A Simple Index for Describing the Efficacy of Chemicals in Breaking Seed Dormancy

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ABSTRACT

When comparing the efficacy of different chemicals on seed dormancy breakage, germination percentage alone is not a good descriptor of the phenomenon because the process can be triggered by high concentrations of a compound while other compound can be highly effective under very low concentrations. Because of that an Efficiency Index (I_{ef}) relating the response (i.e. dormancy breakage) of dormant seeds of Townsville stylo (*Stylosanthes humilis* HBK) to several selenium (Se) compounds at their optimal concentrations was employed by Pinheiro et al. (2008 b). In this paper, the index is revised to a more coherent one (Ef_i), that relates the effect of the chemicals on germination in a more straight-forward way. The innate dimension displayed by the new index (amount of the chemical per unit germination percentage) is an expression of the tissue sensitivity to the chemicals.

EXPERIMENTAL TECHNIQUES

Dormancy breakage of seeds and buds is usually expressed as a percentage of the total number of the organs resuming growth following a period of relative inactivity and as a consequence of perception of environmental cues, changes in internal conditions (e. g. post-maturation) or effect of chemical or physical agents. As a result of dormancy breakage seeds become germinable; thus germination is usually taken to be the end of dormancy, although the two phenomena (germination and dormancy breakage) constitute different processes. From the practical point of view a morphological feature (radicle or cotyledon protrusion) is accepted as representing germination, although the underlying biochemical and physiological processes culminating with organ protrusion had started much earlier.

Germination percentage, the universally employed parameter for quantifying germination, is rendered much more realistic when described simultaneously with the mean rate of germination and variation of this rate (Bewley and Black, 1978). For instance, by combining total germination and germination rate, Timson (1965) proposed the index Σ_{10}. If all seeds germinate in the first day of imbibition, $\Sigma_{10} = 100$; in the second day $\Sigma_{10} = 90$, and if no seeds

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germinate in 10 day $\Sigma_{10} = 0$. Identical values of Σ_{10}, however, can be found with different frequencies of germination along the time (Bewley and Black, 1978). Another index employed is the time required for germination to reach a pre-established value, for instance 50%. This may constitute a problem since the arbitrary germination percentage may not be attained, making the time for germination equal to infinite, a mathematical figure difficult to handle. This can be circumvented by taking the inverse of the time for germination ($time^{-1}$); in this case when the seed does not germinate the time for germination becomes zero. The inverse of time has been used by Witkowska-Żuk and Kapuściński (1969) as a component of their index to describe bud dormancy breakage in *Populus*. The inverse of numbers, however, produces a non-linear relationship with the phenomenon, i.e., large variations in dormancy breakage may be associated with very small variations in the corresponding index.

Although germination percentage is a good way to describe germination, depending on the situation, it can be better described if complemented with additional associated parameters. This is especially true when one is dealing with the effectiveness of different chemical agents that break seed or bud dormancy. Although universally used germination percentage alone is not a good descriptor of the phenomenon since similar germination percentage can be produced by the chemicals at very different amounts (concentrations) and vice-versa.

Dormant seeds of the tropical forage legume Townsville stylo (*Stylosanthes humilis* HBK) were harvested from plants in a green-house, dehulled from their husks and scarified with fine sand paper. Germination tests were conducted in Petri-dishes in the dark at 30 °C in a day/night growth chamber. Ethylene that emanated from the seeds was collected from the atmosphere of 25 cm^3 Erlenmeyer flasks and injected in a Hewlett-Packard 5890 series II chromatograph for quantification. Statistical analysis of results was carried out through the Scott-Knott test at 5%. For further details of experimental conditions see Pinheiro et al. (2008a, b).

