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SIMONA-LAURA LAZAR

SARA MIRA

DORU PAMFIL

JUAN BAUTISTA MARTINEZ-LABORDE

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Germination and electrical conductivity tests on artificially aged seed lots of 2 wall-rocket species

Simona-Laura LAZAR[1], **Sara MIRA**[2,*], **Doru PAMFIL**[1], **Juan Bautista MARTÍNEZ-LABORDE**[2]
[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania
[2]Plant Biology Department, School of Agronomics Engineering, Technical University of Madrid, Madrid, Spain

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Abstract: The aim of this study was to determine whether the loss of germinability and vigour in wall-rocket seed lots was related to electrolyte leakage. Ten seed lots of 2 wall-rocket species (9 of *Diplotaxis tenuifolia* and one of *D. erucoides* (Brassicaceae)) were stored at 45 °C and 70% relative humidity and electrolyte leakage was measured using the conductivity test. Small differences in longevity were found among species or seed lots, suggesting that a similar relative longevity might be expected within the genus *Diplotaxis* after artificial ageing, and that it will be above the average of that found in other species of the same family at similar storage conditions. Electrolyte loss was strongly related to loss of seed viability. There was a negative linear relationship between conductivity and seed germination (R^2 = 0.85), and a positive relationship with T_{50} (R^2 = 0.77). Small differences were found among species or seed lots regarding the correlation between electrolyte leakage and seed germination and vigour. Our results indicate that the conductivity method is able to predict the viability of wall-rocket seeds stored at 45 °C and 70% RH, with the same regression equation of conductivity and germination (–33.97x + 135.59) being able to predict the viability of different seed lots and species. A conductivity level ranging from 1.4 to 2.2 mS g^{-1} DW would indicate that the potential of a *Diplotaxis* seed lot to germinate has decreased to 80%. The correlation between electrolyte leakage and seed germination provides a valuable means for early detection of seed viability in wall-rocket seeds.

Key words: Brassicaceae, conductivity, *Diplotaxis*, seed quality, seed vigour, wall-rocket

1. Introduction

Several remarkable leaf vegetables belong to the family Brassicaceae, including cabbages and their variants (e.g., Brussels sprouts, cauliflower), radish, watercress, and the increasingly widespread arugula or rocket. Rocket (rucola in Italian, roquette in French) is a traditional vegetable crop in Italy and several other Mediterranean countries, as well as an oilseed crop in Asia, that has become in recent years a much appreciated and even fashionable salad ingredient in many Western countries, mostly sold as ready-to-eat packaged leaves. The characteristic pungent, peppery taste of rocket is due to sulphur-rich, volatile isothiocyanates, which in turn arise via hydrolysis from glucosinolates, a group of natural products responsible for the characteristic aroma of many Brassicaceae (Macleod, 1976; Rodman, 1991). These compounds have a relevant role in plant protection (Rosa et al., 1997; Fahey et al., 2001) and human and animal health (Srinibas et al., 2000).

The common name rocket is applied to *Eruca vesicaria* (L.) Cav. subsp. *sativa* (Mill.) Thell., the traditionally cultivated rocket, as well as to other species of the same family, mostly belonging to the genus *Diplotaxis* DC. The 2 main ones are *D. erucoides* (L.) DC., a local source of rocket leaves (Branca, 1995), and in particular *D. tenuifolia* (L.) DC., often referred to as perennial wall-rocket. *Diplotaxis tenuifolia* is currently widely found in markets (Pimpini and Enzo, 1997) and its cultivation on a commercial scale has become well established in the last decades (Bianco and Boari, 1997; Pimpini and Enzo, 1997; Nicoletti et al., 2004). It shares with *E. vesicaria* subsp. *sativa* similar pinnately lobed leaves as well as the same glucosinolates (Fahey et al., 2001), which may account for similarities between these 2 widely used species in taste, flavour, culinary uses, and commercial name. Though sometimes considered annoying weeds or even invaders (Caso, 1972; Martínez Laborde et al., 2007), wall-rocket species should rather be regarded as valuable genetic resources, not just as salad components but also for the potential benefits of their glucosinolate content in cancer protection (D'Antuono et al., 2006).

Establishing the quality of a seed lot is a crucial step, whether for cultivation, breeding, or research activities.

