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Ranges of Vigor Based on the Electrical Conductivity Test in Dehulled Sunflower Seeds

[1]C. Szemruch, [2]O. Del Longo, [1]L. Ferrari, [3]S. Renteria, [4]M. Murcia, [1,5]M. Cantamutto and [1,6]D. Rondanini

[1]Faculty of Agricultural Science, National University of Lomas de Zamora, Route 4 Km 2, (1836) Llavallol, Buenos Aires, Argentina
[2]Faculty of Agricultural Science, National University of Cordoba, Av. Valparaiso and Rogelio Martinez. University City. DC 509 (5000) Cordoba, Argentina
[3]PANNAR Seeds. Av. Mitre 745 (2600) Venado Tuerto, Santa Fe, Argentina
[4]Faculty of Agricultural Science, National University of Mar del Plata EEA INTA, Ruta 226 Km 73, 5 (7620) Balcarce, Argentina
[5]Department of Agronomy, National University of del Sur, San Andres 800 (8000) Bahia Blanca, Argentina
[6]CONICET, Av. Rivadavia, 1917 (1033) Ciudad de Buenos Aires, Argentina

Corresponding Author: C. Szemruch, Faculty of Agricultural Science, National University of Lomas de Zamora, Route 4 Km 2, (1836) Llavallol, Buenos Aires, Argentina

ABSTRACT

Electrical Conductivity (EC) is a promising vigor test since it produces fast laboratory results. In sunflower, the leakage of electrolytes from the pericarp may interfere with exudates from embryo tissues. The aims of this work were (1) To determine the utility of the EC test using dehulled (without pericap) sunflower seeds to evaluate the vigor in different genotypes, exposed to contrasting seed filling period and storage conditions, (2) To explore the relationship between EC and germination values near post harvest and across storage period and (3) To propose ranges of vigor through EC, so as to categorize sunflower seeds lots. Seeds of commercial hybrids differentiated by acid composition (high and standard oleic acid), exploring contrasting seed filling period and storage conditions, were evaluated by EC, Tetrazolium (TZ-V) and Germination (G), near post harvest time and during storage period (1, 5, 9, 13 and 19 months). An independent set of 18 genotypes, stored during 1-108 months, were also analyzed for EC and G. Electric conductivity in dehulled seeds was effective to identify vigor differences of genotypes in different seed filling period and storage conditions. A general relationship between the loss of germination and vigor was established for sunflower. The ranges of vigor based on EC proposed for sunflower seeds classification were <70 $\mu s\ cm^{-1}\ g^{-1}$ for high, 70-110 $\mu s\ cm^{-1}\ g^{-1}$ for intermediate and >110 $\mu s\ cm^{-1}\ g^{-1}$ for low vigor levels. It is the first report based on EC ranges to categorize the seed vigor of sunflower seeds lots.

Key words: *Helianthus annuus*, electrical conductivity, vigor ranges, germination, modeling vigor loss

INTRODUCTION

Seed vigor has been defined as those seed properties that determine a potential for rapid, uniform emergence and development of seedlings under a wide range of field conditions (AOSA., 2002). In sunflower seeds, vigor has been evaluated through seedling size grading, accelerated aging, cold germination, controlled deterioration, energy germination, Hiltner test and tetrazolium staining (Braz *et al*., 2008; Mrda *et al*., 2010; Oliveira *et al*., 2012). However, a standardized test that can be used as an adequate predictor of seed performance at field conditions is not available.

As, sunflower exhibits a high degree of seed dormancy, vigor tests based on the germination process such as accelerated aging and seedlings emergence, have a limited application (Silva *et al*., 2013). Moreover, seed vigor classification can vary greatly depending not only on the test used but on the genotype as well. Indeed, Murcia *et al*. (2003) found that seed tissue damage evidenced by tetrazolium test was not associated with deterioration patterns caused by accelerated aging. In turn Balesevic-Tubic *et al*. (2007) showed the sensitivity of cold test, Hiltner test and seedling classification changed depending on sunflower genotype.

