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Approaches to Quantification of Seed Vigour

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"This paper is most respectfully dedicated to my esteemed teacher the late Professor R. D. Asana of the Indian Agricultural Research Institute, New Delhi, who led us to the firm conviction that authentic experimental data, if properly approached and rationally treated, would be silently but explicitly communicative"- RN Basu.

ABSTRACT: Quantification of seed vigour – a very important qualitative character, has been attempted in this review-cum-reappraisal paper taking into account a number of physiological and biochemical parameters that are positively or negatively correlated with germinability. Efforts have been made to score each of them individually on a scale ranging from 0 to 100 (as with germination percentage) and then, if necessary,combining them into a unified score value for a clear and easy understanding of the ability of a seed lot to make a predictable stand establishment.

Important negatively correlated vigour parameters like electrical conductivity of seed-steep water, leaching of sugars and amino acids from the seed due to loss of integrity of biomembranes, lipid peroxidation and emanation of volatile organic compounds from deteriorating seeds as well as positively correlated parameters such as activity of dehydrogenases and amylases, lesser inhibition of growth of jute seedlings (bioassay material) in presence of germinating high vigour seed stocks than with low vigour ones have been taken into consideration in formulating the vigour scores.

The basic (original) data for the purpose have been selected from mostly published literature on clover, cotton, soybean, wheat, mustard, rice, sunflower and onion from authoritative sources from abroad as well as from the laboratory of the author in the University of Calcutta and then variously transformed before subjecting to correlation-regression analysis. With germination percentage as the independent variate (x), different ways of transformation of the dependent variate (y) such as reciprocal conversion (dividing 1 by the observed variate), deducting from 1 the value of observed parameters expressed in fractions (decimals) less than 1, would convert negative correlations to positive correlations and in certain cases significantly minimize the deviations of the observed values from the statistical estimates predicted by the regression equations. In case of positively correlated traits, necessary conversion to percentage values has been done by taking the value of a very well preserved material of the same kind as the reference or benchmark value (= 100). With the transformations stated in details in the text it has thus been possible to mathematically score seed vigour as percent values such as electrical conductivity resistance score (ECRS), lipid peroxidation resistance score (LPRS), volatiles emanation resistance score (VERS), sugar leaching resistance score (SLRS), amino acid leaching resistance score (ALRS), dehydrogenase enzyme activity score (DEAS), amylolytic enzyme activity score (AEAS), jute seedling bioassay score (JSBS), all with positive scale values ranging from 0 to 100 enabling average or weighted-average calculations to reach a final score value.

The incorporation (by multiplication) of germination factor (GF, obtained by dividing germination percentage by 100) has been shown to further improve the positive correlations (division by GF in case of negative correlations) with considerable lowering of the deviations of observed variate from those predicted by the regression equations. The coefficients of determination (r^2) multiplied by 100 (i.e. $r^2 \times 100$) for vigour scores such as ECRS × GF, LPRS × GF and VERS × GF have gone up to 90-99% in the different materials showing vast improvements in vigour rating that is attributable to data transformation prior to statistical analysis.

The correlations between germination of soybean at planting and the individual vigour scores, ECRS, LPRS, VERS and JSBS, all multiplied by GF, have been found to be highly significant, with the coefficients of determination (r^2) reaching over 90 % coverage of the residual variances in case of a number of GF incorporated vigour ratings, and tentatively a correlative seed vigour score obtained by multiplying r^2 with mean germination percent ($r^2 \times$ G %) has proved useful in comparing the aforesaid individual vigour scores. Interestingly, the correlation with field emergence is still better and in case of LPRS × GF vigour rating it is as high as 0.99. The combination of the aforesaid vigour scores (all with incorporation of germination factor) that has been done taking simple arithmetic averages of the individual scores (combination of 2, 3, and 4 of the above noted four scores), has not given any additional advantage and all of them, with minor exceptions, have shown around 95% value of the coefficient of determination (expressed on a percent scale) suggestion thereby that a single or a selected combination of a few scores such as VERS × GF or JSBS × GF, along with the major score, ECRS × GF, many give reliable prediction of field emergence under usual planting conditions. Most likely, a seed lot with high vigour rating would do better than those with lower vigour scores even under relatively stressful edaphoclimatic conditions.

The present approach, particularly the technique of data transformation prior to bivariate correlation-regression analysis, though specifically made for seed vigour rating, may be useful for similar correlation-based studies in other fields of biology as well.

BACKGROUND

Seed vigour is a vital qualitative character of a seed lot and is of great importance for successful establishment of plants in the field especially under relatively stressful growing conditions. The issue of seed performance *vis-à-vis* seed deterioration and loss of vigour in storage and its assessment has been receiving a lot of attention from researchers over a long time as would be evident from published literature on the subject (Heydecker[11], Heydecker and Coolbear[12], Coolbear[5], McDonald[15,16,17], Hampton[8,9], Hampton and TeKrony[10], Powell[23,24], Wilson[31], Wilson and McDonald[32,33], Dornbos[7], and others), but despite sustained individual, institutional and organizational efforts no vigour test has official sanction in terms of inclusion in the seed testing certificate as an important parameter of seed quality. Only two vigour tests, namely the electrical conductivity of the seed-steep water and the accelerated ageing test are considered to be of sufficient merit by the International Seed Testing Association (ISTA) to be recommended as official but optional tests (Hampton and TeKrony[10]). Perhaps, in future more such tests may have to be seriously considered for acceptance. But more than the tests themselves, the issue regarding quantification of test results to serve as vigour scores (vigour rating) needs critical adjudication in view of the fact that quite often the tests results are masked by undue deviations and variances due to sampling errors, uncontrolled test conditions, etc. Many of the important vigour test parameters are negatively correlated with seed vigour while the morphological vigour test parameters, such as seedling length, dry weight, etc. are positively correlated. Thus, the most acceptable vigour test, electrical conductance, which is one of the easiest vigour testing methods, is negatively correlated, along with tests based on leaching of sugars (Matthews and Bradnock[14], Takayanagi and Murakami[29]) and amino acids (Basu and Rudrapal[2], Rudrapal and Basu[25,26]), from seeds dipped in water. The conductivity and leaching tests are based on sound physiological considerations in that they measure the integrity of semipermeable cell membranes and the loss of integrity is most probably one of the earliest indications of seed deterioration leading to loss of seed vigour, and ultimately viability. If done properly with due consideration to variability in moisture content of seed lots (often requiring moisture stabilization and moderately controlled laboratory conditions), the test results would be free from personal errors. Unfortunately, however, variations in test results have been noted that may be attributed to uncontrollable seed lot differences and often due to confounding of germination percentage and vigour status of those seeds just surviving (many of which may not be included as normal seedlings in a standard germination test) and those with relatively high vigour indices, and commercial seed lots may be heterogenous enough to have variable proportions of such seeds. A large number of samples drawn from a seed lot in conductivity as well as in other tests may partly overcome the problem. Other important physiological and biochemical tests, besides the membrane permeability tests that are negatively correlated with seed vigour include lipid peroxidation (possibly the reason behind loss of membrane integrity), volatile compounds emanation (again, most likely lipid peroxidation byproducts, vide Wilson and McDonald[33], Sur and Basu[28], Sung[27]) which have shown considerable promise as effective parameters for vigour tests.

The positively correlated biochemical parameters that are used for certain specific purposes include dehydrogenase enzyme assay (the basis of topographical tetrazolium, TZ, test as the enzyme turns TZ into red coloured formazan enabling identification of dormant viable seeds, with the additional advantage of eluting the colour from excised incubated embryos with solvents like methyl cellosolve and assay the enzyme colorimetrically, as discussed later), and amylolytic enzyme activity (widely used in the laboratories of brewing industry requiring highly germinable cereal seeds), and bioassay vigour tests (based on the emanation of growth inhibitory volatile substances produced by germinating low vigour seeds).

