

Electrical Conductivity Test For Predict Sunflower Seeds Vigor

Szemruch, C.^{1,2*}, Gallo, C.³, Murcia, M.⁴, Esquivel, M.⁵, García, F.^{1,2}, Medina, J.¹, Magnano L.³

¹ Facultad de Ciencias Agrarias – UNLZ. Ruta 4. Km 2. (1832). Llavallol. Bs. As. Argentina.

² Instituto de Investigación sobre Producción Agropecuaria, Ambiente y Salud (IIPAAS). Ruta 4. Km 2. (1832). Llavallol. Bs. As. Argentina

³ INTA – EEA Oliveros. Ruta 11. Km 353 (2206). Oliveros. Santa Fe. Argentina

⁴ Facultad de Ciencias Agrarias – UNMdP - INTA EEA Balcarce. Ruta 226. Km. 73.5 (7620). Balcarce. Bs. As. Argentina.

⁵ Cámara Arbitral de Cereales de la Bolsa Comercio de Santa Fe. San Martín 2231. Santa Fe. Argentina

Abstract

There is no standardised test for predicting sunflower seed vigor. The electrical conductivity test (EC) produces faster results. Before a test is standardised requires a validation process to ensure reliable and reproducible results. The aims were to analyse the repeatability and reproducibility of EC on sunflower seeds without pericarp and the association between EC and field emergence. Seeds with high (lots 3 and 4), intermediate (lots 1 and 5) and low vigor (lots 2 and 6) were distributed in four laboratories. In each laboratory, EC was measured after 24 h at 3, 6, 9, 13 and 19 months of storage and expressed as $\mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$. Field emergence was evaluated by diverse formulas including chronological days and thermal time. After 19 months, lots 3 and 4 presented high vigor (40.0 at $55.23\ \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$). Lots 1, 2, 5 and 6 ranged between 61.27 and $82.57\ \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ indicating an intermediate vigor. Significant correlation coefficients ($r = -0.67$ and -0.72) were obtained between EC and percentage of emerged seedlings in the field and daily mean emergence, slightly improving with the use of thermal time. The EC test differentiated sunflower seed vigor through reproducible and repeatable results.

Keywords - Electrical conductivity, field emergence, sunflower, seed vigor.

I. INTRODUCTION

Seed vigor is the sum of those properties that determine the activity and performance of seed lots of acceptable germination in a wide range of environments [1]. A seed lot is vigorous if it is potentially able to perform well under environmental conditions not optimal for the species [2].

At present, few species have standardised vigor tests or which are incorporated in the ISTA rules. Such is the case of Electrical Conductivity for *Phaseolus vulgaris* L., *Pisum sativum* L., *Cicer arietinum* L., *Raphanus sativus* L. and *Glycine max*, the Accelerated Ageing and Tetrazolium for *Glycine*

max L., the Controlled Deterioration for *Brassica* sp. and the Radicle Emergence in *Zea mays* L. [2], *Brassica napus* L. and *Raphanus sativus* L. [3].

The electrical conductivity test (EC) is a promising assay because it produces fast results (< 24 h) therefore shortening the decision period for sowing and selling recommendations [4]. Their objective is to evaluate the degree of damage suffered by cell membranes as a result of seed deterioration [5]. Furthermore, it is neither affected by dormancy nor does it require sophisticated equipment or highly qualified personnel [6].

The results of the EC test can be affected by the genotype, seed integrity, seed size and moisture content, as well as by the immersion period and temperature [7]. In sunflower the electrolyte losses from the pericarp or changes in their permeability can interfere with exudates from the tissues of the embryo [8; 9] and derive in contradictory results in the laboratory [10]. Some authors [11] and [12] increased the predictive value of the EC test in sunflower seeds using the dehulled procedure (without pericarp). Due to its selective permeability, the removal of the pericarp and seed coat can be considered as an effective way to break dormancy [13]. Thus, the use of dehulled seeds is useful to eliminate both the interference of pericarp electrolytes, and seed dormancy.

The efficiency of the EC test to discriminate seed vigor was tested in different species such as sweet corn [14], soybean [15] and peanut [16]. In sunflower, the EC allows the classification of lots according to their vigor, identified as high-vigor seeds when EC is < $70\ \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$, medium vigor when found between 70 and $110\ \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$, low vigor when EC is > $110\ \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ and unsuitable for sowing when EC is > $160\ \mu\text{S}\cdot\text{cm}^{-1}\cdot\text{g}^{-1}$ [17]. However, a standardised test is not yet available for sunflower.

Before a test is standardised, it should be well tested by not only intra-laboratory validation, but also inter-laboratory validation [18]. "Validation" is the process of defining an analytical method and its confirmation to measure seed vigor, with an adequate

level of accuracy [19]. Implicit in this definition is the need to evaluate the performance of the method, that is, its reproducibility and repeatability [20]. The EC test has been validated successfully for different species such as *Phaseolus vulgaris* L. [21], *Glycine max* L. [22], *Cicer arietinum* L. [23] and *Raphanus sativu* L. [24].