Tetrazolium and electrical conductivity are promising vigor tests since, they produce fast results (<24 h). These test are not affected by seed dormancy, do not require sophisticated equipment or highly skilled personnel and they could be used to shorten the decision period in the seed industry management (Silva *et al*., 2013). Electrical conductivity test aims to indirectly evaluate the extent of damage caused to cell membranes resulting from seed deterioration (Abreu *et al*., 2011). The genotype, seed integrity, size and moisture content as well as soaking period and temperature affect electrical conductivity results (De Carvalho *et al*., 2009). In sunflower, leakage of electrolytes from the pericarp or changes in its permeability may interfere with exudates from embryo tissues (Del Longo *et al*., 1999; Queiroga and Duran, 2010) and produce contradictory laboratory results (Albuquerque *et al*., 2001). Braz *et al*. (2008) and Oliveira *et al*. (2012) increased the predictive value of the conductivity test using the dehulled procedure of sunflower seeds. Due to their selective permeability, pericarp and seed coat removal could be considered as an effective way to break seed dormancy in sunflower (Szemruch *et al*., 2014). Thus, the electrical conductivity test using dehulled sunflower seeds, seems useful in order to overcome interferences from the pericarp electrolytes and seed dormancy. To prove its usefulness, the electrical conductivity test with dehulled sunflower seeds requires to be tested on seeds of different genotypes exposed to different seed filling period and storage conditions.

Delouche and Caldwell (1960) modeled the general relationship between the loss of germination and vigor as seed deterioration occurs. Nevertheless, such relationship has not yet been established for sunflower. This model could be useful to determine ranges of high, intermediate and low seed vigor levels for sunflower, in a similar way to that mentioned by Vieira *et al*. (1994) in soybean.

The aims of this work were (1) To determine the utility of the EC test using dehulled (without pericap) sunflower seeds to evaluate the vigor in different genotypes, exposed to contrasting seed filling period and storage conditions, (2) To explore the relationship between EC and germination values near post harvest and across storage period and (3) To propose ranges of vigor through EC, so as to categorize sunflower seeds lots.

MATERIALS AND METHODS

Vegetal material: Sunflower seeds were produced at the experimental field of PANNAR Semillas in Venado Tuerto, Santa Fe Province, Argentina (33°44' S, 61°58' W) in three maternal environments: two sowing dates in 2011 (9/25/11; 10/31/11) and one sowing date in 2012 (5/11/12). Controlled pollination was used to produce one three-line (G2) and five two-line (G1, G3, G3´, G4 and G5) commercial hybrids. The G1, G2 and G3 were produced in 2011 and 2012, whereas G3´, G4 and G5 only in 2012. G3 and G3´are high oleic acid hybrids and G4 is *iso*-hybrid of G3´mid-oleic acid. In both years, a completely randomized design with two replicates was applied in the field.

All genotypes explored contrasting environmental conditions, especially during seed filling period, generated from three sowing dates (9/25/11, 10/31/11and 5/11/12) mentioned above. There were no significant differences in the vegetative and reproductive stages length between genotypes for each sowing date (data not shown). Heads from different genotypes were harvested at the same time and physiological stage, after R9 (stage physiological maturity according to Schneiter and Miller, 1981) when seed moisture reached 25.8±4.78% for 2011 and 25.0±2.21% for 2012. Values are Mean±standard deviation and w/w basis (ISTA., 2013). Seeds were placed in an air-forced fluid bed dryer (Econaire 50000, Econaire Inc, Argentina) at room temperature for 48 h and then stored during 19 months in paper bags under two conditions: room temperature (25°C) and cold chamber (10°C) at 50-80% relative humidity. Prior to storage, seed water content was 6-7% and 5-7% (2011 and 2012, respectively), without significant differences among genotypes. Stored seeds were sampled at 1, 5, 9, 13 and 19 months after harvest, for laboratory analysis.

Additionally, an independent data set of seeds samples (n = 420) from 18 commercial sunflower hybrids (including traditional and high oleic genotypes and different deterioration degree) were stored under the same two conditions described above and evaluated from 1 to 108 months after harvest.

Laboratory tests: Seed conditioning: dehulled seeds (without pericarp) and decoated seeds (without pericarp and without seed coat) were obtained by manual removal according to Braz *et al*. (2008) and Szemruch *et al*. (2014), respectively.