A major problem of quantification would be forming a suitable scale (preferably in the range of 0 to 100 as in case of germination) and then, if so required, combining positively as well as negatively correlatedscores into a unified score for a seed lot. A simple approach towards such quantification could be the use of correlation coefficients (r values) obtained from bivariate analysis, and ignoring the + or − signs, multiplying the r value by 100 to get a positive percentage value. Even better would be use of r^2 (coefficient of determination) because that is always positive and gives a better estimate of the coverage of the observed variances by the two variates). The coefficients of determination expressed as percent values (on multiplication by 100) may be amalgamated with other parameters to get a tentative vigour score. However, there are problems such as the question of viability differences (similar correlations may be noted at different levels of viability) and of undesirable deviations that have been discussed later. Prior transformation of the data to improve the correlations and minimize the deviations of the observed variates from those predicted by the relevant regression equations would be necessary for the purpose. With the aforesaid background, a range of vigour related data on membrane functions, notably electrical conductance, sugar and amino acid leaching, lipid peroxidation, volatile inhibitory substances emanation, bioassay vigour scores, dehydrogenase enzyme activity, amylolytic enzyme activity have been variously transformed and scored for subsequent correlation-regression analysis with a view to determine the efficacy and predictability of the different approaches. The data for the study have been obtained from a number of outside sources (published materials in international journals) as specifically mentioned in the tables as well as those originating in the laboratory of the author in the University of Calcutta (mostly published in national journals).

DATA COLLECTION, PROCESSING, TRANSFORMATION AND STATI-STICAL ANALYSIS

Data from several kinds of seeds have been used to have a generalized view on the issue and those included are: crimson clover, cotton, soybean, wheat, mustard, rice, sunflower and onion. The particulars on methods, units, etc. of different vigour parameters are given in the respective publications cited in the tables and in the section on references. Emphasis has been given on electrical conductivity and data for the same have been available for each of the materials. For the other seeds, the available data on parameters such as lipid peroxidation, volatile inhibitor (mostly aldehydes) production, dehydrogenase and amylase activity have been analyzed. Further, an attempt to combine individual vigour scores has been made only for soybean.

The methodology for transformation of data is outlined below for the different parameters starting with electrical conductivity.

Electrical conductivity (EC)

Usually conductance is measured in µmhos; for the purpose of transformation, the value is first converted to millimhos (mmhos). The conductance in mmhos is then deducted from 1 and the remainder is multiplied by 100 to get a percentage value which is named electrical conductivity resistance score (ECRS).

so, ECRS = (1 – 0. millimhos) × 100

Thus for the first value in Table 1, namely 70 µmhos, the transformed value is:

ECRS = (1 – 0.070) × 100 = 93%

The other transformations of electrical conductivity are: EC reciprocal (abbreviated as EC reci), obtained simply by dividing one (1) by EC reci), obtained simply by dividing one (1) by the EC µmhos value and is actually the resistance in ohms. Another transformation of the EC data is division or multiplication by germination factor (GF), which is equal to germination percentage divided by 100, and is 1 for 100% germination and always less than 1 when germination is lower. As would be evident (and expected as well) the inclusion of GF (multiplication in case of positive correlations and division for negative correlations) would considerably improve the value of correlation coefficients and minimize the deviations of the observed variates from those predicted by the regression equations. For EC readings in µmhos, the transformations are thus: ECRS, EC reci (in ohms), EC µmho ÷ GF (value in µmhos), ECRS × GF and EC reci × GF.

Leaching of sugars and amino acids

As mentioned earlier leaching of metabolites such as sugars and amino acids is attributed to loss of membrane integrity (the leaching of metabolites is, however, slower than electrolytes such as K^+ ions).

Sugar leaching is calculated by measuring the optical density (OD) of the anthrone colour reaction product. The transformation is done by deducting from 1 the optical density value and then multiplying the remainder by 100. Thus sugar leaching resistance score (SLRS) for the first item of Table 8(B) for sunflower, namely 0.205 OD would be equal to 79.5% as shown below:

SLRS = (1-0.0 OD)×100 = (1–0.205) × 100 = 79.5%

Although the optical density values for the purpose should be theoretically less than 1, optical density values should preferably lie between 0.5 OD to 0.05 OD, corresponding to transmission percent (% T) approximately from 30 to 80 (that indicates the avoidance of 1, corresponding to log 10% T, as the upper limit of usual OD readings (logarithmic) in the scale of 0 – 100% transmission). Incidentally, the direct transmission reading (that are positively correlated with seed vigour, higher % T values showing lower concentrations of a solute in solution), can be used directly as a parameter for negatively correlated traits as has been tested in case of lipid peroxidation and volatiles emanation.

Amino acid leaching resistance score (ALRS) can be calculated in the same way as sugar leaching.

Thus, ALRS = (1 – 0.0D) × 100; for the first item 8 (B) namely, 0.238 OD for ninhydrin colour reaction it is equal to 76.2%.

Sometimes the amount of sugar or amino acid leached out is given in µg per g of seed. In that case the value has to be converted to mg (upto three decimal places) and deducted from 1 and then the remainder multiplied by 100 as shown below:

SLRS/ALRS = (1 – 0. mg) × 100

The aforesaid transformation would be useful for conversion of violatile aldehyde production data given as µg aldehyde (as formaldehyde) per

Table 1A. Correlation-regression analysis of electrical conductance data of crimson clover seed after various transformations for vigour rating (data from Ching and Schoolcraft[3], as quoted by Heydecker[11]). Data of 10-year-old viable seed lots along with their transformations

Seed lot No.	Germination[a]		EC (μmho per g)	ECRS[b] %	EC reciprocal[c] (ohm × 10^6)	EC μmho ÷GF (μmho)	EC Reciprocal × GF (ohm × 10^6)	ECRS(%) × GF
	G%	GF						
2	98	(0.98)	70	93.0	14285	71.4	13999	91.1
3	98	(0.98)	89	91.1	11235	90.8	11010	89.2
4	98	(0.98)	44	95.6	22727	44.9	22272	93.7
5	99	(0.99)	51	94.9	19607	51.5	19411	93.9
6	94	(0.94)	49	95.1	20408	52.1	19183	89.4
7	86	(0.86)	78	92.2	12820	90.7	11025	79.3
8	89	(0.89)	57	94.3	17543	64.0	15613	83.9
Correlation with germination (%)			-0.1778	0.1778	0.2492	-0.3891	0.4106	0.9532**

(a) Germination factor (GF, germination percentage divided by 100) in parenthesis alongside germination percentage.
(b) ECRS: electrical conductivity resistance score calculated as: (1-0, millimhos) × 100; ECRS × GF: same multiplied by germination factor.
(c) EC reciprocal: reciprocal of EC μmho i.e. 1 ÷ EC μmho, it would give the value of resistance in ohm; EC reci × GF: same multiplied by germination factor.

Table 1B. Summary of correlation-regression analysis with germination percent as the x variate and EC values as the y variate (See Table 1C for detailed analysis of three sets of variates)

y variate	Correlation co-efficient r	Coefficient of determination r^2	Mean of x variate $\bar{x}$	Mean of y variate $\bar{y}$	Sample covariance SC (n=7)	Regression Equation y = A + Bx		Sum of least squares		Mean deviation	
						A	B	$\sum(x-\hat{x})^2$	$\sum(y-\hat{y})^2$	$(x-\hat{x})$	$(y-\hat{y})$
EC μmho	-0.178	0.032	94.6	62.6	-15.38	117.2	-0.578	4489.2 (27.94)	1632.4 (24.41)	23.88 (25.25)	13.80 (22.05)
EC reci	0.249	0.062	94.6	16946	5493.7	-2571.4	206.38	2412.5 (19.63)	103065296 (22.64)	16.88 (17.83)	3474 (22.64)
ECRS	0.178	0.032	94.6	93.7	2.01	88.28	0.058	4889.2 (27.94)	16.32 (1.63)	23.88 (25.25)	1.38 (1.47)
EC μmho ÷ GF	-0.389	0.151	94.6	66.5	-37.61	200.1	1.413	895.1 (11.96)	2299.1 (27.26)	10.29 (10.88)	16.21 (24.38)
EC reci × GF	0.411	0.169	94.6	16073	9270.1	-16861	348.2	778.1 (11.15)	85887636 (21.79)	9.25 (9.78)	3311 (20.60)
ECRS × GF	0.953	0.909	94.6	88.6	26.17	-4.34	0.983	16.06 (1.60)	15.53 (1.68)	1.35 (1.43)	1.34 (1.51)

Table 1C. Detailed correlation-regression analysis of three sets of variates (vide Table 1A); data of Ching & Schoolcraft (Germination (%) versus EC μmho