The vigor tests should also allow estimation of seed field behaviour [5]. EC test adequately correlated with field emergence of *Zea mays* L. [25], *Phaseolus vulgaris* L. [26], *Arachis hipogaea* L. [16] and *Carthamus tinctorius* L. [27]. However, the association between EC and field emergence was not consistently correlated for *Cicer arietinum* L. genotypes [28].

In the case of sunflower there is no standardised test that can be used as an adequate predictor of seed field performance. Also limited research on vigor tests, using large-scale seed lots, has been reported for this crop [29]. The last authors showed higher correlation coefficients between field emergence (measured in seedling percentage) and percentage laboratory emergence ($r=0.8$), cool germination (0.7), or cold test (0.7). Also it has been established a high and significant correlation (-0.85) between the EC (measured in sunflower seeds with pericarp) and the field emergence speed measured in seedlings/day [30]. In seeds without pericarp, high correlation coefficients (0.77 to 0.80) were detected between EC and field emergence speed when it was expressed in mean time of emergence and days for 50% of maximum emergence, including the thermal time calculation [32]. In contrast, other authors [31] showed a low association between EC (in seeds without pericarp) and the number of seedlings / day emerged in the field ($r = 0.41$). Also, for five genotypes of sunflower, the association between EC (in seeds without pericarp) and the percentage of field emergence was not significant [10].

The aims of this study were to i) analyse the repeatability and reproducibility of the EC test on sunflower seeds without pericarp and ii) determine the association between the EC test and different estimators of field emergence.

II. MATERIALS AND METHODS

A. Seed material

Seeds from IL01 sunflower hybrid, obtained in Venado Tuerto, Santa Fe, Argentina (33° 44' S; 61° 58' O) during 2016/2017 were evaluated. To avoid confusion and facilitate reading, the term "lot" will be used hereafter for a defined quantity of seeds (1 Kg) of the IL01 hybrid obtained in similar conditions of production (experimental site, date of sowing, crop practices, type and date of harvest).

B. Treatments

After three months, to create the vigor levels (treatments), the seeds were stored under three conditions:

- (a) 10°C ± 2 °C temperature giving high vigor seeds (lots 3 and 4)
- (b) between 18 and 25 °C temperature giving intermediate vigor seeds (lots 1 and 5)
- (c) same as the previous with 24 h a 38°C each month, giving low vigor seeds (lots 2 and 6)

Until their analysis, seeds were stored in trilaminated paper bags sealed with film paper in moisture-proof containers. Samples were coded independently and distributed for each participating laboratory. The participating laboratories were:

1. Seeds Laboratory of Faculty of Agricultural Sciences, University of Lomas de Zamora, Argentina.
2. National Institute of Agricultural Research, Oliveros Experimental Station, Oliveros, Argentina.
3. Seeds Laboratory of Faculty of Agricultural Sciences of National University of Mar del Plata, Mar del Plata, Argentina.
4. Seeds Laboratory of Arbitral Chamber of Cereals of Santa Fe Commercial Stock Schange, Santa Fe, Argentina.

C. Laboratory test

1) Germination (G)

Germination was calculated through the counting of normal seedlings, on the tenth day after sowing and expressed as percentage. Four replications of 50 seeds were sown in sterilised sand boxes and placed in the germination chamber (IDE, TIPO 40S12, Córdoba, Argentina) at 25°C during 12 hours, alternating light/dark [2]. Abnormal, dead and fresh seeds were evaluated. This variable was calculated only in one laboratory (Seeds Laboratory of Faculty of Agricultural Sciences, University of Lomas de Zamora, Argentina).

2) Electrical conductivity (EC) test

Three replicates of 50 seeds were dehulled in each laboratory. Then, they were weighed and placed in 5 cm diameter, 8 cm high plastic cups with 38 ml distilled water at 25 °C for 23 h. The top of the cups was covered with a film paper. One control cup was used with 38 ml distilled water with $0 \pm 5 \mu\text{S cm}^{-1} \text{ g}^{-1}$ at 25°C temperature. After 23 h the samples were taken out of the chamber and mixed up. EC was measured after 24 h using a conductivity meter and expressed as $\mu\text{S.cm}^{-1} \text{ g}^{-1}$ [11]. Each laboratory completed five runs of the conductivity test at 3, 6, 9, 13 and 19 months of storage, using the same method. At 9 months, only one lot of each vigor category was evaluated (lots 1, 2 and 3) and compared with electrical conductivity measured in seed with pericarp. Electrical conductivity assesses the degree of damage in cell membranes as a result of seed deterioration. A higher deterioration leads to a lower membrane repair capacity and greater amount of solute released during imbibition [5]. Thus, high levels of electrolytes released into the solution (and

higher values of electrical conductivity) involve lower seed vigor [2].