Germination (G): Four replicates of 50 decoated seeds were incubated between paper moisturized whit distilled water (ISTA., 2013) at 25°C and 12 h light/dark. Germination percentage was calculated on the 10th day after sowing by counting only normal seedlings (ISTA., 2013).

Seed vigor: Two tests were applied (1) Electrical Conductivity (EC) and (2) Tetrazolium vigor (TZ-V). For EC, four replicates of 50 pre weighed dehulled seeds were placed in 75 mL distilled water at 25°C for 24 h. Then they were measured using a conductivity meter according to Braz *et al*. (2008) and expressed as µs cm^{-1} g^{-1}. The seeds that were stored in the cold chamber were kept out of the chamber 24 h prior to being analyzed. The TZ-V was performed through a slight adaptation for sunflower form ISTA (2013) methodology with four replicates of 50 decoated seeds imbibed in staining solution of 2, 3, 5-triphenyltetrazolium chloride salt (0.5% w/v) at 25°C during 4 h. The seeds, "viable without defects", were classified as "high vigor" according to Gallo *et al*. (2012) adapted from soybean and expressed as percentage.

Germination and electrical conductivity relationships: The relationship between germination percentage (G) and Electrical Conductivity (EC) was modeled according to Delouche and Caldwell (1960) using three data sets: (1) dehulled seeds near post harvest time, from this study, (2) dehulled seeds near post harvest reported by other authors (Albuquerque *et al*., 2001; Braz *et al*., 2008; Braz and Rosseto, 2009; Abreu *et al*., 2011; Goncalves, 2012; Oliveira *et al*., 2012; Moraes *et al*., 2012) who applied the same methodology for EC test (Braz *et al*., 2008) and (3) Dehulled seeds from 18 genotypes stored during 1-108 months.

Statistical analysis: ANOVA and LSD tests were performed with a 5% significance level. Linear and polynomic regression was fitted for G and EC data measured near post harvest time and during seed storage, respectively. Percentage values were transformed using angular transformation. Infostat statistical software was used (Di Rienzo *et al*., 2008).

RESULTS AND DISCUSSION

Electrical conductivity test: Genotypic variability was observed for G and EC, measured at post harvest time, with the high oleic hybrid (G3) showing the worst performance among all hybrids in both experimental years (Table 1 and 2). The TZ-V expressed a similar behavior to EC among genotypes in both years (Table 1 and 2). The EC test using decoated seeds was useful to discriminate the vigor of sunflower genotypes with a similar ranking of TZ-V, according to Albuquerque *et al*. (2001), Braz *et al*. (2008) and Oliveira *et al*. (2012). The EC test capacity to detect genotypic differences was high, especially in relation to the fatty acid composition, as the high oleic genotype (G3´) showed the worst germination and vigor in both years and statistically differed from their iso-line mid oleic (G4) (Table 2). This was coincident with Sun *et al*. (2014) who also reported the EC test as the most effective to detect vigor differences in peanut and showed a lower vigor in high oleic genotypes.

The EC and TZ-V test reflected seed filling variations (Table 1) showing the lowest seed vigor in the latest sowing date in 2011. On this sowing date the seed growth period was exposed to high temperatures (Szemruch *et al*., 2014) which may have affected seed vigor at harvest time. Similar effects of growing field conditions on seed vigor have been observed in soybean, wheat, barley and groundnut (Egli *et al*., 2005; Samaraha and Alqudaha, 2011; Hasan *et al*., 2013; Sharma *et al*., 2013). In addition, a significant genotype by environment interaction was observed for EC values when G1and G2 grown on all sowing dates were evaluated by ANOVA (data not shown).