G% EC μmho								
$(x-\hat{x})^2$	$(x-\hat{x})$	$\hat{x}$	(x)	(y)	$\hat{y}$	$(y-\hat{y})$	$(y-\hat{y})^2$	
265.195	+16.284	81.715	**98**	**70**	60.590	+9.409	88.541	r = -0.1778
2417.411	+49.167	48.832	**98**	**89**	60.590	+28.410	807.128	r^2=0.0316
824.383	-28.712	126.712	**98**	**44**	60.590	-16.59	275.228	$\bar{x}$=94.57
243.266	-15.597	114.597	**99**	**51**	60.012	-9.012	81.225	$\bar{y}$=62.57
578.826	-24.058	118.060	**94**	**49**	62.90	-13.901	193.254	
328.696	+18.130	67.870	**86**	**78**	67.524	+10.475	109.743	Sample covariance (n=7) = -15.380
231.454	-15.213	104.213	**89**	**57**	65.790	-8.791	77.276	Regression coeff.
$\Sigma(x-\hat{x})^2$ = 4889.23	Σdeviation ÷ n = 23.88					Σdeviation ÷ n = 13.8	$\Sigma(y-\hat{y})^2$ = 1632.39	A = 117.216 B = -0.578
(27.94% of $\bar{x}$)	(25.25% of $\bar{x}$)					(22.05% of $\bar{y}$)	(24.41% of $\bar{y}$)	

Table 1 C continued

(Germination (%) versus ECRS

G% ECRS								
$(x-\hat{x})^2$	$(x-\hat{x})$	$\hat{x}$	(x)	(y)	$\hat{y}$	$(y-\hat{y})$	$(y-\hat{y})^2$	
265.195	+16.284	81.715	**98**	**93.0**	93.941	-0.940	0.885	r = -0.1778
2417.411	+49.167	48.832	**98**	**91.1**	93.941	-2.840	8.071	r^2=0.0316
824.383	-28.712	126.712	**98**	**95.6**	93.941	+1.659	2.752	$\bar{x}$=94.57
243.266	-15.597	114.597	**99**	**94.9**	93.999	+0.901	0.812	$\bar{y}$=93.74
578.826	-24.058	118.060	**94**	**95.1**	93.709	+1.390	1.932	
328.696	+18.130	67.870	**86**	**92.2**	93.247	-1.047	1.097	Sample covariance (n=7) = 2.0095
231.454	-15.213	104.213	**89**	**94.3**	93.421	+0.879	0.772	Regression coeff.
$\Sigma(x-\hat{x})^2$ = 4889.23	Σdeviation ÷ n = 23.88					Σdeviation ÷ n = 1.378	$\Sigma(y-\hat{y})^2$ = 16.322	A = 88.278 B = 0.0578
(27.94% of $\bar{x}$)	(25.25% of $\bar{x}$)					(1.47% of $\bar{y}$)	(1.63% of $\bar{y}$)	

Table 1C continued

(Germination (%) versus ECRS × GF

G% ECRS × GF								
$(x-\hat{x})^2$	$(x-\hat{x})$	$\hat{x}$	(x)	(y)	$\hat{y}$	$(y-\hat{y})$	$(y-\hat{y})^2$	
0.863	+0.929	97.070	**98**	**91.1**	92.013	-0.913	0.834	r = -0.9532
8.190	+2.861	95.138	**98**	**89.2**	92.013	-2.813	7.917	r^2=0.9086
2.941	-1.715	99.715	**98**	**93.7**	92.013	+1.686	2.843	$\bar{x}$=94.57
0.843	-0.918	99.918	**99**	**93.9**	92.997	+0.903	0.815	$\bar{y}$=88.64
1.799	-1.341	95.341	**94**	**89.4**	88.081	+1.318	1.739	
0.867	+0.931	85.068	**86**	**79.3**	80.215	-0.915	0.838	Sample covariance (n=7) = 26.17
0.558	-0.747	89.747	**89**	**83.9**	83.165	+0.734	0.540	Regression coeff. A = -4.338 B =0.9832
$\sum(x-\hat{x})^2 = 16.06$ (1.60% of $\bar{x}$)	∑deviation ÷ n = 1.348 (11.43% of $\bar{x}$)					∑deviation ÷ n = 1.337 (1.51% of $\bar{y}$)	$\sum(y-\hat{y})^2 = 15.528$ (1.68% of $\bar{y}$)	

Note: Values in parentheses indicate average deviations of the individual variates from the estimates, $\hat{x}$ and $\hat{y}$, expressed as percent of respective means ($\bar{x}$ or $\bar{y}$); see text details. In case of simple deviation (+ or -) the summations, irrespective of + or – signs (otherwise the same would be around zero), are divided by n and expressed as the respective percentages of $\bar{x}$ or $\bar{y}$. In case of squared deviations (all positive values), after division by n, the average values are square rooted and expressed as respective percentages of $\bar{x}$ or y. Thus, taking the example of data given in Table 1C (iii) $(x-\hat{x})^2$= 16.06 (n=7), the procedure followed has been: □ $(x-\hat{x}) = 16.06 \div 7 = 2.2943 \rightarrow \sqrt{2.2943} = 1.5147 \rightarrow \div\bar{x}\ (= 94.57) \times 100 = 1.601\%$; the same procedure being followed for the y variate.

g seed (or per 100 seeds) as done by Wilson and McDonald [32].

Lipid peroxidation (LP)

The optical density of the thiobarbituric acid (TBA) colour reaction product is deducted from 1 and then the remainder multiplied by 100 and expressed as LPRS (lipid peroxidation resistance score). Thus the value of LP OD for mustard given as OD × 10^3 = 220 i.e. 0.220 (Table 5, first item) when transformed, has given a score of 78%. Incidentally, LP%T values in wheat (recalculated from OD values in Table 6) also give positively correlated vigour indices in percentages that are mathematically different from LPRS values.

Volatiles (aldehydes) production

Although a range of volatile substances would be emanated from deteriorating seeds, the emphasis is given on low molecular weight aldehydes (presumably products of lipid peroxidation) that are preferentially absorbed by the reagent methyl benzothiazolinone hydrazone (MBTH) and the optical density of the coloured reaction product is read at 635 nm on a colorimeter. This optical density value is deducted from 1 in the same

Table 2

Transformation of electrical conductivity data of seeds of Acala 44 cotton and their correlation regression analysis for seed vigour rating (Data of Andersen, Hart and French[1])

Seed Lot No.	Germination (%)	Conductivity Resistance CR, ohm × 10^6	EC µmho	EC milli-mho	ECRS (%)	CR × GF	EC µmho ÷ GF	ECRS (%) × GF
1	54.5	5580	179	0.179	82.1	3041	328	44.7
4	57.5	6108	163	0.163	83.7	3512	283	48.1
8	60.0	6744	148	0.148	85.2	4046	247	51.1
21	78.0	7450	134	0.134	86.6	5811	172	67.5
13	72.5	7630	131	0.131	86.9	5532	181	63.0
20	90.0	7830	128	0.128	87.2	7047	142	78.5
18	70.0	8420	119	0.119	88.1	5894	170	61.7
9	75.5	8820	113	0.113	88.7	6659	150	67.0
16	80.0	9840	102	0.102	89.8	7872	127	71.8

For abbreviation and details see Table 1 and text; original data of Andersen *et al*[1] in conductivity resistance (CR, in ohms × 10^6). For statistical notations also see Table 1 and text.

