EXPLANINING THE VIGOR (RELATIVE AND ABSOLUTE) IN SEEDS: THE BIOLOGICAL MEANING BEHIND THE MATHEMATICIAN

DESVENDANDO O VIGOR (RELATIVO E ABSOLUTO) EM SEMENTES: O SIGNIFICADO BIOLÓGICO POR TRÁS DA MATEMÁTICA

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ABSTRACT: Seed vigor is the totality of all properties that determine a rapid and uniform emergence and development of normal seedling under a wide range of conditions. However, the physiological quality within a seed lot is not homogeneous, generating a quality gradient between seeds. Thus, the vigor expressed by the final percentage of normal seedlings tends to underestimate the quality of the batch, considering the total number of seeds. One possible method for correcting such an effect would be to weight vigor by germination, generating an index called relative vigor. The index reflects the "success" of viable seeds in maintaining their potential under stress. In this context, this review article proposes the possibility of using a new measure for vigor and new interpretation of relative vigor, as well as providing the mathematical basis for its use.

KEYWORDS: Emergency index in the field. Physiological quality. Stress. Success rate.

INTRODUCTION

Seed vigor is the sum of all properties that determine the fast and uniform emergence and development of normal seedlings under different conditions. However, the physiological quality in a seed lot is not homogeneous, generating a gradient of quality between the seeds (BEWLEY; BLACK,, 1982). Thus, the vigor expressed by the final percentage of normal seedlings tends to underestimate the quality of the lot, because it considers the total number of seeds. A possible method to correct this effect would be to ponder the vigor by germination, generating an index called relative vigor. The index reflects the "success" of viable seeds in maintaining their potential under a stressful situation. In this context, the present review article talks about the possibility of using a new measure, for the vigor, and a new interpretation, the relative vigor, as well as giving the mathematical basis for its use, based on a bibliographical survey and inferences.

In 1876, Nobbe, the founder of modern seed technology, found differences in germination and seedling development within the same seed lot; this differences is currently referred to as "vigor" (Marcos Filho, 2015) and had the concept intensely discussed and refined by the world's leading seed analysis agencies. The American Organization of Seed Analysts (AOSA) defines vigor as the sum of all properties that determine the potential for rapid and uniform emergence of normal seedlings under a wide range of edaphoclimatic conditions (AOSA, 1983; BAALBAKI et al., 2009). For International Seed Testing Assossiation −ISTA, seed vigor is the totality of properties that determine the activity and performance of acceptable germinating lots in a wide range of environments; that is, a vigorous seed lot is one that is potentially capable of germinating well under an ideal environmental conditions. (ISTA, 2014). However, even though not reported by the concepts, sometime ago responses have been contacted throughout the crop cycle as a function of seed vigor, also reflecting yield (TEKRONY et al., 1989; TEKRONY; EGLI, 1991; MEROTTO JÚNIOR, 1999; HÖFS et al., 2004; MIELEZRSKI, 2008; MONDO et al., 2012; CAMELIA, 2014).

Even though the concepts of seed vigor are anchored in seed or seedling performance under non-optimal conditions, it is known to be a consequence of the binomial physiological quality and deterioration. In general, the factors governing seed vigor are inversely proportional to those governing seed deterioration, as physiological quality is still established in the mother plant. (CORBINEAU, 2012). In the latest reviews that address the causes of loss of vigor through different tests, they point out that root deterioration is a consequence of free radical-mediated lipid peroxidation, inactivation of enzymes and proteins, disruption of cell membranes and the damage on genetic materials (BOPPER; KRUSE, 2010; CORBINEAU, 2012; RAJJOU et al., 2012; SHABAN, 2013). It should be noted that the process is dynamic and interconnected. To understand, in the breathing of cells the energy is spent. With