Effects of storage conditions on seed vigor were not evidenced by TZ-V, whereas EC differentiated between room and cold chamber conditions in all genotypes (Fig. 1). The EC test was sensitive to monitoring seed deterioration in storage at 25°C and showed higher values (lower vigor) (Fig. 1). Some authors questioned the ability of EC test for seed deterioration monitoring in

Table 1: Physiological parameters measured near to post harvest time on seeds from sunflower hybrids grown on different sowing dates in the first experimental year (2011)

Parameters	G (%)	EC (μs cm^{-1} g^{-1})	TZ-V (%)
Genotypes			
G1	92^{a}*	32, 9^{b}	98^{a}
G2	93^{a}	30, 0^{b}	96^{ab}
G3	85^{b}	49, 1^{a}	94^{b}
Sowing dates			
9/25/11	91^{a}	33, 8^{b}	98^{a}
10/31/11	90^{a}	40, 9^{a}	96^{b}
CV (%)	6	8	6

*Means with the same letter in each column do not differ by LSD Fisher ($p<0.05$), G: Germination, EC: Electrical conductivity and TZ-V: High vigor seeds by tetrazolium

Table 2: Physiological parameters measured near to post harvest time on seeds from sunflower hybrids grown in the second experimental year (2012)

Genotypes	G (%)	EC (μs cm^{-1} g^{-1})	TZ-V (%)
G1	91^{b}	54, 8^{a}	72^{b}
G2	98^{a}	37, 1^{bc}	87^{a}
G3	88^{c}	55, 7^{a}	76^{ab}
G4	93^{b}	23, 9^{c}	88^{a}
G5	96^{a}	39, 3^{b}	79^{ab}
CV (%)	4	8	9

*Means with the same letter in each column do not differ by LSD Fisher ($p<0.05$), G: Germination, EC: Electrical conductivity and TZ-V: High vigor seeds by tetrazolium at low temperature, suggesting that stabilization of membrane occurs, which results in lower leakage of exudates (Fessel *et al*., 2010). Contrarily other authors recommended this test for vigor evaluation even at low temperature storage (Panobianco *et al*., 2007; Mielezrski and Marcos-Filho, 2013; Marques *et al*., 2014). In our experiments, a greater sensitivity of high oleic G3 on the electrolytes leaching was observed in storage at 10°C (Fig. 1f). This result agrees with Abreu *et al*. (2011), who indicated that the ability of EC test to detect the loss of membrane integrity at low temperature storage varies according to the sunflower genotype employed. As, it is already