Table 2 continued

Summary of correlation-regression analysis with germination % as the x variate

y variate	r	r^2	$\bar{x}$	$\bar{y}$	SC (n=9)	A	B	$\sum(x-\hat{x})^2$	$\sum(y-\hat{y})^2$	$(x-\hat{x})$	$(y-\hat{y})$
Conductivity Resistance (CR)	0.730	0.532	70.9	7602	11385	1678	83.57	956.61 (14.54)	6681548 (11.33)	8.64 (12.20)	722.6 (9.50)
EC µmho	-0.776	0.603	70.9	135.2	-221.6	250.5	-1.63	718.13 (12.60)	1899.5 (10.74)	7.80 (11.05)	12.73 (9.42)
ECRS	0.776	0.603	70.9	86.5	22.16	74.95	0.163	718.13 (12.60)	18.99 (1.68)	7.80 (11.05)	1.27 (1.47)
CR × GF	0.914	0.836	70.9	5530.4	16910	-3268	124.12	213.37 (6.89)	3287394 (10.93)	4.01 (5.66)	497.9 (9.0)
EC µmho ÷ GF	-0.912	0.833	70.9	200	-741.0	585.6	-5.44	200.36 (6.66)	6273.5 (13.30)	4.31 (6.08)	22.59 (11.29)
ECRS × GF	0.996	0.992	70.9	61.48	132.12	-7.26	0.970	9.04 (1.41)	6.94 (1.43)	0.861 (1.21)	0.873 (1.41)

Table 3

Electrical conductivity data of soybean and their transformations for correlation-regression analysis

A. Data of Wilson and McDonald[32]

Lot No.	Germination (%)	EC µmho	EC mmho	ECRS (%)	ECRS (%) × GF
2	93	27	0.027	97.3	90.49
4	79	28	0.028	97.2	76.79
3	92	29	0.029	97.1	89.33
1	71	31	0.031	96.9	68.80
5	62	49	0.049	95.1	58.96

Summary of correlation-regression analysis with germination % as the x variate

y variate	r	r^2	$\bar{x}$	$\bar{y}$	SC (n=5)	A	B	$\Sigma(x-\hat{x})^2$	$\Sigma(y-\hat{y})^2$	$(x-\hat{x})$	$(y-\hat{y})$
EC µmho	-0.797	0.635	79.4	32.8	-97.9	76.15	-0.546	412.5 (11.44)	122.97 (15.12)	8.35 (10.52)	4.56 (13.91)
ECRS	0.797	0.635	79.4	96.7	9.79	92.38	0.055	412.5 (11.44)	1.23 (0.51)	8.35 (10.52)	0.46 (0.47)
ECRS × GF	0.999	0.999	79.4	76.9	180.42	-3.02	1.006	0.453 (0.38)	0.459 (0.39)	0.28 (0.35)	0.28 (0.36)

manner as above, multiplied by 100 and expressed as volatiles emanation resistance score (VERS), the term aldehyde has been excluded to keep uniformity as a 4-letter abbreviation (also partly due to the fact that MBTH colour reaction is not strictly specific and certain other volatile substances may be absorbed). The VERS value for the first lot (T_1) of soybean derived from a VE OD value of 0.30 (given in Table 7 as OD × 10^2 = 30) would be as follows:

$$VERS = (1 - 0.OD) \times 100 = 0.70 \times 100 = 70\%$$

When the volatiles are expressed as µg per g or per 100 seeds, conversion of µg to 0. mg would be necessary for VERS transformation, as mentioned earlier. The other transformations of VE OD data are: VE OD ÷ GF, VE reci, VE reci × GF, VE %T, VE %T × GF, all of which, except the first one, are positively correlated with germinability.

Bioassay vigour score

The jute seedling bioassay score (JSBS) is based on the growth of very high vigour jute seedlings (other similar small seeded, if possible just sprouted, materials characterized by rapid and uniform growth, e.g., lettuce, amaranthus etc. may also be used depending on ambient

Table 3 continued from previous page

B. Data of Van Toai, McDonald and Staby[30]

Cultivars	Germination (%)	EC µmho	EC mmhos	ECRS (%)	ECRS(%) × GF
Amsoy 71	78	187	0.187	81.3	63.41
Pella	85	158	0.158	84.2	71.57
Williams 79	88	153	0.153	84.7	74.54
Cumberland	85	120	0.120	88.0	74.80
Cutler 71	90	107	0.107	89.3	80.37

Summary of correlation-regression analysis with germination % as the x variate

y variate	r	r^2	$\bar{x}$	$\bar{y}$	SC (n=5)	A	B	$\Sigma(x-\bar{x})^2$	$\Sigma(y-\hat{y})^2$	$(x-\bar{x})$	$(y-\hat{y})$
EC µmho	-0.793	0.628	85.2	145	-115	618.3	-5.55	48.94 (3.67)	1510.4 (11.99)	2.76 (3.16)	14.97 (10.32)
ECRS	0.793	0.628	85.2	85.5	11.5	38.17	0.555	48.94 (3.67)	15.10 (2.03)	2.76 (3.16)	1.50 (1.75)
ECRS × GF	0.962	0.926	85.2	72.9	27.15	-38.83	1.312	6.59 (1.35)	11.34 (2.06)	0.99 (1.16)	1.30 (1.79)

See Table 1 for abbreviations and transformations, and also for statistical notations.

expressed as a percentage value, as shown for rice and onion in Table 8.

If no well preserved reference material is available, an estimate of the benchmark enzyme activity may be indirectly obtained by using a set of JSBS (jute seedling bioassay score) data along with corresponding enzyme activity readings of the seed lots being studied and estimating through regression analysis the enzyme activity at 100% JSBS value.

The amylotic activity of usually α- and β-amylases is determined by extracting crude enzymes from duly imbibed and incubated seeds. The extent of hydrolysis of a standard starch solution by the crude enzyme extract is obtained by assaying the amount of unhydrolyzed residual starch using starch-iodine colour (blue) reaction. The amount of starch hydrolyzed, is converted to a percentage value by dividing with the value obtained parallelly from a very high vigour reference material followed by multiplication with 100. The amylolytic enzyme activity score (AEAS) shown for rice in Table 8 can be transformed to improve its vigour rating efficiency by multiplication with GF (i.e. AEAS × GF; the same is true for the dehydrogenase enzyme activity score, DEAS).

It needs to be mentioned here that all vigour rating values for originally negatively correlated

Table 4

Transformations of electrical conductivity data of wheat and onion and their correlation-regression analysis

Wheat[a]								Onion[b]				
Seed Lot	Germination (%)	EC μmho	EC reci	ECRS (%)	EC μmho ÷ GF	EC reci × GF	ECRS(%) × GF	Seed Lot No.	Germination (%)	EC μmho	ECRS (%)	ECRS(%) × GF
T_1	71	96	10416	90.4	135.2	7396	64.2	T_1	22	597	40.3	8.9
T_2	80	89	11236	91.1	111.2	8989	72.9	T_2	55	566	43.4	23.9
T_3	85	86	11628	91.4	101.2	9884	77.7	T_3	68	557	44.3	30.1
T_4	88	65	15385	93.5	73.9	13538	82.3	T_4	77	533	46.7	36.0
T_5	100	58	17241	94.2	58.0	17241	94.2	T_5	79	420	58.0	45.8
								T_6	80	450	55.0	44.0
								T_7	84	357	64.3	54.0

(a) Data of Rudrapal and Basu[25]

(b) Data of Choudhuri and Basu[4]

Table 4 continued

Summary of correlation-regression analysis with germination % as the x variate

	y variate	r	r^2	$\bar{x}$	$\bar{y}$	SC (n=5)	A	B	$\Sigma(x-\hat{x})^2$	$\Sigma(y-\hat{y})^2$	$(x-\hat{x})$	$(y-\hat{y})$
Wheat	EC μmho	-0.923	0.852	84.8	78.8	-161.3	199.1	-0.142	79.25 (4.69)	159..5 (7.17)	3.28 (3.87)	4.65 (5.91)
	EC reci	0.917	0.840	84.8	13181	28987	-8438	254.9	86.24 (4.90)	5621569 (8.04)	3.65 (4.30)	930 (7.06)
	ECRS	0.923	0.852	84.8	92.1	16.13	80.1	0.142	79.25 (4.69)	1.59 (0.61)	3.28 (3.87)	0.46 (0.50)
	EC μmho ÷ GF	-0.969	0.938	84.8	95.9	-315.4	331.4	-2.77	29.96 (2.89)	230.6 (7.08)	1.90 (2.24)	5.26 (5.49)
	EC reci × GF	0.959	0.920	84.8	11410	40.54	-18824	356.5	39.49 (3.31)	4280366 (8.11)	2.59 (3.05)	922 (8.08)
	ECRS × GF	0.999	0.998	84.8	78.3	118.78	-10.32	1.045	1.074 (0.55)	1.17 (0.62)	0.40 (0.48)	0.42 (0.54)
Onion	EC μmho	-0.742	0.550	66.4	497.1	SC(n=7) -1344.7	692.5	-2.811	2345 (27.56)	18540 (10.18)	15.29 (23.02)	42.97 (8.50)
	ECRS	0.742	0.550	66.4	50.3	134.47	30.75	0.281	2345 (27.56)	185.4 (10.41)	15.29 (23.02)	4.30 (8.69)
	ECRS × GF	0.943	0.889	66.4	34.7	303.67	-8.18	0.635	359.3 (10.78)	144.84 (13.37)	6.32 (9.03)	3.81 (11.20)

Note: With a wide range of germination in case of onion (22-84%) the deviations $(x-\hat{x})$ or $(y-\hat{y})$ though considerably reduced in ECRS × GF still remain quite high indicating reduced predictability; this has since been attributed not to viability but to usually high EC μmho readings in the original data set which should better be around 100-150 or so for the efficacy of data transformation and GF integration.