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compromised breathing capacity, it reduces both production and energy distribution to the seed / seedling (ATP). This energy comes from the seed reserve compounds and is intensified when the cells are soaked. However, this "breakdown" of compounds while producing energy for the system to stay alive produces free radicals that degrade the membrane system. (CORBINEAU, 2012; RAJJOU et al., 2012; SHABAN, 2013; BOPPER; KRUSE, 2010). At first, these reactions have a slow but cadenced rhythm, which, due to the species-genotype-environment association, can reach an accelerated rhythm, with sigmoid pattern for vigor (absolute) and paraboloid for germination, and determine the intense fall of the plant. quality, culminating in the death of seeds (DELOUCHE; CALDWELL, 1960; MARCOS FILHO, 2015;)

The factors that give the seed more or less vigor are also linked to its composition; In general, seeds with starches, soluble sugars and soluble proteins result in greater vigor because energy is readily available to the embryo (CARVALHO; NAKAGAWA, 2000; HENNING et al., 2010, CARVALHO et al., 2014). This process is latent and its measurement can take time and resource and are therefore impractical commercially. However, the use of seed stresses or the measurement of physiological / biochemical characteristics of seedlings / seeds or secondary inferences of seed metabolism allows to elucidate seed deterioration and this type of test is called the vigor test.

In short, vigor tests aim to detect differences between lots associated with seed performance regarding the establishment of the stand in the midst of multiple stresses, therefore being comparative (FICH-SAVAGE; BASSEL, 2015; MARCOS FILHO, 2015). So the question is, why then does not the vigor test only apply to verify the physiological quality of the seeds? What is the relationship between germination and vigor?

The answer to these questions foresees the establishment of the shelf, being associated with the investigation of the germinative potential of the seeds. The germination test is the reference, since the factors in its conduction are controlled and with this there is reproducibility (BRASIL, 2009; MARCOS-FILHO, 2015). In fact, it is known that the results of vigor tests given by calculating the percentage of normal seedlings are related to seedling emergence in the field (MARTINS; SILVA, 2005; MARCOS-FILHO, 2015). Although the concept of seed vigor, expressed as the final percentage of normal seedlings is correct, a marginally germinating lot (close to the commercial standard) may have a high percentage of seedlings in the vigor test, as well as a high germinating lot. (≈100%), may present low percentage of seedlings in the vigor test (CORBINEAU, 2012; FICH-SAVAGE; BASSEL, 2015). This statement is based on the fact that vigor test is dependent on seed viability that is intrinsic to seed and verified by germination test (FICH-SAVAGE; BASSEL, 2015). It is known that warned that seed aging occurs at various rates within the batch, leading to inhomogeneity, culminating with vigor discrimination within the batch itself (VESELOVA; VESELOVSKY; OBROUCHEVA, 2015)

Based on these assertions it is assumed that the vigor of a seed lot should be expressed weighted by the germination percentage, which is then called relative vigor. Thus the resulting value would be the "success" of viable seeds in maintaining their germination in the face of stress. An outline of this formula was made in 1995 by Egli; Tekrony, to verify the suitability of the vigor test for field emergency. For this, they related the field emergence by laboratory germination and called this the Field Emergency Index (FEI). In this aspect, the closer to 100 the index reached, the better and more adequate the conditions of the field were. The fact is, there are few studies with this measure, and a primordial step is, like germination measures, to understand the biological and mathematical meaning of the expression (SANTANA; RANAL, 2004; DORNELES ET AL., 2005; RANAL; SANTANA, 2006). In this context, this review article proposes the possibility of using a new measure for vigor and new interpretation of relative vigor, as well as providing the mathematical basis for its use.

The origin of relative vigor: the Field Emergency Index

After advances in the elaboration of mathematical calculations that characterize the physiological processes of germination, several measures and interpretations were listed and discussed. Among them, those that characterize the time, rate or speed, uniformity, uncertainty and synchrony of the germinal process, besides the capacity, with emphasis on germinability (SCOTT et al., 1984; BROWN; MAYER; 1988; SANTANA; RANAL, 2004 ; RANAL; SANTANA, 2006; MCNAIR et al., 2012; SOLTANI et al., 2015). Regardless of botanical or agronomic criteria, germination measures are applied when a morphological marker of germination occurs.