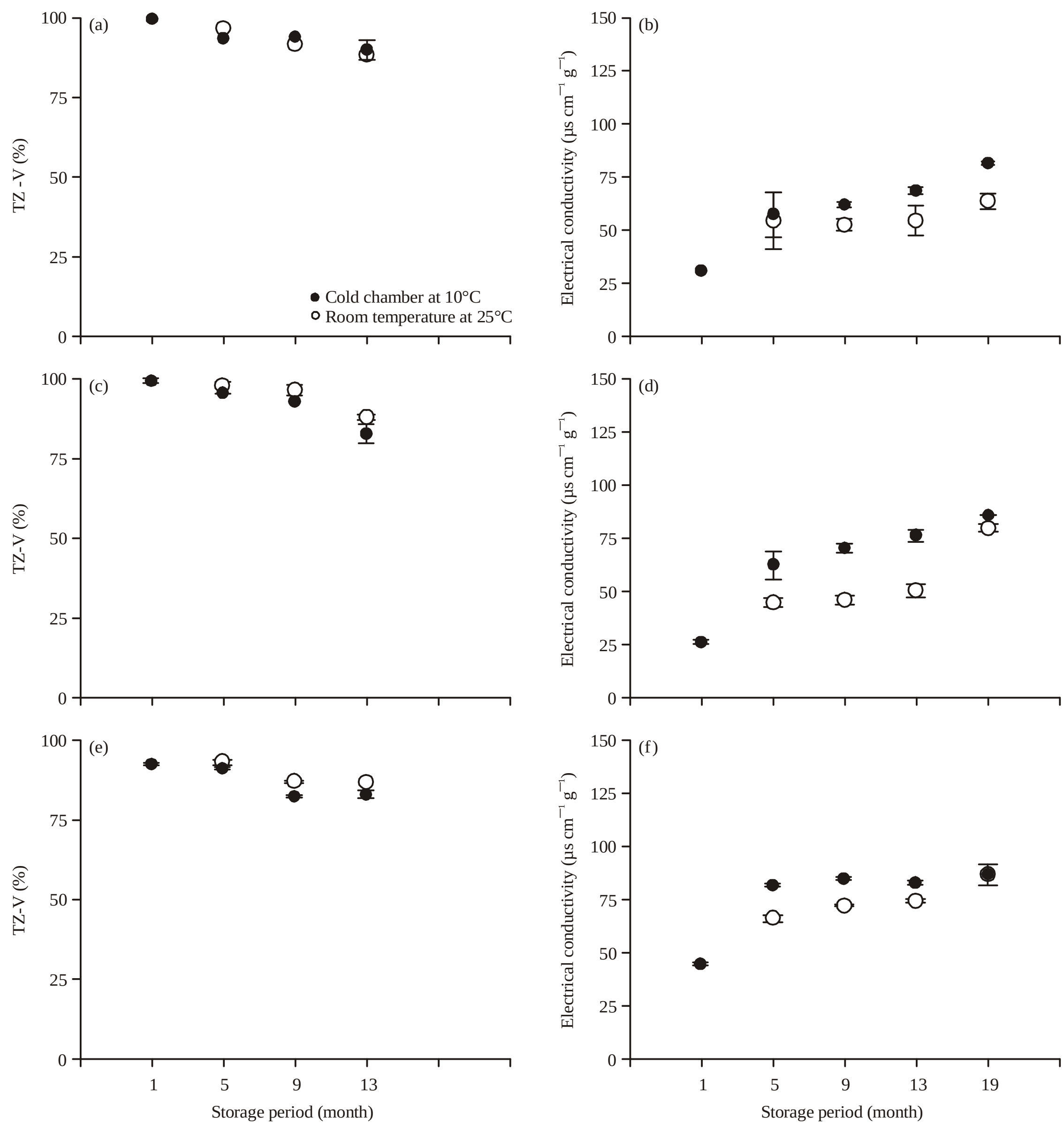

Fig. 1(a-f): Evolution of tetrazolium vigour (TZ-V) and electrical conductivity of dehulled seeds on three sunflower hybrids stored in a cold chamber at 10°C or under room temperature at 25ºC. (a, b) G1, (c, d) G2 and (e, f) G3 from the first experimental year (2011). Bars are±1 standard error

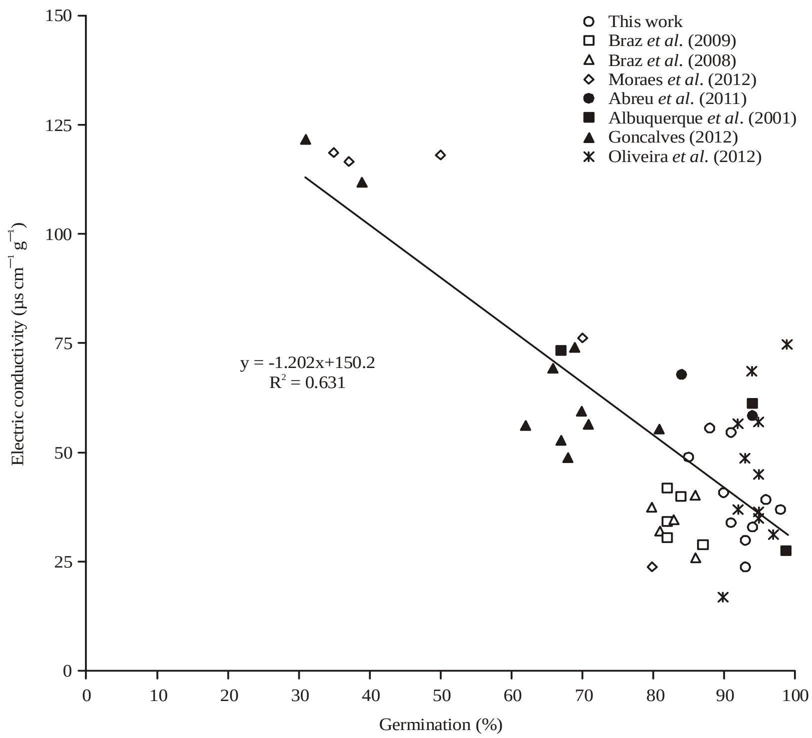

Fig. 2: Germination and electrical conductivity relationship for sunflower dehulled seeds, analyzed near at harvest time, including this work and data found in the literature

established, lipid peroxidation and oxidative damage affect membrane functionality in sunflower (Corbineau *et al*., 2002). So, it would be interesting to assess how changes in the fatty acid composition of the seeds can interact with changes in membrane fluidity at low temperature storage.