D. Field emergence test

The field emergence tests were performed on a Typic Argiudoll soil in the experimental field of Agronomy, University of Lomas de Zamora (34° 45' S; 58° 29' W), Argentina. Three sowings were conducted on 09/08/2017 (experiment 1), 06/15/2018 (experiment 2) and 07/11/2018 (experiment 3), coinciding with 3, 6 and 19 months of storage, respectively. One hundred seeds were sown in each 1 × 1 m plot with 4 rows separated by 0.25 m and at 5 cm soil depth. These plots were free from weeds, diseases and pests and without fertilization and supplementary irrigation. Field emergence was evaluated counting the emerged seedlings at intervals of 2 or 3 days after sowing, considering the following variables:

i) Final percentage of emerged seedlings (FPES), resulting from the relationship between the total number of seeds sown, relative to those which indeed showed cotyledons above the soil surface (emergence state [33]).

ii) Days for 50% of maximum emergence (SE50) were calculated using the following formula:

$$SE50 = \frac{\left[\left(\frac{E_{MAX}}{2} \right) - E_1 \right] \times (D_2 - D_1)}{E_2 - E_1} + D_1$$

where,

SE50: days to reach 50% of seedlings emergence

EMAX: maximum number of seedlings emerged

D1: beginning of the interval measured in days where 50% of seedling emergence occurs

D2: end of the interval where 50% of seedling emergence occurs

E1: number of emerged seedlings in D1

E2: number of emerged seedlings in D2

iii) Mean field emergence rate (ER) calculated as the inverse of SE50 and expressed in 1.d-1

iv) Mean time of emergence (TME) was calculated according to Nakagawa formula [34] and expressed in days (d).

v) Daily Mean emergence (DME) was calculated according to Maguire's formula [35] and expressed by seedling per chronological day (i.e., seedlings.d⁻¹). Also, DME was calculated expressing the chronological days in thermal time, calculated as the sum of the average air temperature - the base temperature, to obtain DME (seedlings.°Cd-Air⁻¹). The thermal time was calculated according to [36], with a base temperature of 6 °C. DME variable was also analysed considering the soil temperature in the thermal time calculation to express DME (seedlings.°Cd-Soil⁻¹).

E. Statistical analysis

Laboratory and field tests were studied by means of a complete randomised design (CRD) with 4 replicates and two factors: the participating laboratories and the lots (treatments). The analysis contemplated a random effect model for a factorial arrangement with interaction for each run (3, 6, 9, 13 and 19 months of storage). Analysis of variance and LSD Fisher tests were performed with a 5 % significance level. Also z-scores were calculated (37). Repeatability and reproducibility were analysed with the statistical tool based on ISO 5725-2 (38). This allowed the calculation of h- and k-values. The h-values showed the tendency for a laboratory to overestimate or underestimate the mean values compared to the mean of all the results available, whereas the k-values give a measure of the variability of the repeats. Significant h-values indicate an over or underestimation of the mean value compared with the overall mean. Alternatively, significant k-values indicate greater variability among lots within a laboratory. Both were compared with statistics critical value at 1 or 5 % significance. Pearson coefficient correlations were calculated between laboratory and field data. Percentage values were transformed using angular transformation [39]. Infostat statistical software was used [40].

III. RESULTS

A. Laboratory test

1) Germination (G)

All lots reached 99 % germination (normal seedlings) after 3 months of storage. These percentages remained high (> 96 %) without significant differences during the 6, 9 and 13 months of storage (table I). Even after 19 months, four lots (1, 3, 4 and 6) still retained germination above 90 % (92 – 96 %) and two lots (2 and 5) presented 89 % germination.

2) Electrical conductivity (EC) test

The minimum EC values (ie, the high seed vigor) were observed prior to treatments' application (different temperatures) at 3 months of storage. The mean EC across the laboratories was 45.66 µs cm⁻¹ g⁻¹ without significant differences among them (data not shown).

3) Differences between lots

The evolution of EC over time in different laboratories (figure 1) revealed that storage treatments did not generate differences in lot vigor through time.

Considering runs at 6, 9 and 13 months of storage, EC means of lots were not different (figure 1). Only at 19 months of storage, the differences among lots were clearer. Lots 3 and 4 showed the highest vigor (ie, lower EC values: 43.25 at 55.23 µs cm⁻¹ g⁻¹) without significant differences between them, in all laboratories (table II). Comparing with the rest of the lots, these differences were significant in most of the

cases, in laboratories 3 and 4, regarding lot 6 (table II).