Recently, advances in understanding stricto sensory germination have broadened the way of measuring the physiological process still in the seed phase. In this way, the rate, acceleration, entropy, uniformity, diffusion coefficient and velocity of seed water dynamics in germination process were calculated (RIBEIRO-OLIVEIRA, 2015). Even with all these advances, the use of vigor has always been prominent in studies of the germinal process.

Originally called the field emergency index (FEI), relative vigor was proposed to verify the adequacy between sand emergency methodologies (EGLI; TEKRONY, 1995). In this study, the authors submitted seed lots to different emergence conditions in sand and having the result, verifying, through the quotient with the germination obtained in the laboratory, which increment / decrement was obtained. Therefore, the higher the value reached by a given sand emergency methodology, the more appropriate it was; whereas if the FEI was less than 100, the methodology would be deleterious to express the physiological potential of seeds. It is worth noting that this interpretation is correct, but centered on the methodology, while the new approach is to relativize the germination potential of the batch. When weighting absolute vigor by germination (Equation 1), the index gives a probability of "success" in the face of stress, which is the concept adopted from then on. Relative vigor represents the percentage of seeds that withstood stress and germinated, from the total to germinate (estimated by the germination test); whereas absolute vigor is the very proportion of seeds that germinated under stress conditions.

Relativization is not unique to these authors, a similar formula was proposed by Karim et al. (1992), which relates the germination percentage of a given treatment with the witness, however, the gain / loss of a treatment in relation to the witness is seen. This calculation, however, is apparently based on the Abbott (1925) index to verify insecticide efficiency. In this the variable of interest is insect mortality, while in the adaptation of Karim et al. (1992) interest is in germinated seeds. Recently, a paper on the theme of relativization in the germination process was published (SANTANA et al., 2018), which proposed the use of relative germination. Relative germination has as denominator, not the number of seeds put to germinate (n), but the number of seeds that the embryo has. This equation is similar to Egli's expression; Tekrony (1995), but it is not wise if this was the inspiration. Its use was proposed to make a comparison between individuals of a species that presented seed formation problems (empty seeds). There is similarity of the FEI equation, relative vigor and relative germination and the index of Karim et al. (1992), however the interpretation is different, as well as the situation in which each one is suitable for use.

The mathematical basis of relative vigor

Possibly, the development of the FEI was in order not to use Pearson correlation, since Egli; Tekrony (1995) warn about fragility in correlation between laboratory testing and emergency in the field. It should be noted that the values obtained when using this statistic are questionable, because the significance only represents that the association between the variables is not null, but not necessarily relevant (MILLER, 1994; SANTANA et al., 2010). Moreover, lots with different germination and vigor quantities could give the same correlations, since this statistic takes into account only variances (PEARSON, 1930). Thus, the FEI was proposed to evaluate the best sand emergency methodology, as it had the most intuitive and straightforward interpretation (EGLI; TEKRONY, 1995).

Different Authors have identified conceptual and statistical problems in different measures of germination, and thus suggested a refinement in interpretation or even in the mode of calculation (GOODCHILD; WALKER, 1971; SCOTT et al., 1984; BROWN; MAYER, 1988; SANTANA; RANAL, 2004; RANAL; SANTANA, 2006), Based on this, the new interpretation of the FEI is just a breakthrough, a contribution, with unprecedented context. The use of expressions hitherto used in other areas of knowledge to understand the germinal process is common and, thus, acquires new interpretations. In this aspect there is uncertainty and synchrony; the first was used by Labouriau; Valadares (1976) to quantify the variation of germination over time, through the uncertainty associated with the distribution of relative germination frequency, or informational entropy. The development and application of uncertainty is credited to Shannon (1948) for measuring informational entropy. this measure is usual for ecologists to measure the diversity of an environment (RANAL; SANTANA, 2006). Thus, high values indicate high diversity and numbers towards zero indicate low diversity. When applied to seed germination, the conventional interpretation is in the opposite direction, ie low values indicate synchronic germination (RANAL; SANTANA, 2006). The same authors also made an adaptation of the sync, "Z" of Primack (1980). This index initially assessed the degree of overlap between individuals in a flowering population, and the same meaning extends to seeds, that is, the "Z" only generates a number if two seeds germinate at the same time. Thus, the Z value, in fact, measures the synchrony, or the degree of germination overlap (RANAL; SANTANA, 2006). These adaptations for the germinal process reinvigor the viability of the new interpretation for the expression of Egli; Tekrony (1995), aiming to determine relative vigor.

