using the SVIS[®], indicates the viability in using this software for analysis of seeds in a consistent and fast manner. For Gomes Junior et al. (2009), seed vigor assessments using the software SVIS[®] has advantages, such as simplicity of execution and swiftness, once results may be obtained within two to three minutes, for a sample of 50 seeds; besides allowing eliminating human errors, by increasing reliability on data obtained for comparisons, for archiving of images for further analysis and lowering costs, which in this case is equivalent to the cost of germination assessment.

By means of computerized image analysis of seedlings through the SVIS[®], it was still possible verifying that wheat seedling assessed on the third period, have had lower GI than those seedling assessed in the earlier period. Although these comparisons had been performed among assessment periods, it is possible inferring that likely such decrease is associated to a vigor reduction of seeds. An example that can be cited is the growth index of seedlings of lot 1, which was 742, on the first assessment period, and has reached 317, in the third assessment period. This reduction on vigor has not been evident on the remaining assessment performed; except in the test of accelerated ageing. This indicates potential of the use of computerized image analysis of seedling through the SVIS[®], for monitoring physiological quality of seeds during storage. Especially considering that, within this study, the tests of germination and emergence of seedlings, used to assess physiological potential of seeds, did not detect losses of vigor. It is important to emphasize that in the process of seed deterioration, the loss of vigor precedes a swift decline of germination and for such reason, and it is not possible monitoring performance of seed lots

stored by means of assessment of germination percentage. Marchi et al. (2011) have verified that, in peanut (*Arachis hypogaea* L.) seeds, the reduction of physiological potential has been detected in advance by computerized imaging analysis of seedlings through the SVIS[®].

The use of software SVIS[®] has contributed for the assessment of physiological potential of seeds of cucumber (Chiquito et al., 2012), rattle pods (*Crotalaria* spp.) (Silva et al., 2012), castor bean (Kobori et al., 2010), soybean (Marcos-Filho et al., 2009) and corn (Otoni and McDonald, 2005) and for assessment of physiological conditioning of sweet corn seeds (Gomes Junior et al., 2009) and mutamba (Brancalion et al., 2010). Such system may be a supplementary tool for interpretation others tests as accelerated aging, germination in low temperature, germination speed and growth of seedling, or even in identifying effects of chemical treatments and physiological conditioning; besides other situations in which monitoring of germination and growth of seedlings, at regular intervals, is of interest (Marcos-Filho et al., 2009). In addition, future perspectives of interest of researchers and seed technology analysts are focused in introducing computerized systems in seed laboratories to integrate data obtained by human inspection and imaging processing to seed analysis standard procedure (Dell'Aquila, 2007).

It was possible to verify the efficiency of computerized imaging analysis of seedlings, under conditions in which this study was performed. Such technology has been routinely used in laboratories of seed certification entities and seed producing enterprises in USA, particularly in Ohio State, being considered a promising procedure for safe evaluation of seed vigor of several cultivated plant species (Marcos-Filho et al., 2009); once the use of growth and vigor indexes supplied by the software SVIS[®] can provide fast, precise, and reproducible information for safe analysis of seed lots.

Conclusion

The computerized imaging analysis of seedling, by means of growth and vigor indexes is efficient for the assessment of vigor of wheat seeds.

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