TABLE I

Germination percentage for six lots of sunflower seeds at 3, 6, 9, 13 and 19 months of storage. Lower case in line indicate significant differences between months of storage and upper case in each column between lots ($P < 0.05$, LSD)

Lot	Months of storage				
	3	6	9	13	19
2	99 A a	95 AB a	96 A a	96 A a	89 B ab
6	99 A a	95 AB a	-	99 A a	92 B ab
3	99 A a	95 B a	98 A a	99 A a	96 AB a
4	99 A a	98 A a	-	97 A a	96 A a
1	99 A a	95 AB a	97 AB a	99 A a	92 B ab
5	99 A a	96 A a	-	98 A a	89 B b

Lots 1, 2, 5 and 6 showed EC values ranging between 61.27 and 82.57 $\mu\text{s cm}^{-1} \text{g}^{-1}$, indicating an intermediate vigor. Differences among them were not significant in most of the cases, except for laboratory 1, in which lot 2 differed from 1 and 5 (table II). Laboratory 2 showed a similar tendency among lots but with much higher EC values, which could show an over-estimation of these values (see section Differences between laboratories below). The z-score indicated that most of the data fell within the acceptable range of -2.00 to 2.00, according to ISTA proficiency tests (table II), except for laboratory 2, which showed four of them above.

EC in seeds with pericarp ranged between 124.73 and 147.54 $\mu\text{s cm}^{-1} \text{g}^{-1}$, being significantly higher than seeds without pericarp (table III). In most laboratories, lots vigor did not match with storage conditions, since lot 3 (stored at low temperature) showed the highest values of conductivity (table III).

4) Differences among laboratories (reproducibility and repeatability)

Respect to reproducibility (h values), at 1%, no significant h-values were found in most of the runs (6, 9, 13 and 19 months), indicating that EC measurements were not over or underestimated (figure 2). Only at 19 months, lot 6 in laboratory 2 had h-value above this significance level (figure 2 d). At 5%, there were two significant h-values, one for the run at 13 months for lot 1 in laboratory 1, which was underestimated (figure 2 c) and the other for the run at 19 months for lots 4 and 6 in laboratory 2, which were overestimated (figure 2 d). Within the 82 sets of data analysed, only 3 were outside the statistical critical value at 1 and 5 % significance.

Respect to repeatability (k values), at 1%, no significant k-values were found in most of the runs (6, 9, 13 and 19 months) and only one (lot 1 in laboratory 4) was above this critical level for the run at 13 months (figure 3c).

TABLE II

Comparison of EC test for six lots of sunflower seeds tested by four laboratories (19 months run). Lower case in each column indicate significant differences between laboratories and upper case in each line between lots ($P < 0.05$, LSD)

Laboratory	19 Months of storage					
	Lot					
	1	2	3	4	5	6
1	61.27 c B	74.62 b A	40.87 b C	40.00 b C	64.69 b B	68.01 b AB
2	113.20 a A	103.82 a A	80.01 a BC	74.74 a C	105.09 a A	96.70 a AB
3	77.90 b A	68.00 b A	47.64 b BC	46.16 b C	69.04 b A	64.68 b AB
4	81.33 b A	82.57 ab A	55.23 b BC	43.25 b C	80.66 b A	65.40 b AB
Means	74.27	73.34	50.05	45.81	69.15	66.18
SD	19.2	14.1	15.0	14.1	18.4	13.8
Z-SCORE						
1	-0.676	0.092	-0.611	-0.413	-0.242	0.133
2	2.025	2.169	1.993	2.059	1.951	2.219
3	0.189	-0.380	-0.161	0.025	-0.592	-0.109
4	0.367	0.657	0.344	-0.182	0.625	-0.056