Why the use of relative vigor is centered on the limitations of vigor tests, whose recommendation is that results can only be compared when batch germination is similar (MARCOS FILHO et al., 1984; CARVALHO; NAKAGAWA, 2000; MARCOS FILHO, 2015). In order to verify this condition, the authors submit the germination percentages of the seeds to the variance analysis, in order to make the difference not significant (MARCOS FILHO et al., 1984; CALIARI; SILVA, 2001; MARTINS; SILVA, 2005). In doing this procedure, the researchers intuitively weight the vigor percentage by the germination percentage (MARCOS FILHO et al., 1984), even without making any algebraic manipulations, making the comparison possible.

In short, the relativization of vigor allows the comparison of vigor of seed lots with distinct germinative potentials, a gargle in seed studies. Moreover, for seed industry the relative vigor will allow the reduction of batches that fall within the absolute vigor considered marginal, but still have vigorous seeds. In biological studies may clarify the contribution of the individual in the adaptive aptitude of the offspring under stressful conditions. Through this measure it will still be able to identify "elite" genetic materials in terms of vigor, which materials would be discarded from the breeding programs because of their low germination percentage. This is possible because relativization minimizes problems with underestimation due to germination. In this sense, Santana et al. (2018) postulate that relative germination minimizes underestimation of germination potential when seeds with embryonic problems (empty / malformed seeds) are present in the sample. This is also true for relative vigor, however, by minimizing underestimation of vigor potential when seeds are not viable and able to germinate. In this work a numerical proof was made, in which, when comparing different individuals, they found that the individual 7 was erroneously classified as not efficient in the absolute germination (12%), while this one had a high relative germination capacity (78.1%; *vide* SANTANA *et al.*, 2018). Therefore, it cannot be inferred that the seeds of this individual have low germination potential, but that these individuals produce large amounts of seeds without embryos, but when the embryo is present, the seeds have high germinability.

Relative vigor was calculated as a percentage, which, from a practical point of view, facilitates interpretation (EGLI; TEKRONY, 1995). In fact, this approach is correct because both absolute vigor and germination are given in percentages of normal seedlings and, in making the ratio, resulting in a dimensionless index converted to a percentage by multiplying by one hundred (Equation 1).

$$V_r = {}^{V}\!/_{G}\,100, \text{ ou } V_r = \left[\left(\frac{\sum V_i}{n_v}\,100\right) \Big/ \left(\frac{\sum G_i}{n_G}\,100\right)\right] 100 \qquad \begin{array}{l}\text{Limits (\%):}\\ V_r\text{: [0; 100]}\\ V\text{: [0; 100]}\\ G\text{:]0; 100]}\end{array} \qquad [1]$$

where *V* is absolute vigor obtained as a percentage of the vigor test; *G* is the percentage of germination obtained by the standard germination test, *Vi* is the number of normal seedlings of the vigor test; *Gi* is the number of normal seedlings in the germination test; n_v is the vigor test test sample size and *nG* is the germination test test sample size.

Germinability and absolute vigor expressed as a percentage is essentially important for the design of relative vigor because it nullifies the effect of sample size (BROWN; MAYER, 1988; RIBEIRO-OLIVEIRA, 2011; RIBEIRO-OLIVEIRA et al., 2013; RIBEIRO-OLIVEIRA; RANAL, 2015). It should be remembered that germination and absolute vigor originate from "n" which may vary between samples or between experiments, and in this situation the comparison remains applicable because it is proportional (SANTANA; RANAL 2004). Moreover, of all germination measures, the most robust to variations in sample size and seed quality is germinability (RIBEIRO-OLIVEIRA, 2011; RIBEIRO-OLIVEIRA et al., 2013; RIBEIRO-OLIVEIRA; RANAL, 2015), that is, in the face of variations there is no compromise of the physiological inference of the sample. By analogy, relative vigor seems to be robust as well, by involving in the calculation absolute vigor and germination, both derived from the same equation.