RESULTS AND DISCUSSION

As shown in Table 1 (mostly reproduced from Pinheiro et al., 2008b), all soluble Se compounds employed broke dormancy of Townsville stylo seeds. The optimal concentrations of the compounds promoting maximal germination showed a 10-fold variation (0.2 to 2 mM), whereas the highest (86%) and the lowest (64%) germination exhibited only a 1.34-fold variation. This much wider range in concentration than in germination shows that the variation in the efficacy of Se compounds in breaking seed dormancy is very large. Among other reasons this might result from different sensitivities of the seed tissues to the several compounds, i.e., the higher the tissue sensitivity the higher its response to the compound (Trewavas, 1982). Hence, when dealing with the efficiency of a compound in breaking dormancy not only total germination but also its chemical concentration is of utmost importance.

In an attempt to group the Se compounds according to their efficacy in breaking dormancy of Townsville stylo seeds Pinheiro et al. (2008b) used an

Table 1. Germination of dormant seeds of Townsville stylo exposed to some Se compounds at optimal concentrations in Petri-dishes (90 mm), five replications. Average of eight different experiments (Data used with permission from *Seed Science and Technology*).

Compound – Optimal concentration	Germination (%)								
	1	2	3	4	5	6	7	8	Mean ± SE
Sodium selenate (Na_2SeO_4) – **2.0 mM**	66.8	79.2	83.2	77.2	71.6	73.2	87.2	85.2	78.0 ± 3.5 a
Sodium selenite (Na_2SeO_3) – **0.2 mM**	49.2	81.2	81.2	79.2	80.0	64.4	77.2	73.6	73.3 ± 4.0 a
Selenic acid (H_2SeO_4) – **1.0 mM**	68.0	73.2	86.6	76.8	74.8	80.0	88.0	76.0	77.9 ± 3.4 a
Selenious acid (H_2SeO_3) – **0.6 mM**	70.8	70.0	86.0	79.2	72.8	87.6	86.8	78.4	79.0 ± 2.6 a
Selenourea (SeU) (CH_4N_2Se) – **1.0 mM**	61.6	84.8	95.2	87.2	90.0	90.8	89.6	87.2	85.9 ± 3.6 a
Selenomethionine (SeM ($C_5H_{11}NO_2Se$) – **1.0 mM**	55.6	68.4	56.8	70.4	70.4	60.4	74.8	56.4	64.2 ± 2.7 b
Selenium tetrachloride ($SeCl_4$) – **0.2 mM**	47.6	77.2	86.4	80.8	84.0	78.4	78.0	79.4	76.5 ± 4.3 a
Selenium dioxide (SeO_2) – **0.2 mM**	49.2	74.8	88.4	79.6	78.4	72.0	84.4	68.0	74.4 ± 4.3 a
Control (dormant)	14.0	10.0	1.6	9.2	17.2	7.6	11.6	2.0	9.2 ± 1.9 c

Efficiency Index (I_{ef}) relating the amount of the chemical to germination as follows:

$$I_{ef} = C^{-1}\left(\frac{\Delta G}{100}\right) \qquad [1]$$

or its equivalent

$$I_{ef} = \frac{1}{C}\left(\frac{G_x - G_o}{100}\right)$$

where C is the optimal concentration (pre-chosen experimentally), G_x the maximal germination promoted by the chemical, G_o germination in the control, and ΔG (= $G_x - G_o$) the net germination. This equation has been successfully worked out by Pinheiro et al. (2008b). Their data of germination (Table 1) were used to calculate the I_{ef} values in Table 2. This index displays the following characteristics: 1) The greater the efficacy of the compound, the greater its value; 2) It can be used to evaluate the effects of chemicals that inhibit germination, in which case it assumes negative values; 3) I_{ef} can be used to monitor the degree of dormancy of a seed or bud; as the structure is coming out of dormancy its values becomes lower and lower (and see below); and 4) The equation is very easy to manipulate (once two parameters of the equation are known the third one is easily calculated).