* Correspondence: sara.mira@upm.es

In the case of nondomesticated species, assessing seed viability by laboratory germination tests as established by International Standards (ISTA, 2009) is highly time-consuming and meaningless if they are not accompanied by parallel studies on dormancy, because seeds might present primary dormancy, or develop secondary dormancy during storage, which would confound the viability of results (Pérez-García et al., 2007; Mira et al., 2011b; van Hintum and van Treuren, 2012; van Treuren et al., 2013). The few available studies on *D. tenuifolia* (Kleemann et al., 2007; Sakcali and Serin, 2009; Hall et al., 2012; Lazar et al., 2012) and *D. erucoides* (Pérez García et al., 1995; Martínez Laborde et al., 2007) germination show that viable, nondormant seeds of both species can reach high (≥80%) germination percentages. However, previous works also suggest that *D. erucoides* seed germination may decline during storage due to the development of secondary dormancy, which could be mistaken for seed viability loss (Pérez García et al., 1995; Martínez Laborde et al., 2007). Therefore, seed quality cannot always be easily evaluated by germination tests. On the other hand, the use of the tetrazolium test or the cut test to assess seed viability does not rely on seed germination, but implies seed death, which is usually not desirable when dealing with seeds of nondomesticated species or that are otherwise scarce.

Finding seed quality indicators that do not require seed destruction would save enormous amounts of time, money, and seed material. The conductivity test has been validated as a vigour test for peas (ISTA, 2009), and previous works reported the potential application of the conductivity test as a vigour test in Brassicaeae seeds, both wild (Mira et al., 2011a) and cultivated (Fessel et al., 2005; Mirdad et al., 2006; Demir et al., 2008; Matthews et al., 2009; Nery et al., 2009) stored at high humidity. The aim of the present study was to determine whether the loss of seed viability during storage in 2 *Diplotaxis* species was related to electrolyte leakage, measured using the conductivity test.

2. Materials and methods

2.1. Plant material

Experiments were performed on seeds of 2 *Diplotaxis* species, *D. tenuifolia* and *D. erucoides*, from different origins (Table 1). A total of 10 seed lots were evaluated: 9 seed lots of *D. tenuifolia*, obtained in 2010 from seed companies (Tozer Seeds, UK, and AdvanSeed, Denmark), and one seed lot of *D. erucoides*, obtained in 2010 at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca from an accession of IPK Gatersleben Seed Bank. Seeds were stored at 5 °C with silica gel (7% RH) until their use, and showed high germination levels at the beginning of the experiments in February 2011, at the Technical University of Madrid.

2.2. Accelerated ageing treatment

Before being stored, seeds were equilibrated for 3 days at room temperature in airtight glass jars with a LiCl solution set to obtain a 70% RH, as described by Hay et al. (2008). After that, seeds were stored at 45 °C over the LiCl solution. Seed samples were removed periodically and set to germinate. The number of days required for seed germination to be reduced to 80% and 50% of maximum germination (P80 and P50) was determined graphically.

2.3. Water content determination

Seed water content was evaluated twice during the ageing process, at the beginning and at the end of the assay. The control of water content was performed using the low-constant-temperature-oven method (ISTA, 2009) on 2 replicates of 0.5 g seeds. Water contents were expressed as g H_2O g^{-1} dry weight (DW), and the results corresponded to the mean ± standard error (SE).

2.4. Germination assays

Germination assays were performed by placing 3 replicates of 50 seeds in 9-cm petri dishes on a layer of paper moistened with deionised water at 25 °C and 16/8 h of light. The criterion for germination was 1 mm radicle protrusion, and it was quantified daily for up to 15 days. Results are expressed as germination percentage (%) and as germination speed, measured as the number of days required to reach 50% of final germination (T_{50}), which was estimated graphically from each of the cumulative germination curves (Figure 1).

2.5. Electrolyte leakage

Electrolyte leakage was determined by placing 3 replicates of 0.1 g of seeds into 10 mL of deionised water at 25 °C and measuring the conductivity of the medium with a conductivity meter (EC-Meter GLP 31) after 24 h. Results are expressed as mS g^{-1} DW and represent the mean of 3 measurements ± standard error (SE).