Initially one might think that relative vigor is a mixed measure because it involves two characteristics, but it is not, since the unit is a percentage. The mixed measures present a dependence between the evaluated characteristics, being dubious (GOODCHILD; WALKER, 1971; BROWN; MAYER, 1988, SANTANA; RANAL, 2000). Maguire speed, or emergency speed index (Maguire, 1962) is an example. The value obtained is influenced by the balance of germinability and speed (beginning, end, process uniformity and sample size), so much so that the unit is seedlings per day (SANTANA; RANAL, 2000; RIBEIRO-OLIVEIRA, 2011; RIBEIRO-OLIVEIRA et al. ., 2013; RIBEIRO-OLIVEIRA; RANAL, 2015).

For the determination of relative vigor one must take into consideration the theoretical limits of the variables involved in its determination. For absolute vigor, the lower limit is zero and the upper limit is 100; whereas for germination the lower is greater than zero and the upper is 100. This is because a non-germinating plot will not have seedlings to analyze vigor. As well as relative vigor, other measures that depend on germination to be calculated also do not exist in its absence, generally those which in the calculation use the term germination (RANAL; SANTANA, 2006). Relative vigor should be considered as zero limits for when absolute vigor is zero and 100 for when germination and absolute vigor are equal (Figure 1). A particular feature of the FEI is that it reaches values greater than 100%, ie in these cases the field was better than laboratory conditions (EGLI; TEKRONY, 1995; NERY-SILVA, 2012).

Although designed from the FEI, the relative vigor for its calculation uses the germination percentage given by the germination test, which is done under optimal conditions, so it makes no sense for the relative vigor to be much higher than 100. These values above this limit elucidate methodological errors in either germination or vigor testing, may only be due to simple variation and chance. In the latter case it is expected that due to sampling (BRASIL, 2009) the addition of the maximum theoretical limit will be small, so it is recommended to convert the percentage to 100% (maximum limit).

How does relative vigor relate to absolute means?

Regarding the calculation of relative vigor, it is a fact that keeping the absolute vigor fixed, the addition of one unit in the germination percentage will impact the reduction of relative vigor (Figure 1). This is because the weighting is the inverse of germination, however the impact of this reduction is not fixed (linear) as observed in figure 1.