Table 5

Transformations of electrical conductivity and lipid peroxidation data of mustard and their correlation-regression analysis
(Data of Rudrapal and Basu[27])

Seed lot No.	Germination %	EC µmho	ECRS (%)	EC µmho − GF	ECRS (%) × GF	LP OD × 10^3	LPRS (%)	LPRS (%) × GF
1	47	203	79.7	432	37.45	220	78	36.66
2	52	190	81.0	365	42.12	208	79.2	41.18
3	60	179	82.1	298	49.26	200	80	48.0
4	68	150	85.0	220	57.8	180	82	55.76
5	75	138	86.2	184	64.65	170	83	62.25
6	65	135	86.5	208	56.225	176	82.4	53.56

Details of transformations and abbreviations same as in Tables 1 and 6; for further details see text.

Table 5 continued

Summary of correlation-regression analysis with germination % as the x variate

y variate	r	r^2	$\bar{x}$	$\bar{y}$	SC (n=6)	A	B	$\sum(x-\hat{x})^2$	$\sum(y-\hat{y})^2$	$(x-\hat{x})$	$(y-\hat{y})$
EC µmho	-0.923	0.852	61.2	165.8	-274.97	321.90	-2.551	93.23 (6.44)	606.9 (1.64)	2.75 (4.50)	7.02 (4.23)
ECRS	0.923	0.852	61.2	83.4	27.50	67.81	0.255	93.23 (6.44)	6.07 (1.21)	2.75 (4.50)	0.702 (0.84)
EC µmho ÷ GF	-0.971	0.944	61.2	284.5	-993.5	848.4	-9.219	20.83 (3.05)	2736.16 (7.51)	1.69 (2.76)	15.55 (5.47)
EC µmho × GF	0.998	0.996	61.2	51.2	106.12	-8.978	0.985	2.34 (1.02)	2.26 (1.20)	0.46 (0.75)	0.45 (0.88)
LP OD × 10^3	-0.9667	0.9345	61.2	192.3	-200.07	305.89	-1.856	37.79 (4.10)	130.23 (2.42)	1.90 (3.10)	3.52 (1.83)
LPRS	0.9667	0.9345	61.2	80.8	20.007	69.411	0.186	37.79 (4.10)	1.30 (0.58)	1.90 (3.10)	0.35 (0.44)
LPRS × GF	0.999	0.999	61.2	49.7	98.926	-6.581	0.918	0.57 (0.50)	0.56 (0.62)	0.22 (0.35)	0.20 (0.40)
LP OD versus EC µmho	0.989	0.978	192.3	165.8	565.67	- 107.89	1.423	44.24 (1.41)	89.6 (2.33)	2.29 (1.19)	3.26 (1.97)
LPRS × GF versus ECRS × GF	0.999	0.999	49.7	51.2	99.467	-3.806	1.107	0.3 (0.45)	0.37 (0.49)	0.19 (0.38)	0.21 (0.42)

Table 6

Transformations of lipid peroxidation data of wheat and their correlation-regression analysis for vigour rating (data of Rudrapal and Basu[25])

Seed lot No.	Germination (%)	LP OD × 10^3	LPRS (%)	LP reci × 10^3	LP %T	LP OD ÷ GF	LPRS(%) × GF	LP reci × GF	LP %T × GF
T_1	71	155	84.5	6.45	69.98	218.3	59.99	4.58	49.69
T_2	80	144	85.6	6.94	71.78	180.0	68.48	5.55	57.42
T_3	85	142	85.8	7.04	72.11	167.1	72.93	5.98	61.29
T_4	88	148	85.2	6.75	71.12	168.2	74.98	5.95	62.59
T_5	100	137	86.3	7.29	72.95	137.0	86.3	7.30	72.95

Abbreviations: LP, lipid peroxidation, LPRS, lipid peroxidation resistance score; LP reci., reciprocal of LP OD; LP%T, Lipid peroxidation % transmission (%T) recalculated from LP OD; the rest are GF (germination factor) incorporated. For further details on transformations see text.

Table 6 continued

Summary of correlations and regressions with germination (%) as the x variate

y variate	r	r^2	$\bar{x}$	$\bar{y}$	SC (n=5)	Value of A	Value of B	$\Sigma(x-\hat{x})^2$	$\Sigma(y-\hat{y})^2$	$(x-\hat{x})$	$(y-\hat{y})$
LP OD × 10^3	-0.852	0.727	84.8	145.2	-61.45	-0.540	-61.45	171.0 (6.90)	49.9 (2.18)	5.10 (6.01)	2.75 (1.90)
LPRS	0.852	0.727	84.8	85.5	6.14	80.89	0.054	171.0 (6.90)	0.499 (0.37)	5.10 (6.01)	0.27 (0.32)
LP reci × 10^3	0.862	0.743	84.8	6.90	2.92	4.720	0.026	156.9 (6.60)	0.103 (2.08)	4.89 (5.76)	0.12 (1.82)
LP % T	0.853	0.728	84.8	71.6	10.12	64.04	0.089	169.7 (6.90)	1.343 (0.72)	5.07 (5.98)	0.45 (0.63)
LP OD÷GF	-0.977	0.954	84.8	174.0	-304.75	401.29	-2.68	22.09 (2.48)	158.71 (3.24)	1.99 (2.34)	5.33 (3.06)
LPRS × GF	0.999	0.999	84.8	72.5	102.41	-3.84	0.901	0.41 (0.34)	0.33 (0.35)	0.24 (0.29)	0.21 (029)
LP reci × GF	0.991	0.982	84.8	5.87	10.34	-1.84	0.091	8.47 (1.53)	0.07 (2.014)	1.06 (1.30)	0.10 (1.70)
LP %T × GF	0.998	0.997	84.8	60.8	90	-6.33	0.791	1.43 (0.63)	0.89 (0.70)	0.46 (0.54)	0.36 (0.60)
LPRS × GF versus ECRS × GF	0.998	0.997	72.4	78.2	105.9	-5.78	1.160	1.09 (0.64)	1.46 (0.69)	0.34 (0.47)	0.40 (0.51)

Table 7

Transformations of data on volatiles release from soybean seeds for correlation-regression analysis along with data on jute seedling bioassay score for seed vigour rating

(Data of Mukhopadhyay, Pal and Basu[20])

Seed lot No.	Germination (%)	VE OD × 10^2	VERS (%)	VE reci × 10^2	VE %T	VE OD ÷ GF (× 10^2)	VERS (%) × GF	VE reci × GF (×10^2)	VE %T × GF	JSBS (%)	JSBS (%) × GF
T_1	40	30	70.0	3.333	50.118	75	28	1.333	20.05	74	29.6
T_2	69	18	82.0	5.555	66.069	26.087	56.58	3.833	45.59	83	57.3
T_3	82	12	88.0	8.333	75.857	14.634	72.16	6.833	62.2	90	73.8
T_4	77	14	86.0	7.143	72.443	18.181	66.22	5.5	55.78	88	67.8
T_5	68	17	83.0	5.882	67.608	25	56.44	4	45.97	86	58.5
T_6	73	16	84.0	6.25	69.183	21.918	61.32	4.562	50.5	88	64.2
T_7	47	25	75.0	4	56.234	53.191	35.23	1.88	26.43	81	38.1
T_8	90	9	91	11.111	81.283	10	81.9	9.999	73.15	93	83.7

Abbreviations: VE, volatiles emanation, in OD × 10^2; VERS, volatiles emanation resistance score (%); VE reci × (10^2), reciprocal transformation of VE OD × (10^2); VE %T, volatiles emanation in % transmission; GF, germination factor for division or multiplication; JSBS, jute seedling bioassay score.