3. Results

3.1. Effects of ageing treatments on seed germination

The changes in seed germination during ageing at 45 °C and 70% RH for *D. tenuifolia* and *D. erucoides* are shown in Figures 1 and 2, and the longevity parameters in Table 1. The water content of seed samples equilibrated at 70% RH did not change along storage time (with standard error of water content measurements ≤ 0.002), nor among seed lots or species (Table 1). The average water content for all lots at 45 °C and 70% RH was 0.094 ± 0.001 g H_2O g^{-1} on a dry weight basis (DW). Control nonaged seeds from all lots germinated within 2–4 days, with maximum germination percentage above 92% for all lots, except for DT6 with 77%. Germination speed (T_{50}), was approximately 0.6–1.2 days for control nonaged seeds. For all seed lots, ageing reduced the final germination percentage and markedly slowed germination; longer ageing treatments led to

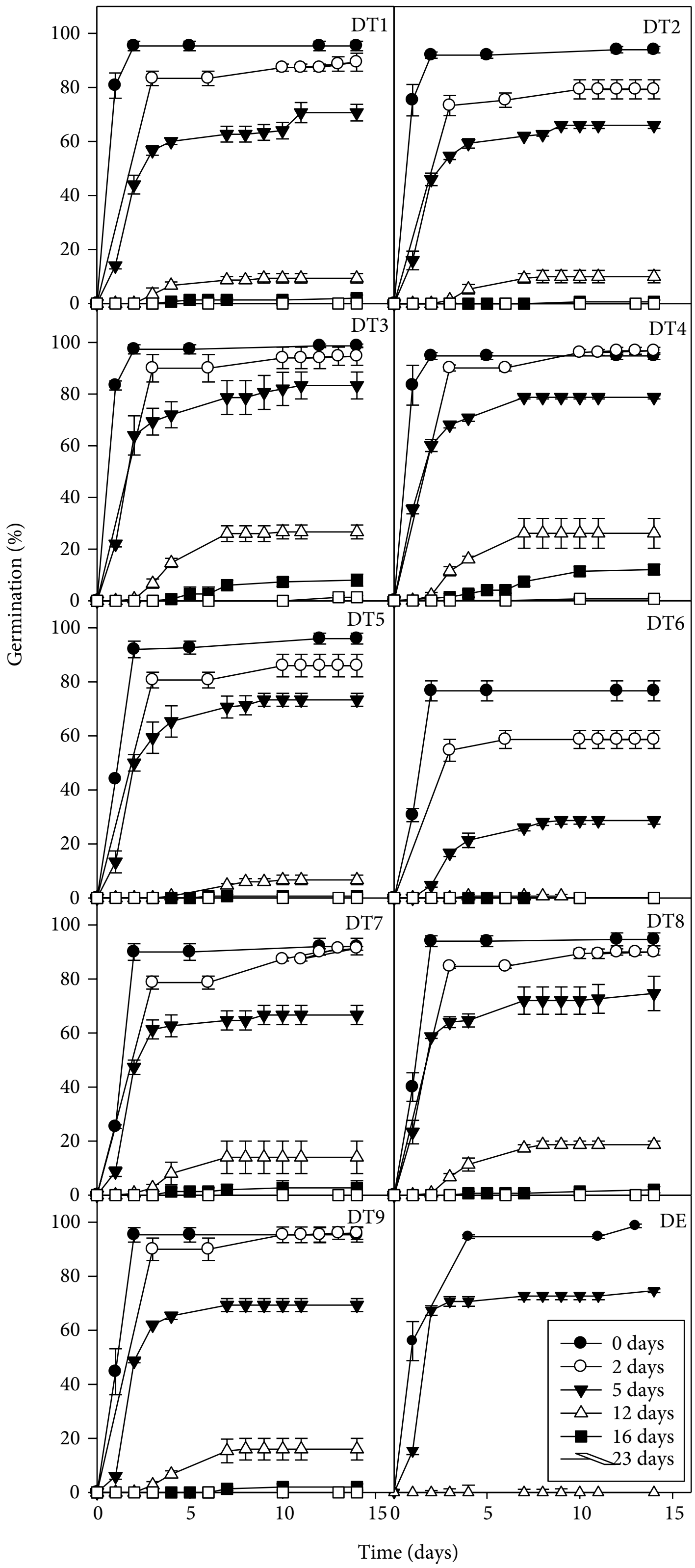

Figure 1. Time courses of germination of 9 *Diplotaxis tenuifolia* (DT) and 1 *D. erucoides* (DE) seed lots under ageing treatment at 45 °C and 70% RH. Values represent the average of 2 replicates ± standard error.