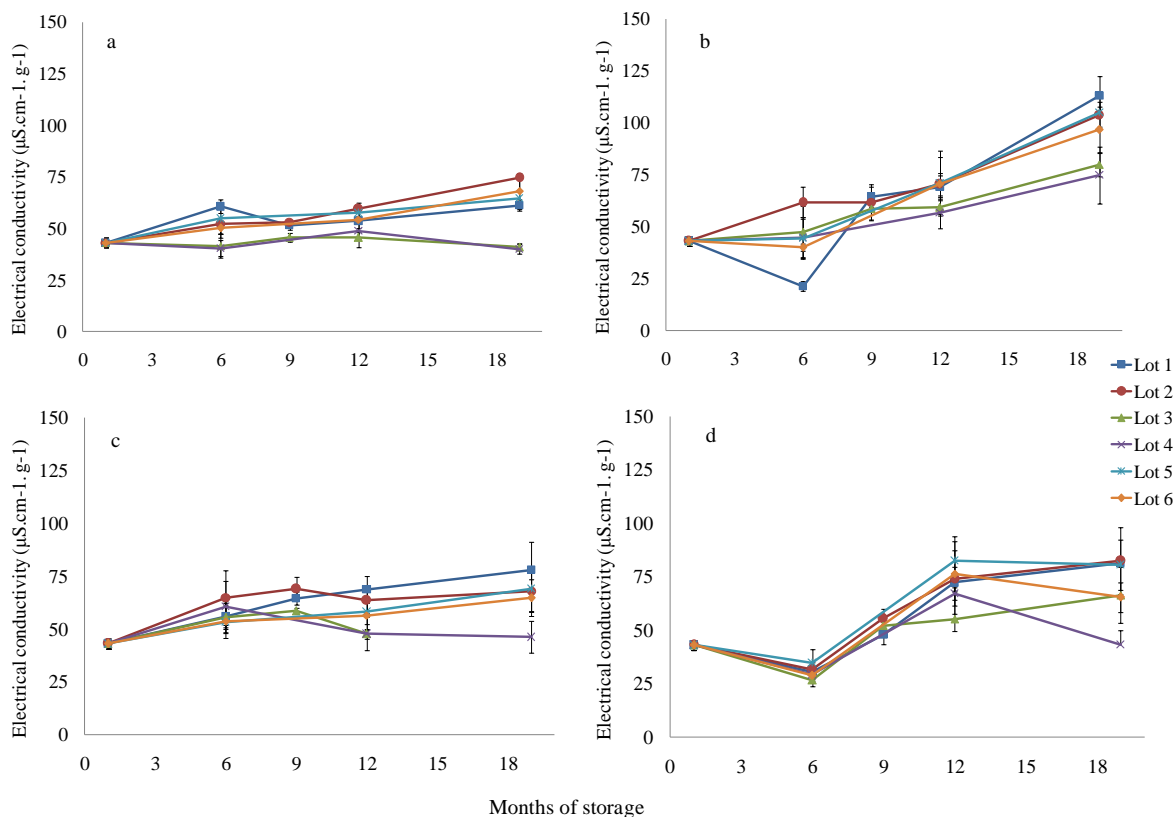


Figure 1. Evolution of EC during 6, 9, 13 and 19 months of storage for six lots of sunflower seeds tested by four laboratories: Seeds Laboratory of Faculty of Agricultural Sciences. University of Lomas de Zamora (a); National Institute of Agricultural Research, Oliveros Experimental Station, Oliveros (b); Seeds Laboratory of Faculty of Agricultural Sciences of National University of Mar del Plata (c) and Seeds Laboratory of Arbitral Chamber of Cereals of Santa Fe Commercial Stock Exchange (d). Vertical bars indicate ± 1 SD. Two points differ significantly when the standard deviation bars do not touch each other.