Germination and electrical conductivity relationships: Our results for G and EC relationship in sunflower seeds near post harvest time for both our results and those from the literature, showing a clear upward trend in EC as germination decreased (Fig. 2). Germination in the range of 80-100% was associated with EC values from 25-75 µs cm^{-1} g^{-1}. Germination percentages between 70-60% were associated with EC values from 50-80 µs cm^{-1} g^{-1} and germination between 50-30% was associated with EC values from 90-110 µs cm^{-1} g^{-1}.

The analysis of G and EC relationship from independent data set of seeds during storage (Fig. 3) allowed to classify the seeds samples in two classes: "high quality" (n = 232) when G exceeded 85% at 13 months from post harvest and "low quality" (n = 188) when G was below 75%, at 13 months from post harvest. In high quality seeds, EC was 75 and 78 µs cm^{-1} g^{-1} at the time when G fell 85% in 10°C and room storage, respectively (Fig. 3a-b). Under both storage conditions, there were no significant differences between 5, 9 and 13 months. For low quality seeds, EC was 87 and 100 µs cm^{-1} g^{-1} for cold chamber and room storage respectively, when G fell below 75% (Fig. 3c-d).

Thresholds for sunflower seed vigor: Data provided by Fig. 2 and 3 allowed to adapt the Delouche and Caldwell (1960) model in order to propose values for categorizing sunflower