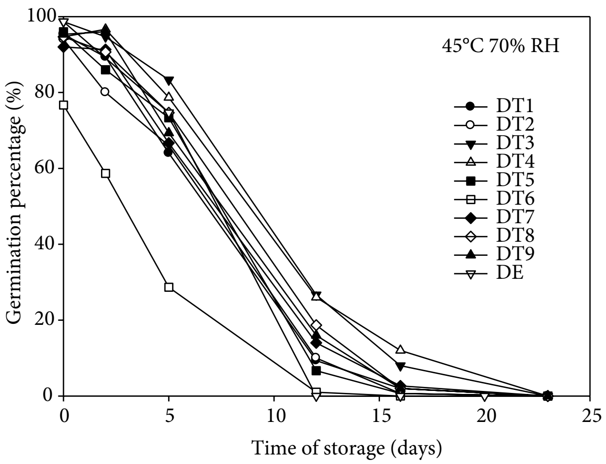

Figure 2. Seed viability loss during storage at 45 °C and 70% RH for 9 *Diplotaxis tenuifolia* (DT) and one *D. erucoides* (DE) seed lots. Values represent the average of 3 replicates ± standard error.

stronger inhibitory effects (Figure 1). The development of secondary dormancy during storage at 45 °C and 70% RH was dismissed since nongerminated seeds were clearly unfirm, mouldy and, therefore, unviable.

During storage at 45 °C and 70% RH, all seed lots lost germination capacity completely after 12–23 days of ageing (Figure 2). The average length of time required for germination to decrease to 80% or 50% of maximum germination (P80 and P50) for all lots was 4.1 and 7.5 days, respectively. We found little difference among the lots, except for DT6, which lost 50% of germination after only 3.7 days of storage at 45 °C and 70% RH (Table 1). *D. erucoides* seeds behaved close to the average, with P80 = 4.5 days and P50 = 7.4 days.

3.2. Electrolyte leakage

The relationship between storage time and conductivity is shown in Figure 3; 4 *D. tenuifolia* and one *D. erucoides* seed lots are shown as an example of the complete data set. Before ageing, the initial value of seed electrolyte leakage

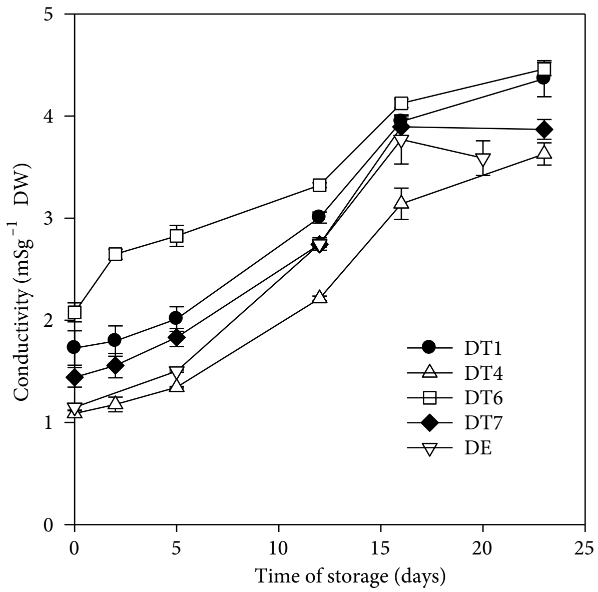

Figure 3. Effect of the duration of seed storage at 45 °C and 70% RH on electrolyte leakage (mS g^{-1} DW) of seeds from 4 *Diplotaxis tenuifolia* (DT) and 1 *D. erucoides* (DE) seed lots. Values are the average of 3 replicates ± standard error. Linear regression curves were fitted for all datasets with $R^2 \geq 0.89$.

Table 1. Seed lots of *Diplotaxis tenuifolia* (DT) and *D. erucoides* (DE) studied and their origin, water content, and seed longevity at 45 °C and 70% RH, expressed as the storage time required for viability to be reduced to 80% and 50% (P80 and P50) of the maximum germination.