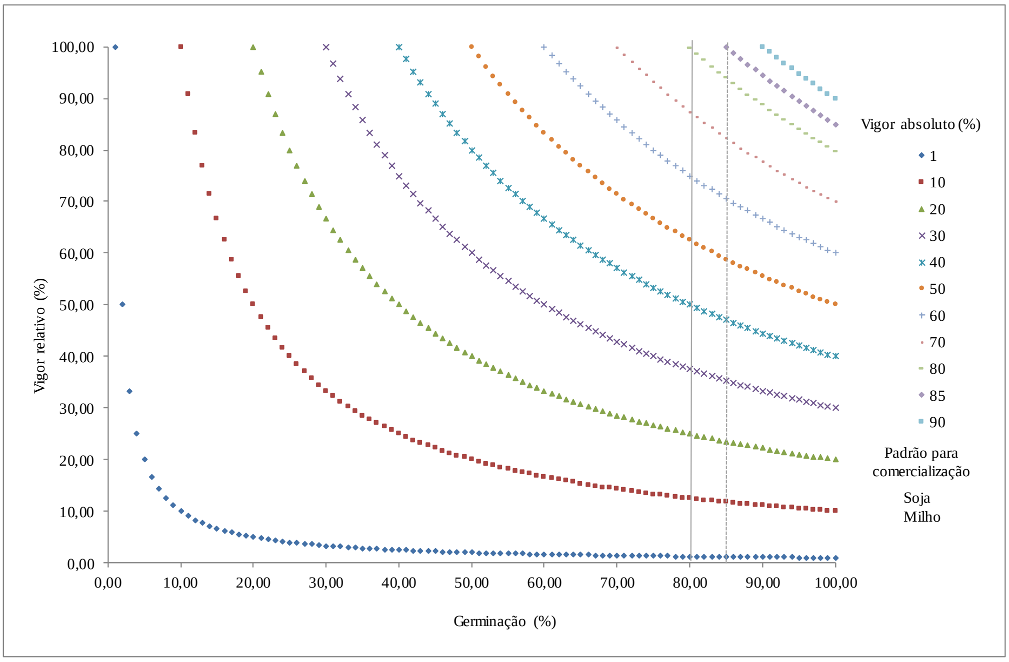

Figure 1. Relative vigor variations in favor of fixed absolute vigor and variable germination.

Ranal; Santana (2006), studying various germination measures, in which the weighting was the inverse of germination, found that in these cases the measures are not linearly associated, but proportionally inverse. This is clearly observed through the mean time and mean velocity measurements, where the second is obtained only by the inverse of the first, and disjunction occurs in the mean test categories (DORNELES et al., 2005; BERGER et al., 2014 ; RIBEIRO OLIVEIRA; RANAL, 2015). As expected, the impact of absolute vigor on relative vigor is direct and proportional (Figure 2) and relative vigor is never greater than absolute. The application of relative vigor is centered on the fact that the tested lots do not have similar germination.

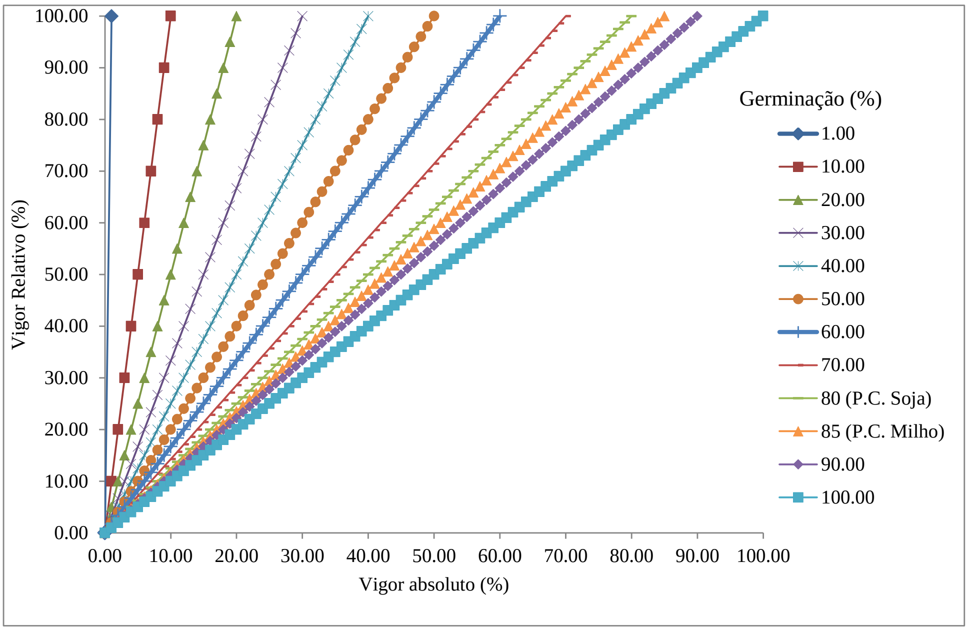

Figure 2. Relative vigor variations in favor of fixed germination and variable absolute vigor.

The Figure 1 illustrates this condition by setting vigor and varying germination. The models initially fit the exponential function of three parameters (Table 1), with two very characteristic patterns (Figure 1). The first with a large decrease for each unit of increase in germination percentage and a second stationary. For example, setting absolute vigor at 1% when germinating at 1% gives relative vigor of 100%, increasing a unit at germination changes it to 50%.

Table 1. Regressions regarding relative vigor when maintaining absolute vigor and changing germination.