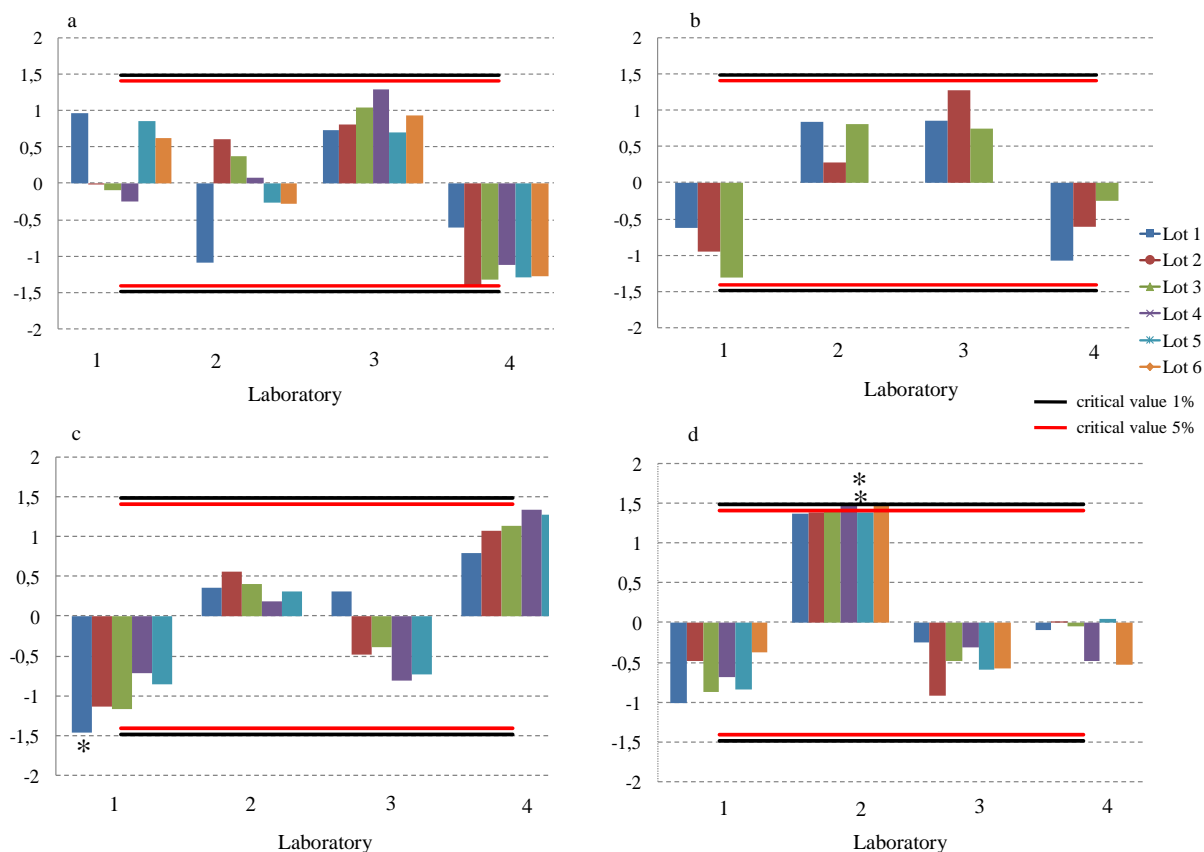


Figure 2. H values for 6 lots of sunflower seeds using the EC test in four laboratories at 6 (a), 9 (b), 13 (c) and 19 (d) months storage runs. * indicates a significant difference at 1% or 5% critical value.

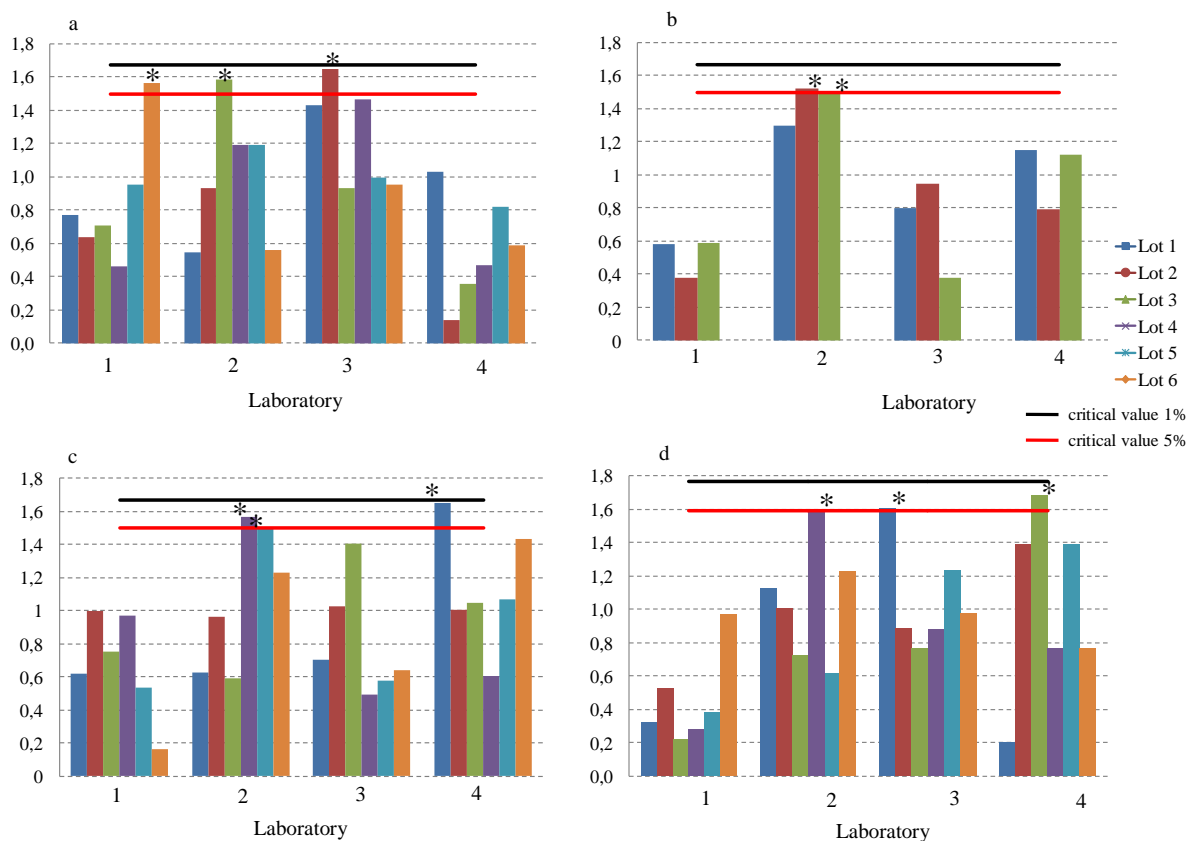


Figure 3. k values for 6 lots of sunflower seeds using the EC test in four laboratories at 6 (a), 9 (b), 13 (c) and 19 (d) months storage runs. * indicates a significant difference at 1% or 5% critical value.