Seed lot	Provider	Origin	Harvest year	Water content ($g H_2O g^{-1}$ DW ± SE)	Longevity parameters	
					P80 (days)	P50 (days)
DT1	Tozer Seeds	UK	2009	0.095 ± 0.000	3.5	7.0
DT2	Tozer Seeds	UK	2009	0.097 ± 0.001	3.2	7.3
DT3	AdvanSeed	Denmark	2009	0.095 ± 0.002	5.5	9.2
DT4	AdvanSeed	Denmark	2009	0.090 ± 0.001	5.4	9.0
DT5	AdvanSeed	Denmark	2009	0.092 ± 0.001	4.4	7.6
DT6	AdvanSeed	Denmark	2009	0.098 ± 0.000	1.8	3.7
DT7	AdvanSeed	Denmark	2009	0.093 ± 0.001	4.0	7.5
DT8	AdvanSeed	Denmark	2009	0.095 ± 0.001	4.9	8.4
DT9	AdvanSeed	Denmark	2009	0.093 ± 0.001	4.1	7.6
DE	University of Cluj-Napoca	Rumania	2010	0.092 ± 0.001	4.5	7.4

ranged between 1.1 and 2.1 mS g^{-1} DW. Seed ageing at 45 °C and 70% RH resulted in a progressive increase of electrolyte leakage up to 3.6–4.8 mS g^{-1} DW over 23 days of treatment for all seed lots.

Electrolyte loss was related to a decrease in seed germination capacity for all seed lots. Figure 4 shows the relationship between electrolyte leakage and germination (Figure 4A) and germination speed T_{50} (Figure 4B). There was a negative linear relationship between electrolyte leakage and seed germination (R^2 = 0.85) (Figure 4A), and a positive relationship with T_{50} (R^2 = 0.77) (Figure 4B). When looking at each individual seed lot, the correlation between electrolyte leakage and seed germination (R^2 = 0.80–0.94) or T_{50} (R^2 = 0.74–0.99) was even higher (Table 2). Though the relationship between electrolyte leakage and seed germination seems to be strongly affected by data obtained from dead seed samples (0% germination), the correlation holds even if those data points are not taken into account (R^2 = 0.84). Moreover, the conductivity levels at which seed germination decreased to 80% ranged from 1.4 to 2.2 mS g^{-1} DW, with 1.7 mS g^{-1} DW being the average value for all lots (Table 2; Figure 4).

4. Discussion

Research has shown that seed quality is influenced by genotype, environment during seed development, and seed storage conditions (Clerkx et al., 2004; Fessel et al., 2006; Açıkgöz et al., 2013; Hampton et al., 2013; Hay et al., 2013; Uddin et al., 2013). Our results suggest significant differences in longevity could not be found between either different species of the same genus (*Diplotaxis tenuifolia* and *D. erucoides*), or different seed lots of the same species (*D. tenuifolia*). The only lot with a significantly lower longevity was DT6, but this difference could be explained by its initial germination percentage, also considerably lower (77%) than in the remaining lots (above 92%), which indicates that DT6 had already deteriorated at the start of the experiment. While seed longevity has been found to be relatively similar within some genera and families (Walters et al., 2005; Probert et al., 2009), there are previous reports of highly variable seed longevity even among related species (Walters et al., 2005; Pérez-García et al., 2009; van Treuren et al., 2013), lots of the same cultivar (Nagel et al., 2009; Niedzielski et al., 2009; Nagel and Borner, 2010), or wild seed lots (Kochanek et al., 2009). Within Brassicaceae, longevity was found to vary greatly among wild species stored both at high and low humidity (Mira et al., 2014). Differences in viability results among species or seed lots may sometimes result from some of the seeds used to compare being particularly deteriorated, as was the case with DT6 in the present study, or having developed secondary dormancy during storage (Mira et al., 2011b; van Treuren et al., 2013).