Table 2. Efficiency Index (Ef_i – optimal concentration of the chemicals per unit germination percentage, pct^{-1}) of some Se compounds in triggering dormancy breakage of Townsville stylo seeds calculated with data from Table 1, according to Eq. [2]. Average of eight different experiments. In the far right column I_{ef} (Eq. [1], dimensionless) is also presented.

	Ef_i ($\mu M \cdot pct^{-1}$)									
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Mean ± SE**	**I_{ef}**
Na_2SeO_4	30.49	28.90	24.51	26.46	36.76	29.41	37.88	24.04	**29.80 ± 1.83 e**	**0.34 ± 0.02 e**
Na_2SeO_3	3.52	2.81	2.51	3.05	3.18	2.86	5.68	2.79	**3.30 ± 0.36 a**	**3.21 ± 0.24 a**
H_2SeO_4	13.81	15.82	11.76	13.09	17.36	14.79	18.52	13.51	**14.83 ± 0.80 c**	**0.69 ± 0.04 c**
H_2SeO_3	7.50	10.00	7.11	7.98	10.79	8.57	10.56	7.85	**8.80 ± 0.51 b**	**1.16 ± 0.07 b**
SeU	12.02	13.37	10.68	12.82	13.74	12.82	21.01	11.68	**13.52 ± 1.12 c**	**0.77 ± 0.05 c**
SeM	18.94	17.12	18.12	15.82	18.80	16.34	24.04	18.38	**18.44 ± 0.90 d**	**0.55 ± 0.02 d**
$SeCl_4$	2.82	2.98	2.36	3.01	2.99	2.79	5.95	2.58	**3.19 ± 0.40 a**	**3.37 ± 0.27 a**
SeO_2	3.11	3.09	2.30	2.75	3.27	2.84	5.68	3.03	**3.26 ± 0.36 a**	**3.26 ± 0.26 a**

The usefulness of I_{ef} in determining the efficacy of a compound is clearly seen in Tables 1 and 2. Whereas selenourea (SeU) produced the highest seed dormancy breakage (Table 1), its efficiency as a dormancy-breaking agent was marginally different from that of selenomethionine (SeM), a compound which promoted the lowest germination (Tables 1 and 2). These markedly different effects were caused by the same concentration (1.0 mM) of both compounds.

Although useful and operative I_{ef} was deliberately left dimensionless by Pinheiro et al. (2008b), because one of its components was the inverse of concentration (C^{-1}). The real concentration can nevertheless be found from Eq. [1] by using two or three simple calculation steps. However, this small problem can be eliminated by improving Eq. [1] as follows:

$$Ef_i = C\,(\Delta G)^{-1} \qquad [2]$$

or its equivalent

$$Ef_i = \frac{C}{G_x - G_o}$$

Equation [2] is more simple and much easier to handle than Eq. [1]. Besides displaying all the useful aspects of Eq. [1], it relates the compound concentrations directly with its effects (germination), thus being more rational. In doing so it describes the sensitivity of the seeds to the chemicals (Trewavas, 1982). In other words, the more efficient the compound in eliciting dormancy breakage the lower Ef_i (Table 2).

Actually both indexes (I_{ef} and Ef_i) operate in an inverse way in relation to each other: a very efficient chemical produces an I_{ef} tending to infinite and an Ef_i approaching zero. It should be stressed that in this situation the infinite number is a result of an operation contrarily to the presumable infinite of

Witkowska-Żuk and Kapuściński (1969) that takes an active part in the calculations to produce their index. As Ef_i (Eq. [2]) values approach zero, this means that the compound is becoming highly effective in breaking seed dormancy. This index, however, never reaches zero value because C (optimal concentration), an experimentally pre-chosen parameter, obviously never assumes the zero value. An increase in Ef_i value means that dormancy breakage and the action of the particular chemical are becoming decoupled. That is the only aspect in which I_{ef} shows an advantage over Ef_i: a closer relationship of its meaning with a modular number instead of with an inverse value.