This indicates that in all laboratories the EC measurements had a good repeatability for this statistical level (figure 3). At 5%, there were significant k-values for three lots in the run at 6 months of storage (lot 6 in laboratory 1, lot 3 in laboratory 2 and lot 2 in laboratory 3) (figure 3 a). For the run at 9 months of storage, only lots 2 and 3 in laboratory 2 were significant at 5% (figure 3 b). For the run at 13 months of storage significant k-values (5%) were found for three lots (lots 4 and 5 in laboratory 2 and lot 1 in laboratory 4) (figure 3 c).

The run at 19 months of storage, presented significant k-values (5%) for three lots (lot 4 in laboratory 2, lot 1 in laboratory 3 and lot 3 in laboratory 4) (figure 3 d).

B. Field emergence

Out of the three sowing dates, only the results of experiment 3 (19 months of storage) were correlated, because at that moment the differences in the vigor among lots was evident. After 19 months of storage, the relation between EC values and field emergence was significant ($p < 0.05$) for FPES and DME with

TABLE III
Comparison of EC test in sunflower seeds with and without pericarp (dehulled) tested for three lots and four laboratories (9 months run). Lower case in each line indicates significant differences between lots and upper case between seeds with and without pericarp ($P < 0.05$, LSD).

9 Months of storage						
Laboratory	Lot 1		Lot 2		Lot 3	
	With pericarp	Dehulled	With pericarp	Dehulled	With pericarp	Dehulled
1	141.57 ab	51.61 c	138.27 b	53.11 c	147.54 a	45.52 c
2	141.63 b	64.26 c	135.92 b	61.83 c	155.07 a	58.75 c
3	134.44 a	64.50 cd	125.42 b	69.03 c	124.73 b	58.42 d
4	130.51 a	47.77 b	128.25 a	55.50 b	134.73 a	52.18 b
Means	137.04 A	57.03 B	131.96 A	59.87 B	140.52 A	53.72 B
SD	9.21	8.42	9.56	8.16	13.63	6.43