Among the longevity parameters used in this work, the length of time required for germination to decrease to 80% of maximum germination (P80) was calculated as a measure of the onset of viability loss and is a useful parameter to assess the moment when a seed lot in a seed

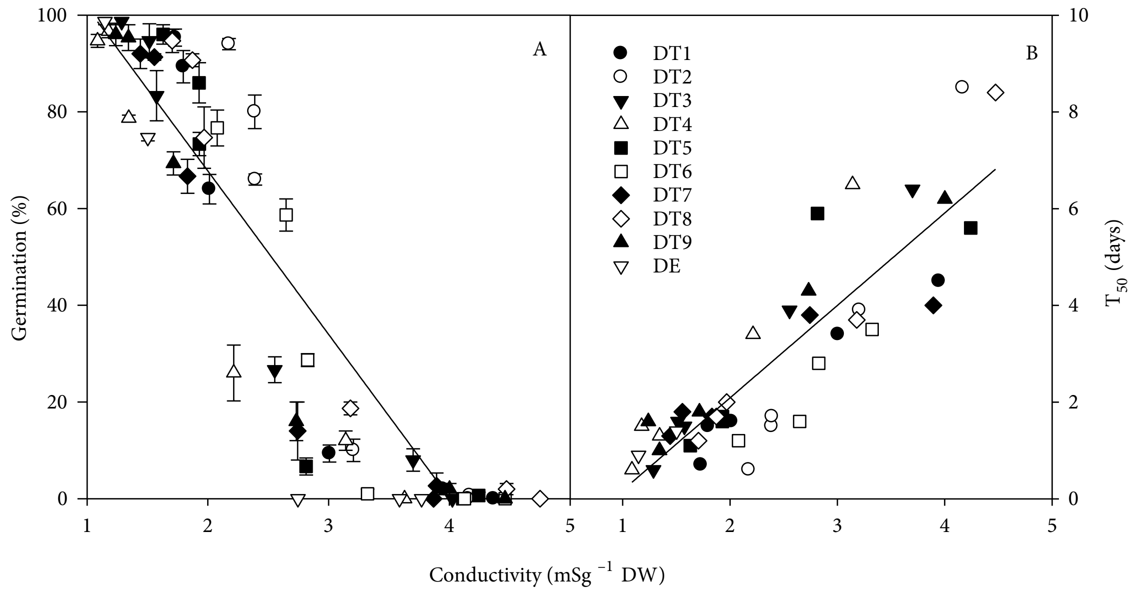

Figure 4. Relationships between electrolyte leakage (mS g^{-1} DW) and germination percentage (A) or seed vigour (T_{50}) (B) of seeds from 9 *Diplotaxis tenuifolia* (DT) and 1 *D. erucoides* (DE) seed lots stored at 45 °C and 70% RH. Values are the average of 3 replicates ± standard error. Linear regression was fitted for the complete dataset, R^2 = 0.85 (A) and R^2 = 0.77 (B).

Table 2. Regression parameters obtained between electrolyte leakage (mS g^{-1} DW) and germination percentage (A) or seed vigour (T_{50}) (B) of seeds from 9 *Diplotaxis tenuifolia* (DT) and 1 *D. erucoides* (DE) seed lots stored at 45 °C and 70% RH, and values of electrolyte leakage achieved when viability decreases to 80% germination (determined from Figure 4A).

Seed lot	Germination			Vigour (T_{50})			Electrolyte leakage at 80% germination (mS g^{-1} DW)
	slope	intercept	R^2	slope	intercept	R^2	
DT1	–36.52	145.93	0.88	1.60	–1.66	0.97	1.8
DT2	–44.59	179.15	0.89	3.81	–7.69	0.99	2.2
DT3	–37.06	142.41	0.94	2.33	–2.16	0.99	1.7
DT4	–38.87	132.91	0.94	2.69	–2.16	0.98	1.4
DT5	–36.47	145.01	0.84	1.90	–1.58	0.74	1.8
DT6	–32.80	133.87	0.80	1.94	–2.99	0.88	1.6
DT7	–37.35	139.92	0.92	1.15	–0.12	0.87	1.6
DT8	–31.59	141.30	0.93	2.45	–3.06	0.96	1.9
DT9	–31.25	127.13	0.90	1.86	–1.13	0.97	1.5
DE	–38.24	132.21	0.89	1.41	–0.72	1.00	1.4

bank should be regenerated or to indicate to farmers which seed lots should not be sown. Longevity parameters (P80, P50) were similar for all lots, with 4.1 and 7.5 days, respectively, being seed longevity in *Diplotaxis* above the average of that found in species in the family stored in similar conditions (Probert et al., 2009; Mira et al., 2014).