Table 7 continued

Summary of correlations and regressions with germination (%) as the x variate

y variate	r	r^2	$\bar{x}$	$\bar{y}$	SC (n=8)	Value of A	Value of B	$\Sigma(x-\hat{x})^2$	$\Sigma(y-\hat{y})^2$	$(x-\hat{x})$	$(y-\hat{y})$
VE OD × 10^2	-0.995	0.989	68.2	17.62	-115.75	45.11	-0.403	21.56 (2.40)	3.50 (3.75)	1.30 (1.91)	0.52 (2.97)
VERS	0.995	0.989	68.2	82.4	115.7	54.88	0.403	21.56 (2.40)	3.50 (0.80)	1.31 (1.91)	0.52 (0.64)
VE reci	0.929	0.862	68.2	6.541	38.85	-2.78	0.135	320.56 (9.27)	5.86 (13.27)	5.3 (7.76)	0.72 (11.1)
VE %T	0.995	0.989	68.2	67.3	170.75	26.79	0.594	21.95 (2.43)	7.75 (1.46)	1.37 (2.01)	0.81 (1.21)
VE OD ÷ GF	-0.970	0.942	68.2	30.5	-364.8	117.15	-1.269	123.99 (5.77)	199.86 (16.39)	3.41 (5.00)	4.33 (14.21)
VERS × GF	0.998	0.997	68.2	57.2	304.70	-15.14	1.060	6.10 (1.28)	6.86 (1.62)	0.76 (1.11)	0.80 (1.40)
VE reci × GF	0.929	0.862	68.2	4.74	43.64	-5.62	0.152	321.1 (9.28)	7.41 (20.29)	5.27 (7.72)	0.80 (16.87)
VE %T × GF	0.994	0.987	68.2	47.5	295.55	-22.74	1.028	25.65 (2.62)	27.13 (3.88)	1.55 (2.28)	1.60 (3.37)
JSBS	0.959	0.920	68.2	85.3	96.75	62.40	0.33	175.13 (6.85)	19.85 (1.84)	3.57 (5.24)	1.20 (1.41)
JSBS × GF	0.997	0.995	68.2	59.1	302.28	-12.67	1.052	10.8 (1.70)	7.01 (1.80)	0.83 (1.22)	0.87 (1.48)

For other details on statistical notations and abbreviations see Table 1 and text.

characters (*vis-a-vis* original/basic data) which have become positively correlated on transformation, such as ECRS, EC reci, SLRS, ALRS, LPRS, LP reci, LP %T (recalculated from LP OD), VERS, VE reci, VE %T (recalculated from OD), each with or without GF incorporation (multiplication by GF), would actually give an estimate of physiological resistance of the seed to the occurrences of the untoward processes and events leading to the loss of vigour and viability and, as such, would measure the intrinsic vigour of the seed. The use of the term 'resistance' (with 'R' as its abbreviation) must not be mixed up with electrical resistance which has a specific connotation in being the reciprocal of "mho" (i.e. ohm); the term resistance (meaning physiological resistance) has been preferred to other synonyms such as deterrence, hindrance, opposition, etc. because of its easy understandability and general but more meaningful appeal in the present context. The CR value in Table 2 (Andersen *et al.*[1]) that stands for conductivity resistance would be equivalent to EC reci values in ohms and ECRS is a totally different mathematical entity. The scores, namely ECRS, LPRS, VERS, JSBS, DEAS, etc. and all such values in the present discourse with their abbreviations ending with a capital 'S' (S for score), with or without GF, are actually percentage values strictly within the scale ranging from 0-100 (the upper limit will not be exceeded even on GF incorporation as the maximum value of the latter is 1). As such, the term "score" in the context of this paper has a specific connotation that singles it out from the general term "parameter" of vigour which stands for all measurements and indices of vigour including scores.

Statistical calculations

Correlation-regression analysis of the original data obtained from the different sources, and of their various transformations, have been done following standard methods (Panse and Sukhatme[22]) with special emphasis on bivariate analysis. The regression equation, y = A + Bx (where y is the dependent variatey is the independent variate, A and B are the two regression coefficients) would yield information on the deviations of observed values from those predicted by regression. A perusal of data in Table 1 dealing with the original data of Ching and Schoolcraft[3] and their transformations and correlation-regression analyses would be of help in understanding the approach. Table 1(A) shows the original data and their transformation ready for statistical analysis, Table 1(C) gives examples of three sets of data with detailed correlation-regression analysis that has been presented only in case of the first table (only the summarized versions of the analyses are shown in Tables 2 – 7), Table 1 (B) gives the summary of the correlation-regression analysis for critical comparative study and as mentioned earlier, the same is available for all the materials covered in Tables 1 to 7. The statistical terms, notations and abbreviations mentioned in the tables and in different places in the text are as follows (full name in parentheses): r (coefficient of correlation), r^2 (coefficient of determination), x(mean of x variate), y (mean of y variate), SC (sample covariance), Reg. Eqn. (regression equation), Reg. coeff. A (regression coefficient A), Reg. coeff. B (regression coefficient B),x(estimated value of x), y (estimated value of y), x–x (deviation of a x variate from the estimate), y-y(deviation of a y variate from the estimate), ©$(x–x)^2$, ©$(y–y)^2$(sum of least squares for the x and y variates, respectively). Below the columns of sum of least squares and also of mean deviations, certain values have been given in parentheses which indicate the extent of deviation as % of x and y. Those below the sum of least squares column were obtained as follows: ©$(x–x)^2$÷ n, the mean sum of squares is square–rooted (√) and then expressed as % ofxi.e. (mean deviation ÷ x) × 100; the procedure is exactly the same in case of ©$(y–y)^2$. The values under the simple deviation column ©(x–x) or ©(y–y) have been obtained from mean deviations (summing up the mean deviations ignoring the positive or negative signs and dividing by n, as otherwise the summation would give zero) and expressed as % of x or y for the respective variates; however, for obvious mathematical reasons, the latter estimates are always lower than those obtained from the sum of least squares; they nevertheless, serve as useful checks. All these % deviation values in parentheses have been calculated for easy understanding of general seed scientists regarding the effect of data transformations on the deviations of observed values of the variates from those predicted by the regression equations especially when the x and y values are very different.

In bivariate correlation-regression analysis, if all the data in either x or in y set of variates are exactly the same, the calculation will be subjected to mathematical error. Sometimes that may happen in very high vigour seed lots, all showing 100% germination; in that case r may be calculated taking the seedling length as the x variate (which may even be converted to percent values against a reference or benchmark value) or by taking corresponding JSBS values as the x variate.

The regression analysis for most of the data has been done keeping values upto 3-4 places of decimals for accuracy but for presentation in the tables, those have often been rounded off to a fewer places to save space and, as such, may show minor differences on recalculation.

THE FINDINGS AND IMPLICATIONS

The data analyzed in this paper literally speak for themselves. Beginning with the data of Ching and Schoolcraft[3], the need for transformation and analysis has been clearly brought about in all the materials considered in Tables 1 to 9. The salient findings are very briefly narrated hereunder for a general overview of their relevance in seed vigour rating:

(1) The original data of Ching and Schoolcraft[3] on electrical conductance in EC µmhos have shown negative correlation with germination percent but the r value is very low and statistically nonsignificant. When the conductivity data are reciprocally converted to ohms (resistance) there is a marginal improvement in r value which becomes positive but still far below the level of statistical significance (Table 1A). ECRS transformation does not show any change in r value but it greatly reduces the deviations of the observed values of dependent y variates from the statistical estimates provided by the regression equation (Table 1C) and with the incorporation of the germination factor (GF), the correlation with germination in ECRS × GF versus germination % has reached the level of statistical significance at 0.01P. The inclusion of GF has improved the r value in EC reci × GF and EC µmho ÷ GF approaches as well but those are still statistically nonsignificant (Table 1B). Further, ECRS × GF transformation has greatly minimized the deviations of observed and statistically estimated values of both x and y variates that show a fall from around 25% in the original data to less than 2% demonstrating a vast improvement in predictivity (Table 1 B, 1C).

(2) In cotton, the original data of Andersen, Hart and French[1] have been provided in ohms as a conductivity resistance value (Table 2). There has been no change in the value of r with ECRS or EC µmho (the sign becomes negative) transformations but the former (ECRS) considerably minimizes the deviations between observed and estimated values of y variates. Following incorporation of GF, the correlation has improved and the ECRS × GF transformation has reduced the deviations between the observed values of the two variates (x and y) and the respective predicted values to less than 1.5% of x and y.