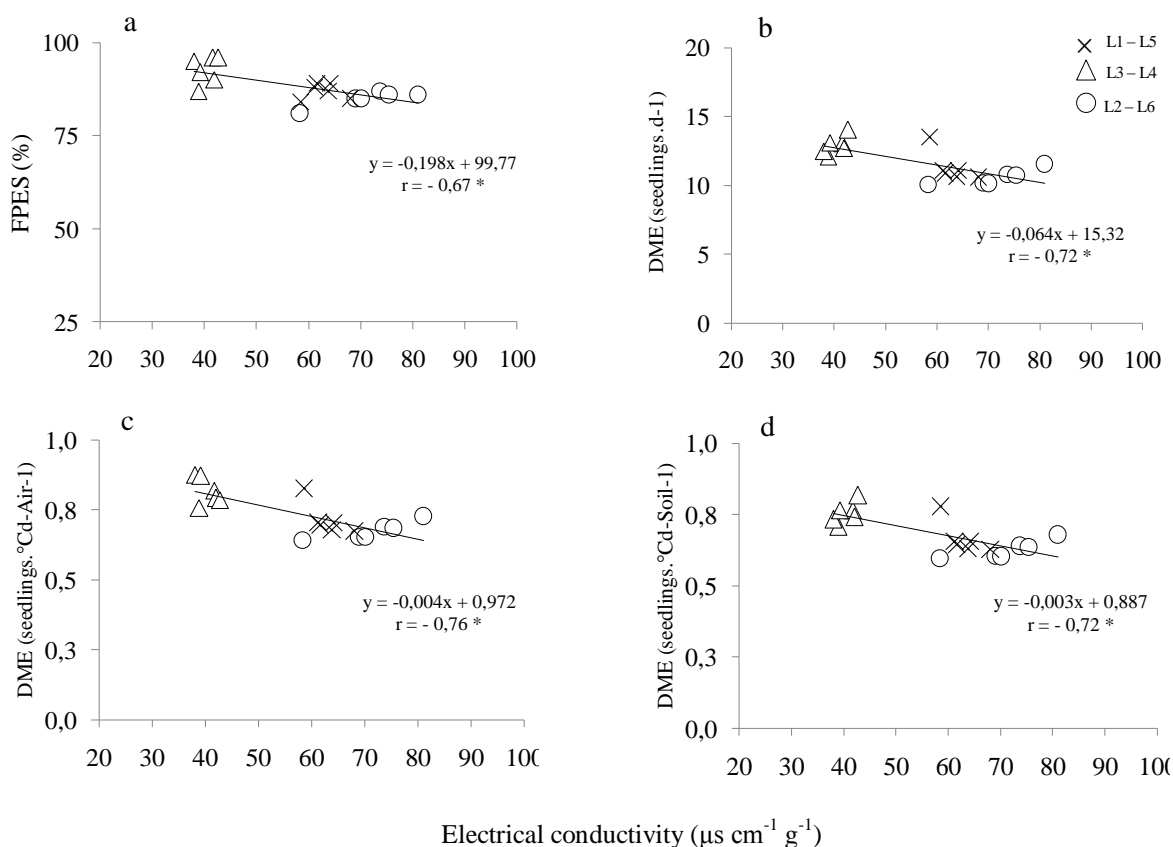


Figure 4. Relation between EC (test) and Field emergence measured in FPES (Final percentage of emerged seedlings) (a) and DME (Daily Mean emergence,) expressed in seedlings.d-1 (b), in thermal time considering the air (seedlings.°Cd-Air-1) (c) and soil (seedlings.°Cd-Soil-1) (d) of high (lots 3 and 4, triangle symbol), intermediate (lots 1 and 5, cross symbol) and low vigour seeds (lots 2 and 6, circle symbol) at 19 storage months. * indicates a significant values at $p < 0.05$

-0.67 and -0.72 correlation coefficients, respectively (figure 4 a,b).

It was evident that the greater vigor of lots 3 and 4 was maintained during the field emergence (figure 4), with high percentage of emergence and number of seedling per day (figure 4 a,b). This relation improved slightly when the DME was expressed in thermal time, seedlings.°Cd-Air-1 and seedlings.°Cd-Soil-1 (figure 4 c,d).

IV. DISCUSSION

Among seed quality attributes, germination expresses its performance under optimum conditions. The sunflower hybrid analysed showed high germination after 19 months, even in the worst storage conditions (high storage temperature, lots 2 and 6). This deterioration rate is not compatible with some sunflower modern hybrids which have a significant decrease in germination after 4 - 6 months, on average [41; 42]. The genetic background would be explaining the differences in seed quality and their deterioration rate [43]. Therefore, it would be necessary to analyse the evolution of EC test with different genotypes, especially those that have a higher level of deterioration.

The effect of storage conditions on vigor was also evident at 19 months, especially for cold stored seeds (lots 3 and 4), which had the lowest EC levels and maximum vigor. The benefits of cold storage for sunflower seeds agree with previous studies [41; 44; 42]. Low temperatures minimise chemical reactions in general [5] and oxidation in particular [43], so that the deterioration process is delayed and seed viability is extended [46].

The differences in the levels of vigor between seeds stored at 25 °C (lots 1, 5) and those stored at the same temperature with periods of 38 °C during 24 h (lots 2, 6) were not significant in any period. This shows that these temperatures are not high enough to generate a significant deterioration in sunflower seeds. There are reports of sunflower deterioration centred on tests such as accelerated aging or controlled deterioration [47; 48; 49; 50; 51; 52]. In these cases, the seeds are subjected to a rapid stress which can generate metabolic reactions different from those occurring during natural seed aging [53; 54]. Therefore, the loss in vigor of sunflower seeds under natural high temperatures should be further investigated, establishing with greater precision

which is the storage maximum temperature which would exert a significant deterioration in their quality.

Under the conditions of this work, the EC test consistently identified differences among six lots, being reproducible and repeatable within and among four laboratories over 4 storage times. These indicate the potential for EC as vigor method evaluation. Significant k-values were found in some cases, especially at 5%, indicating a small variability between replicates. The EC obtained in seeds with pericarp (9 months' run) indicates an important electrolyte release by this structure, which masks the vigor classification of sunflower seeds. These were in accordance with [8; 9; 10] and could be explained by the presence of hairs (which can accumulate diverse particles among them) or presence of different chemical compounds in pericarp tissues like phytomelanin layers or oil waxes [55; 56].

The reproducibility of EC test in sunflower seeds without pericarp is comparable with previous EC validations in other species [21; 22; 23; 24]. That EC test has shown a high capacity to detect genotypic differences among sunflower hybrids, especially in iso-lines, which differ only in fatty acid composition [17]. However, this ability to differentiate between diverse genetic materials must be repeatable and reproducible among and within different laboratories. Therefore, it is necessary to continue with the validation process, using different hybrids, to strengthen its potential use and recommendation.

The soil seedbed is a complex environment in which seeds and seedlings are exposed to multiple stresses [57], which may complicate the selection of field emergence indices. In spite of this, there was a satisfactory correlation of the EC test with field emergence, expressing the same order among the lots (lots 3 and 4 presented higher percentage and emergency speed). These relations improved slightly when the DME was expressed in thermal time, seedlings.^{°Cd}-Air-1 and seedlings.^{°Cd}-Soil-1. Although the correlation coefficients were lower than those previously found in sunflower and other species [26; 25; 16; 27; 29; 32], the use of indices that include thermal time and air –soil temperature allows minimising the effect of such environmental variables, which would represent a novel approach to express the field emergence in sunflower.

V. CONCLUSION

The EC test differentiated the sunflower seed vigor through reproducible and repeatable results, providing a reliable methodology.

The EC test was capable to estimate sunflower field emergence satisfactorily, which can be improved through the appropriate selection of field estimators.

VI. ACKNOWLEDGEMENTS

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