Absolute vigor (%)	Equation of relative vigor (%)	^{2}(%)	[1]*F*
1%	$Y= 2,7111+141,9418\ e^{-0,4531x}$	95,42	1032,6553
10	$Y= 12,4785+154,5837\ e^{-0,0668x}$	98,96	4273,3161
20	$Y= 19,7288+188,8379\ e^{-0,0453x}$	99,71	13411,0009
30	$Y= 25,4830+218,1018\ e^{-0,0367x}$	99.90	34776,2040
40	$Y= 30,3173+244,1441\ e^{-0,0317x}$	99,96	84784,7257
50	$Y= 34,5114+268,0088\ e^{-0,0283x}$	99,99	207756,9476
60	$Y= 41,5716+311,3629\ e^{-0,0239x}$	100,00	1625140,0786
	$Y= 167,3152-0,9886x$	99,07	3200,4204
70	$Y= 178,9126-0,9958x$	99,62	5228,2197
85	$Y= 184,4238-0,9979x$	99,78	7090,4922
90	$Y= 189,7661-0,9992x$	99,90	10388,5343

[1] Bold values represent significant regression models at 0.01 significance by the Snedecor F test.

Using soybean and corn as the minimum germination pattern of 80 and 85% (BRASIL, 2009), , respectively, it is clear that up to 50% absolute vigor levels, the relative vigor is in the syrup of the curve, close to stylization. Analyzing the tail end at 99% germination gives 1.01% relative vigor and increasing one unit yields 1% relative vigor. In this case, one unit at the beginning of the curve had an impact of 50% and at the end of 0.01%. In the 1% absolute vigor curve, stabilization was reached close to 20% of germination and from this point the increments were low (R^2 = 95.42%).

From the estimated curve for absolute vigor of 30% the model does not have a stationary point, but the increments tend to continue to be smaller in the syrup. Finally, the slope is very small, characterizing a linear model (Figure 1), even though the exponential function of three parameters being significant and the perfectly fitting one (R^2 = 100%). In these cases, parsimonious models that explain the phenomenon with as few parameters as possible should be chosen. Thus we opted for the linear (R^2 = 99.07%). On the other side, there is relative vigor against the variation of absolute vigor and maintaining the fixed germination. In this case, all lines fit the linear function (Figure 2).

FINAL CONSIDERATIONS

The history of science is full of epiphanies, in which research groups in different parts of the globe present similar ideas for the same process. In this sense, another work, with a biological focus, proposed to relativize germinability in a particular context of empty seeds, given the relevance given to the parent (SANTANA et al., 2018). Similarly, this text proposes to relativize another seed physiological attribute in order to provide inferential accuracy to the vigor process, providing information that even complements the work of Santana et al. (2018). So the question that remains is, has seed science entered a phase of relativity to attribute accuracy to experiments that were once inaccurate? This author believes that, yes! And it is in this context that the present revision can enter the history of sowing, as north for a new beginning. Moreover, this measure is not intended to replace the usual (absolute vigor) but is to add a new index that characterizes the individual capacity of seeds, therefore an index of "success".

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RESUMO: O vigor de sementes é a totalidade de todas as propriedades que determinam uma rápida e uniforme emergência e desenvolvimento de plântulas normais sob uma vasta gama de condições. Todavia, a qualidade fisiológica dentro de um lote de sementes não é homogênea, gerando um gradiente de qualidade entre as sementes. Assim, o vigor expresso pela porcentagem final de plântulas normais tende a subestimar a qualidade do lote, por levar em consideração o número total de sementes. Um método possível para corrigir tal efeito seria ponderar o vigor pela germinação, gerando um índice denominado vigor relativo. O índice reflete o "sucesso" das sementes viáveis em manter seu potencial sob uma situação de estresse. Neste contexto, o artigo de propõe a possibilidade de uso uma nova medida, para o vigor, e uma nova interpretação, o vigor relativo, além de dar as bases matemáticas para seu uso.

PALAVRAS-CHAVE: Estresse. Índice de emergência em campo. Índice de sucesso. Qualidade fisiológica.

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