(3) In case of soybean, the data from McDonald's group (Table 3, A, B) have shown similar improvements in correlation and predictive abilities. The data of Wilson and McDonald[32] on ECRS transformation show reduced deviation of y variate to around 0.5% and on inclusion of GF (i.e. ECRS × GF), to lessthan 0.4% for both x and y variates. The r values as well as the coefficient of determination have nearly touched the theoretical maximum of 1. The deviations in the y variate side of the original data of Van Toai, McDonald and Staby[30] were less than in the former study but on ECRS × GF transformation there has been further overall improvement in correlation and predictive efficiency.

(4) The data of Rudrapal and Basu[25] on electrical conductivity of wheat seeds vis-à-vis germination percent fully confirm the benefit of transformation, and in ECRS × GF, the value of r and r^2 (coefficient of determination), similar to

that of Wilson and McDonald[32], have almost reached the theoretical limit of 1 along with minimization of deviations between observed values and statistical estimates (0.48 – 0.62% of x and y). Unfortunately, in the case of onion, the original data of Choudhuri and Basu[4] show very high deviations between observed values and statistical estimates. ECRS × GF transformation has not minimized the deviations to the level seen in the other seed materials (only to half in case of x variate) though correlation coefficient has gone up to +0.943 from the original -0.742 and the coefficient of determination (r^2) from 55% to about 90% (both in percentages on multiplication by 100). A close scrutiny of the data would reveal certain shortcomings of the data for the purpose of transformation for correlation-regression analysis. The very wide range of germination (22 – 84%) and very high EC µmho value could not be duly taken into account by the present ECRS × GF transformation. Perhaps, a relatively narrow range of germination and lower EC readings (with greater dilution of the leachates) would have surmounted the problem. That would, however, not be in conformity with the objectives of the study of Choudhuri and Basu[4] the source from which the data have been collected.

(5) The transformation and analysis of electrical conductance data of mustard (Rudrapal and Basu[26]) once more categorically bring out the benefits of transformation and incorporation of germination factor (Table 5).

(6) In mustard, an important oil seed crop of the country, the role of lipid peroxidation in seed deterioration was studied with the view to control the process by exposing the seeds to low concentrations of iodine vapour to stabilize unsaturated bonds in lipid fractions with great success (Basu and Rudrapal[2]), the data from that project, as well as those of Rudrapal and Basu[25] as presented in Table 5, very clearly show that lipid peroxidation can be used as an important indicator of seed vigour and lipid peroxidation resistance score (LPRS) is very highly correlated with germination (r and r^2 values nearly reaching the theoretical limit of 1); with the incorporation of GF (i.e. LPRS × GF), the deviations between observed values and statistical estimates have been further minimized (Table 5). As would be statistically and logically expected the correlation between LPRS × GF and ECRS × GF is highly significant along with concomitant lowering of deviations between observations and estimates. From the physiological viewpoint, this correlation would most likely indicate a cause-effect relation between lipid peroxidation and loss of membrane integrity. The highly significant correlation between LPRS × GF and ECRS × GF in wheat also would indicate such a possibility (Table 6).

(7) Further confirmation of the benefits of transformation of LP data prior to regression-correlation analysis is brought out by the findings in wheat (Rudrapal and Basu[25]). The transformation of LP OD values to the lipid peroxidation resistance score has not shown any effect on r value except changing its sign from negative to positive but has greatly reduced the deviation in the y variate. However, LPRS × GF has vastly improved the correlation with concomitant minimization of deviations in both x and y variates. LP %T × GF transformation is also almost equally good.

As in case of mustard (Table 5) and soybean (Table 7), the possibility of using LPRS × GF for vigour rating of wheat seed is very strong at least for specific purposes.

(8) The data presented in Table 7 originating from a paper of Mukhopadhyay, Pal and Basu[20] on soybean bring out the benefits of transforming volatiles emanation (VE) data in the same manner as in case of lipid peroxidation (LP) data. The VERS × GF score giving the best correlation amongst the seven approaches made on the original VE OD data. It needs to be emphasized here that in all correlation-regression analyses on soybean, the r values have always been over 0.9 and it is the minimization of deviation of observed values of variates from the statistical estimates that has been the major advantage of the transformations. Incidentally, a testing of the efficiency of transformation has been done in case of active absorption by MBTH of volatile aldehyde released by soybean seeds (Wilson and McDonald[32]) during 27-48 hr versus field emergence (%) after VERS × GF transformation of the same (data in µg converted to 0.mg,

Table 8

Correlations between germination and some vigour parameters of rice, sunflower and onion seeds to evaluate the use of coefficient of determination as possible vigour scores

A. Rice: Data of Pal and Basu[21]

Seed lot No.	Germination %	EC µmho	ECRS (%)	ECRS (%) × GF	AEA µg starch hydrolyzed	AEAS (%)	AEAS (%) × GF	DEA OD	DEAS (%)	DEAS (%) × GF
T_1	67	117	88.3	59	82	54.7	36.8	0.090	31.4	21.0
T_2	91	59	94.1	85	100	66.7	61.0	0.140	48.2	43.9
T_3	93	52	94.8	88	108	72	67.0	0.148	51.0	47.5
T_4	96	56	94.4	91	116	77.3	73.9	0.161	55.5	53.3
T_5	91	59	94.1	86	98	65.3	59.1	0.161	55.5	50.5
T_6	98	58	94.2	93	150	100	98.0	0.290	100	98.0
(Refrigerated material)										
Correlation coefficient (r)		-0.967	0.967	0.999	0.743	0.736	0.851	0.692	0.692	0.747
Coeff. determination (r^2)		0.936	0.936	0.999	0.552	0.542	0.724	0.479	0.479	0.559
$r^2 \times 100$		93.6	93.6	99.9	55.2	54.2	72.4	47.9	47.9	55.9
r^2 × □ % ($CSVS_1$)		89.3	89.3	89.3	49.3	48.4	64.7	42.8	42.8	49.4

Abbreviations: AEAS -amylase enzyme activity score; DEAS - dehydrogenase enzyme activity score, also see Table 1 and text.

deducted from 1 and multiplied by 100 and then by GF of 7-day germination test); the transformation has shown a r value of +0.878** (against the original correlation of -0.69 that was statistically non-significant).

The jute seedling bioassay score (JSBS) is by itself a percentage value; it has shown very good correlation with germination and demonstrates still higher correlation with the incorporation of GF (Table 7) with requisite minimization of deviations in both x and y variates. When laboratory conditions are inadequate for other biochemical tests, with a little practice this test can be done to get a very reliable vigour score. Techniques being developed in the laboratory of the author suggest that even autobioassay (the same seed acting as a test material) would give an effective vigour score.

Taking advantage of the transformation techniques, data on several vigour parameters of rice, sunflower and onion have been subjected to bivariate correlation analysis with the view to evaluate the relative efficacy of several vigour scores employing the coefficient of determination (r^2) as a measure for the purpose. For the sake of easy understanding of the comparative efficiency of the different scores, the r^2 values have been converted to percentage values by

Table 8 continued

B. Sunflower: Data of Dey and Basu[6]

Seed lot No.	Germination %	EC µmho	ECRS (%)	ECRS (%) × GF	Sugar leaching OD 580	SLRS (%)	SLRS (%) × GF	Amino acid leaching OD 610	ALRS (%)	ALRS (%) × GF
T_1	48	381	61.9	29.7	0.205	79.5	38.2	0.238	76.2	36.6
T_2	59	354	64.6	38.1	0.201	79.9	47.1	0.225	77.5	45.7
T_3	68	342	65.8	44.7	0.148	85.2	57.9	0.215	78.5	53.4
T_4	79	302	69.8	55.1	0.136	86.4	68.2	0.198	80.2	63.3
T_5	75	299	70.1	52.6	0.155	84.5	63.4	0.218	78.2	58.6
T_6	65	338	66.2	43	0.171	82.9	53.9	0.220	78	50.7
T_7	100	290	71.0	71.0	0.090	91.0	91.0	0.095	90.5	90.5
Correlation coefficient (r)		-0.920	0.920	0.998	-0.967	0.967	0.998	-0.902	0.902	0.990
Coeff. determination (r^2)		0.847	0.847	0.997	-0.936	0.936	0.996	0.813	0.813	0.980
$r^2 \times 100$		84.7	84.7	99.7	93.6	93.6	99.6	81.3	81.3	98.0
$r^2 \times 100\%$ ($CSVS_1$)		59.8	59.8	70.3	66.1	66.1	70.3	57.4	57.4	69.2

Abbreviations: SLRS, sugar leaching resistance score; ALRS, amino acid leaching resistance score, also see Table 1 and text; $CSVS_1$, correlative seed vigour score, calculated by multiplying r^2 with mean germination percent (□%), also see note below Table 9.