Intuitive as Ef_i is, it is very surprising that it is not being used widely. Ef_i has an advantage over I_{ef} of expressing how much of a unit percentage (of seed germination) is being affected by a given amount of the chemical, transducing in this way the sensitivity of the seeds to the compound, and automatically imprinting in the index an innate dimension. This fact per se may obscure whatever advantage I_{ef} may have over Ef_i. The latter can also monitor the dormancy state of the seeds. An increasing Ef_i (and a decreasing I_{ef}) indicates that seeds are coming out of dormancy; in this case, however, it expresses the capacity of response of the tissue rather than its sensitivity to the chemical. Similarly to I_{ef}, Ef_i can also be used to describe the inhibitory power of chemicals to germination of non-dormant seeds, producing negative values.

Tables 1 and 2 shows that the compounds triggering the highest dormancy breakages (SeU, H_2SeO_3, Na_2SeO_4 and H_2SeO_4) are not necessarily the most efficient affecting that phenomenon (shown by $SeCl_4$, SeO_2, Na_2SeO_3 and H_2SeO_3), whichever index is employed. The least efficient compounds were Na_2SeO_4, SeM, H_2SeO_4 and SeU; from these only SeM caused a relatively low germination. This highlights the usefulness of relating the concentrations of the chemicals to germination percentage.

Ethylene seems to constitute an absolute requirement for the breakage of dormancy of Townsville stylo seeds (Ribeiro and Barros, 2006). Pinheiro et al. (2008a, b) demonstrated that Se compounds broke dormancy of Townsville stylo seeds by inducing ethylene biosynthesis through the accumulation of its immediate precursor 1-aminocyclopropane-1-carboxylic acid (ACC). On a molecular basis, these findings recently received strong support from the fact that upregulation in the transcription of genes codifying for ACC synthase (16-fold) and for ACC oxidase (4-fold) were observed in roots of *Arabidopsis thaliana* plants treated with selenate. Additionally upregulations of some genes from the ethylene signalling pathway were also observed in the same study (Van Hoewyk et al., 2008). The amounts of ethylene (pmol $seed^{-1}$) that emanated from Se-stimulated dormant seeds were: H_2SeO_4 – 217.5, H_2SeO_3 – 143.6, SeO_2 – 196.0, $SeCl_4$ – 214.5, SeM – 285.6, SeU – 260.5, Na_2SeO_4 – 215.3, Na_2SeO_3 – 194.1 and dormant untreated – 43.5 (Pinheiro et al. 2008a). Following uptake, transport, metabolism and arrival of the chemicals to target cells, ethylene production is the ultimate and decisive step for seed dormancy breakage. Comparison of the amounts of ethylene produced by seeds, on one side, with both Ef_i and I_{ef} (Table 2) does not produce a straight-linear correlation between them. For instance, SeM induced the largest seed ethylene production,

but was shown to be one of the least efficient compounds in breaking dormancy (a very high Ef_i and a very low I_{ef}). Thus, the indexes were capturing the post-ethylene production effects and working in an integrative way. This can also be deduced from the effects of Na_2SeO_3, SeO_2, and $SeCl_4$ which exhibited the highest efficiencies (Table 2) but did not promote the highest dormancy breakages (Table 1). This was likely due to limiting factors resulting from treatments (e.g. Se toxicity, see Pinheiro et al., 2008a, b). By capturing and computing all this information and integrating it on a per unit response (germination percentage) the indexes are likely to be operating through algorithms. In summary both indexes are useful in comparing the effectiveness of chemicals in eliciting seed dormancy breakage. Furthermore, such indexes are much more associated with the underlying mechanisms of action of the chemicals than is germination percentage. However, Ef_i describes a more direct effect of the chemicals, is simpler than I_{ef}, is much easier to manipulate and is associated with the sensitivity of the tissues to the chemicals. Altogether these features make Ef_i a more coherent index than I_{ef}.

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