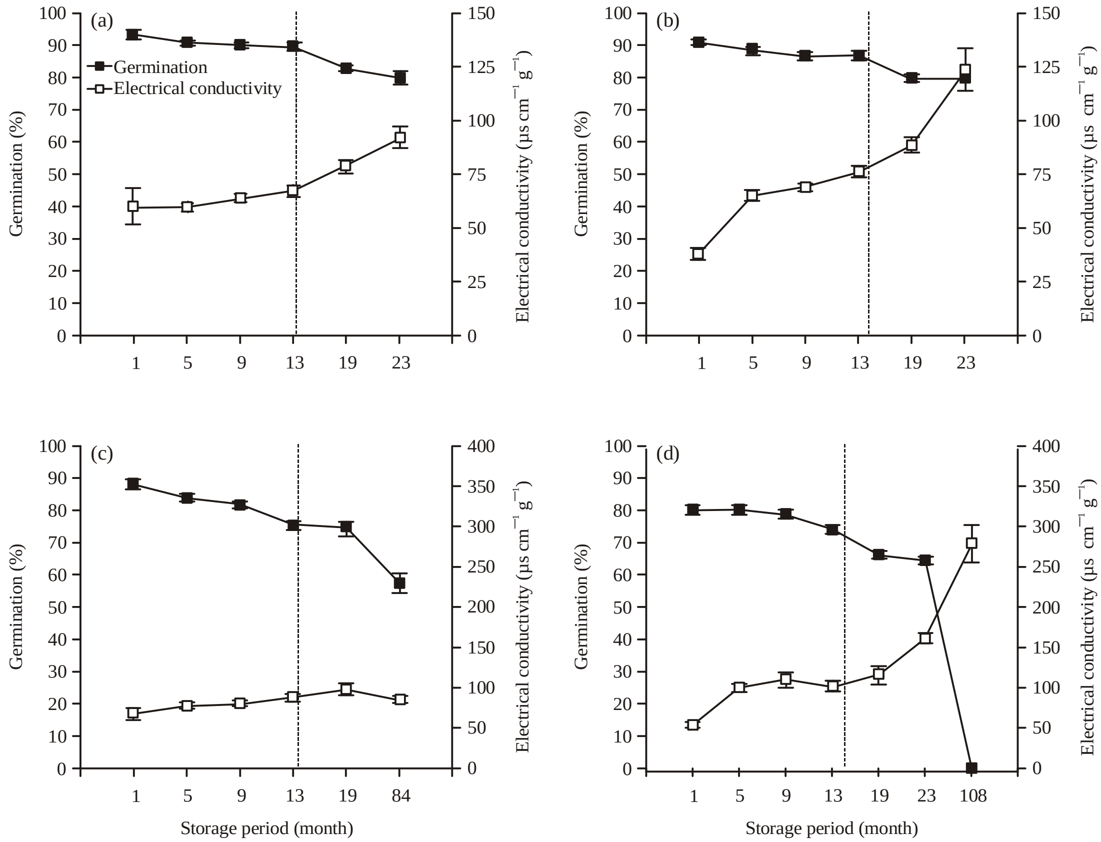

Fig. 3(a-d): Germination and electrical conductivity relationships during storage for sunflower seeds classified as (a, b) high and (c, d) low quality stored in a cold chamber (a, c) at 10°C or (b, d) under room temperature at 25°C conditions. Values are Mean±1 standard error from 18 different commercial sunflower hybrids. Note the different scales on the electrical conductivity axis among upper and lower panels. Vertical dotted lines show the moment (13 months from harvest) when germination descended below 85% (high quality seeds) or below 75% (low quality seeds)

seeds by their vigor (Fig. 4). When G exceeded 85%, EC was <70 µs cm^{-1} g^{-1} and the seeds would be considered as "high vigor", when G was between 85 and 75%, EC was between 70 and 110 µs cm^{-1} g^{-1} and it would be considered as "intermediate vigor", when G was between 75 and 50% EC was >110 µs cm^{-1} g^{-1} which would be considered as "low vigor" and when G fell 50% the seeds were unsuitable for sowing with EC values >160 µs cm^{-1} g^{-1}. The thresholds proposed in Fig. 4 for sunflower vigor classification were close to those mentioned for soybean by Vieira *et al*. (1994) and AOSA (1983), which indicate that seed lots with EC values between 60 and 70 µs cm^{-1} g^{-1} are classified as high vigor, values between 70 and 80 µs cm^{-1} g^{-1} are presented as intermediate vigor and seeds with EC values higher than 150 µs cm^{-1} g^{-1} are classified as low vigor and considered inadequate for sowing. This is the first report on seed vigor ranges for sunflower. To extend the utility of the proposed ranges of seed vigor, further studies should explore a broader range of seed lots within the same genotype and validate the correlation of seed vigor measured in laboratory with seed performance under field conditions. The selection of seed vigor tests in the field is not trivial, as significant variability caused by genotype and different

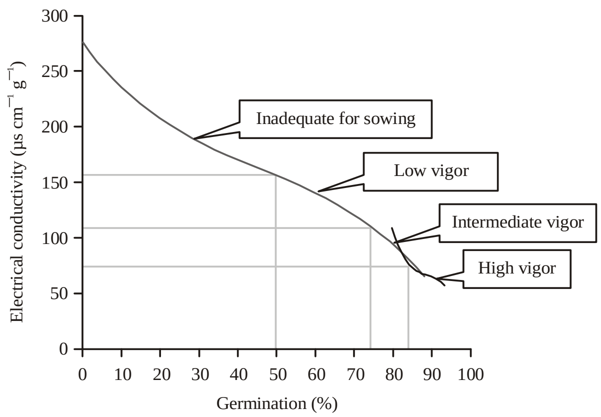

Fig. 4: Germination and electrical conductivity relationship and proposed vigor thresholds for sunflower seeds (horizontal dotted lines). Black line corresponds to high quality seeds (Fig. 3a, b) and grey line, to low quality seeds (Fig. 3c, d)

adverse soil conditions (temperature, water content, soil texture, etc) have been stated in previous works (Anfinrud and Schneiter, 1984; Santorum *et al*., 2013). Studies are being conducted in order to validate the EC thresholds proposed here for sunflower seeds. If the correlation with field performance is confirmed, thresholds of seed vigor measured by the electrical conductivity test from dehulled seeds should be a useful tool for seed industry and farmers.

CONCLUSION

Electrical conductivity test in dehulled seeds was able to identify the vigor of genotypes exposed to different seed filling period and storage conditions. A general relationship between the loss of germination and vigor was established for sunflower, suggesting EC ranges for high, intermediate and low seed vigor. Although, this should be validated measuring seed vigor under field conditions, it suggested as a guide in order to classify lots of sunflower seeds by vigor.

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