Table 8 continued

C. Onion: Data of Choudhuri and Basu[4]

Seed lot No.	Germination %	LP OD	LPRS (%)	LPRS (%) × GF	DEA OD	DEAS[(a)] (%)	DEAS (%) × GF
T_1	97	0.170	83.0	80.5	0.242	99.2	96.2
T_2	96	0.155	84.5	81.1	0.235	96.3	92.4
T_3	95	0.166	83.4	79.2	0.240	98.4	93.5
T_4	99	0.142	85.8	84.9	0.240	98.4	97.4
T_5	71	0.243	75.7	53.7	0.154	63.1	44.8
T_6	89	0.173	82.7	73.6	0.222	91.0	81.0
T_7	92	0.193	80.7	74.2	0.215	88.1	81.0
T_8	93	0.155	84.5	78.6	0.228	93.4	86.9
Correlation coefficient (r)		-0.926	0.926	0.993	0.980	0.979	0.989
Coeff. determination (r^2)		0.857	0.857	0.986	0.960	0.960	0.979
$r^2 \times 100$		85.7	85.7	98.6	96.0	96.0	97.9
$r^2 \times 100\%$ ($CSVS_1$)		78.4	78.4	90.2	87.8	87.8	89.6

Abbreviations: LPRS, lipid peroxidation resistance score, others same as in Table 6 and Tables 8A & Table 8B.

(a) A refrigerated (non-aged) material of the same stock giving an optical density of 0.244 at 470 nm was used as a reference (DEAS value = 100).

Table 9

Comparison of different vigour scores of soybean after data transformations (basic data for transformation from Mukhopadhyay, 2001)

A. Transformations of basic data

Seed lot No.	Germina -tion %	Field emer- gence (%)	EC μmho	ECRS (%)	ECRS (%) × GF (a)	LP OD × 10^3	LPRS (%)	LPRS (%) × GF (b)	VE OD × 10^3	VERS (%)	VERS (%) × GF (c)	JSBS (%)	JSBS (%) × GF (d)
T_1	84	64	155	84.5	71.0	120	88.0	73.9	96	90.4	75.9	75	63.0
T_2	89	81	84	91.6	81.5	48	95.2	84.7	32	96.8	86.1	88	78.3
T_3	86	75	99	90.1	77.5	72	92.8	79.8	45	95.5	82.1	82	70.5
T_4	86	76	113	88.7	76.3	60	94.0	80.8	43	95.7	82.3	86	74.0
T_5	87	71	125	87.5	76.1	96	90.4	78.6	42	95.8	83.3	79	68.7
Correlations of vigour parameters with germination: (germination % versus vigour parameters)													
r	-	0.850	-0.832	0.832	0.941	-0.754	0.754	0.909	-0.854	0.854	0.956	0.761	0.86
r^2	-	0.722	0.692	0.692	0.885	0.569	0.569	0.827	0.730	0.730	0.914	0.580	0.74
$r^2 \times 100$	-	72.2	69.2	69.2	88.5	56.9	56.9	82.7	73.0	73.0	91.4	58.0	74.0
r^2 × □% ($CSVS_1$)	-	-	59.8	59.8	76.5	49.2	49.2	71.4	63.1	63.1	79.0	50.1	63.9
Correlations of vigour parameters with field emergence: (field emergence % versus vigour parameters)													
r	0.85	-	-0.972	0.972	0.961	-0.984	0.984	0.990	-0.896	0.896	0.909	0.969	0.983
r^2	0.722	-	0.943	0.943	0.923	0.969	0.969	0.980	0.803	0.803	0.826	0.938	0.966
$r^2 \times 100$	-	-	94.3	94.3	92.3	96.9	96.9	98.0	80.3	80.3	82.6	93.8	96.6
$r^2 \times \overline{FE}\%$ ($CSVS_2$)	-	-	69.2	69.2	67.7	71.1	71.1	71.9	58.9	58.9	60.6	68.8	70.9

multiplying with 100 or with mean germination or field emergence percent values (Table 8, 9). It is revealed that the transformed EC data are much more reliable as vigour parameters of rice than dehydrogenase or amylase-based indices. In sunflower also, the ECRS × GF and ALRS × GF, both concerned with membrane integrity, would prove useful. In onion, however, LPRS × GF and DEAS × GF have shown good efficiency as vigour scores. The vigour scores for soybean (Table 9) have demonstrated good correlations with germination singly as well as in combinations of two, three and four scores. But more important is the high r and r^2 values when correlations are worked out with field emergence. The percentage values of coefficient of determination ($r^2 \times 100$) have shown 89-98% efficiency for most of the calculated scores. The present statistical treatment would not, however, allow the final determination of superiority of any particular vigour score or a combination of two or more vigour scores over other scores for which there could be

Table 9 continued

B. Different combinations of GF-incorporated vigour scores and their correlations with field emergence
(a) ECRS × GF, (b) LPRS × GF, (c) VERS × GF, (d) JSBS × GF

Different combinations of vigour scores, a, b, c, d (see respective columns in Table 9A).

Lot No.	abcd	abc	abd	adc	bcd	ab	ac	ad	bc	bd	cd
T_1	70.9	73.6	69.3	70.0	70.9	72.4	73.5	67.0	74.9	68.5	69.5
T_2	82.7	84.1	81.5	82.0	83.1	83.1	83.8	79.9	85.4	81.5	82.2
T_3	77.5	79.8	75.9	76.7	77.5	78.6	79.8	74.0	81.0	75.2	76.3
T_4	78.3	79.8	77.0	77.5	79.0	78.6	79.3	75.1	81.6	77.4	78.1
T_5	76.7	79.4	74.5	76.1	76.9	77.4	79.7	72.4	81.0	73.7	76.0
Correlations with field emergence: (field emergence % versus vigour scores in combination)											
r	0.983	0.968	0.990	0.981	0.983	0.982	0.945	0.990	0.965	0.988	0.979
r^2	0.967	0.937	0.981	0.961	0.967	0.964	0.894	0.981	0.931	0.977	0.958
$r^2 \times 100$	96.7	93.7	98.1	96.1	96.7	96.4	89.4	98.1	93.1	97.7	95.8
$r^2 \times \overline{FE}\%$ ($CSVS_2$)	71	68.8	72.0	70.5	71.0	70.5	65.6	72.0	68.3	71.7	72.3

Abbreviations and other details same as in Tables 1, 5, 6 and 7.

Note: The two tentative correlation based vigour scores, provisionally named as 'correlative seed vigour scores', viz. $CSVS_1$ and $CSVS_2$ obtained by multiplying r^2 with mean germination percent (□%) and mean field emergence percent ($\overline{FE}\%$), respectively are found useful for comparing the individual vigour scores and their combinations (the number of pairs of variates, n, the degrees of freedom should be same in all cases for such comparisons) with the potential to predict final field performance in terms of agricultural productivity.

several options. More detailed analysis with appropriate computer-based software packages, rather than ordinary scientific calculators, would be required for the purpose. For the present, however, EC data with required transformation, namely ECRS × GF is recommended for practical use in seed and crop production oriented practices, and if necessary in seed trade and commerce. In due course, there would be a possibility of combining ECRS × GF score with VERS × GF or JSBS × GF score for even more reliable vigour rating of crop seeds.

Lastly, this paper highlights the very important role of germination percentage in vigour rating; if germination factor is not incorporated in vigour scores, the predictions would be unreliable, thereby vindicating the emphasis given to standard germination tests by the two major international organizations deeply concerned with research and development in seed science and technology - the ISTA and AOSA.

Although the present paper deals specifically on the issue of seed vigour rating, it is hoped that some of the data transformation techniques may be useful to other fields of biology in which masking of data due to various factors has been a long standing problem in correlation related studies.

Note: *The individual vigour scores have, in general, successfully brought out the differences between seed lots (in case of materials from the author's laboratory attributable to seed invigoration and ageing conditions). However, the details of issues concerning differences between kinds of seeds, differences in seed lots within a kind and the statistical treatments of interactions between vigour scores and kinds of seeds and seed lots, have been taken up separately, elsewhere. – Author.*

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