Electrolyte leakage was strongly related to loss of seed viability. Conductivity increased linearly with storage time as seeds lost viability, and a stabilisation of the conductivity values could be observed between 16 and 23 days of storage, coincident with the time by which seeds had lost most of their viability (Figure 3). Therefore, by the time most of the seeds within a sample had lost their ability to produce a radicle, electrolyte leakage had achieved its maximum level. The increase of electrolyte leakage from bulk samples of seed with increasing deterioration time was also detected in cultivated *Brassica* species (Fessel et al., 2005; Mirdad et al., 2006; Demir et al., 2008) and in wild Brassicaceae, in which small changes were found in measured conductivity after germination had decreased to 0% (Mira et al., 2011a). Furthermore, electrolyte loss was linearly related to both seed germination and vigour (Figure 4). As germination percentage decreased with ageing, electrolyte leakage increased linearly. Likewise, as seeds lost vigour—measured as germination speed—electrolyte leakage also increased linearly. A correlation between germination and conductivity had been previously reported for some cultivated (Elias and Copeland, 1997; Verma et al., 2001; Bedi et al., 2006; Mirdad et al., 2006; Demir et al., 2008; Matthews et al., 2009) and wild (Mira et al., 2011a) Brassica species. Seed vigour can be measured by a number of parameters; vigour tests based on germination behaviour are the most common ones (Hampton and TeKrony, 1995). In *Brassica oleracea* var. *capitata*, seed conductivity did not identify low vigour (measured as seeds being capable of germinating (Demir et al., 2008) or as field emergence (Matthews et al., 2009)), while there was a positive correlation when vigour was measured as seedling emergence (Matthews et al., 2009). Our results suggest that there is a high correlation between *Diplotaxis* seed vigour, measured as germination speed (T50), and conductivity measurements, as also found in other Brassicaceae (Mira et al., 2011a), when seeds are stored at high temperature and humidity.

No differences among species or lots were found regarding the correlation between electrolyte leakage and seed germination or vigour. There was a negative linear relationship between electrolyte leakage and seed germination (R^2 = 0.85), and a positive relationship with T_{50} (R^2 = 0.77) in both *D. tenuifolia* and *D. erucoides*. In other Brassicaceae, not only differences among species (Mira et al., 2011a) but also great differences among cultivars (Demir et al., 2008) were found, suggesting that predictive regression equations should be produced for individual genotypes. Our results indicate, however, that the same regression equation relating conductivity and germination (–33.97x + 135.59) predicts the viability of different seed lots of both studied species of *Diplotaxis*, *D. tenuifolia* and *D. erucoides*. Moreover, a conductivity level of 1.7 mS g^{-1} DW indicates when seed germination during storage has decreased to 80%, and is similar to the 2 mS g^{-1} DW value previously obtained for 4 different Brassicaceae species (Mira et al., 2011a).

In conclusion, the conductivity method is able to predict the viability of wall-rocket seeds stored at high humidity. The conductivity test has been validated as a vigour test for peas (ISTA, 2009). Several authors have reported the potential application of the conductivity test as a vigour test for cultivated species of *Brassica* (Fessel et al., 2005, Mirdad et al., 2006; Demir et al., 2008; Matthews et al., 2009), and previous results also suggest that it could be used to assess seed deterioration in Brassicaceae species of different genera, such as white mustard, watercress, and radish (Nery et al., 2009; Mira et al., 2011a). Commercial seeds are often kept under uncontrolled storage conditions from harvest to sale, and ambient conditions such as those studied here could be the result of bad storage during sale distribution among small-scale farmers and local seed markets. Therefore, the conductivity test could be used to evaluate the quality of wall-rocket seeds stored at these conditions until sale or sowing planning. Moreover, previous data suggest that germination of *D. erucoides* seeds may decline during storage due to the development of secondary dormancy, which would confound the viability results (Pérez García et al., 1995; Martínez Laborde et al., 2007). The conductivity test could allow the identification of a dormant (nongerminating) seed lot as viable. Our work presents evidence that points to the potential of a standardised conductivity test for wall-rocket seeds to detect poorly germinating and nonvigorous seed lots after storage at high temperature and humidity, i.e. a quick, inexpensive, and uncomplicated test to detect seed lots of with unacceptably low germination levels.

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