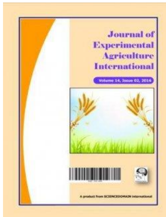

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Assessment of Seed Vigour Tests for Efficiently Determining the Physiological Potential of *Tetrapleura tetraptera* (Schum. & Thonn.)

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Authors' contributions

This work was carried out in collaboration between all authors. Author HSS designed the study, collected data, performed the statistical analysis and wrote the first draft of the manuscript. Authors JMA, EAG, JSA, JJT and FAKS supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Seed vigour tests were conducted to identify differences in physiological potential among seed lots of *Tetrapleura tetraptera* (Schum. & Thonn.) with the potential to perform well after sowing and/or during storage. The present study aimed at investigating rapid vigour testing methods for estimating the relative physiological potential of *T. tetraptera* (Schum. & Thonn.) to provide relevant information to guide its domestication and ex-situ conservation. Eight (8) seed lots of *T. tetraptera* were subjected to the accelerated ageing and the electrical conductivity tests to determine their physiological potential. For the accelerated ageing tests, the traditional accelerated ageing (TAA)

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and the salt-saturated accelerated ageing (SSAA) tests were performed. In the TAA tests, seed lots were exposed to 100% RH at 38 and 41°C for 48, 72 and 96 h, respectively. In the SSAA tests, seed lots were exposed to saturated NaCl solution with RH of 76% at temperatures and durations similar to the TAA tests. For the electrical conductivity tests, leachates conductivity of the 8 seed lots, were measured after 20 seeds of each lot had been placed in beakers and soaked in 50 ml of de-ionized water for 1,2,3,4,5,6, and 24 h at room temperature (24-28°C). Seed moisture content was determined and seed germination tests of seed lots were conducted before and after the accelerated ageing tests. Seedling root, shoot and total length as well as seedling dry weights were also recorded after accelerated ageing and germination of the seeds. The experimental design was 8 x 7 factorial (lots x soaking period) for the conductivity test and 8 x 2 x 3 factorial (lots x temperature x exposure time) for the accelerated ageing tests, in a completely randomized design. Results showed that seed emergence and seedling length were effective to distinguish the physiological quality of *T. tetraptera* seeds while seedling dry weights were not. Accelerated ageing test influenced the percentage of germination, but showed low sensitivity in lots differentiation. For the electrical conductivity test, 24 h was the most promising soaking period for effective stratification in determining the physiological quality of *T. tetraptera* seed lots and was significantly correlated with seed emergence (-0.76, $p < 0.01$) and seedling length ($r = -0.72$, $p < 0.01$). Electrical conductivity test may therefore be a more feasible option for vigour testing of *T. tetraptera* seeds.

Keywords: Seed quality testing; Tetrapleura tetraptera; vigour; seed storability; ex-situ conservation.

1. INTRODUCTION

The contributions of forests to peoples' welfare are particularly vast and far-reaching. Forests play an important role in reducing rural poverty, guaranteeing food security and supporting economic growth [1,2]. However, the global rate of forest deforestation is still high in many parts of the world particularly in the tropics [1]. The tropical rainforests are the most genetically diverse terrestrial ecosystems on earth, and stocked with many tree species that produce numerous edible, highly nutritive and medicinal fruits, seeds, leaves, twigs nuts and bark ([3], [4]). Negative human activity and change in climate have exposed the rainforests to an over-exploitation of their resources, leading to a progressive depletion of their genetic diversity.

Planting of indigenous trees is an effective rehabilitation method for degraded tropical rainforests because of their socio-economic importance [5,6]. Unfortunately, the use of highly valuable indigenous tree species in reforestation and conservation programmes is hampered by problems related to seed collection, handling and storage [7,8]. Besides, studies on tropical forest tree seeds, in general, remain more difficult compared to those on agricultural species and the gaps in our understanding of tropical seeds include seed testing [9,10].

Tetrapleura tetraptera (Schum and Thonn), commonly known as Aridan, is a highly valuable indigenous species from the Fabaceae family. It has a wide natural distribution across a large part of tropical Africa, especially in the rainforest belts of West, Central and East Africa [11]. Known as *Prɛkɛsɛ* in the Twi language of Ghana, *T. tetraptera* exhibits numerous nutritional and medicinal properties and has an undeniable socio-economic importance for the local communities. *T. tetraptera* pharmacological activities include cardio-vascular, neuromuscular, hypotensive, anti-convulsant, trypanocidal, hirudinicidal, schistomiasis control, anti-ulcerative, ectoxicity, anti-inflammatory, hypoglycaemic, anti-microbial, emulsifying property, birth control, and the control of intestinal parasites [11]. The compounds isolated from the leaves were found to exhibit strong molluscicidal properties [12]. The fruit possesses an insect repellent property due to its pungent aromatic odour and is used as spices and aroma (exotic tropical scents) [11]. The species is also valued in timber as fairly hard heartwood [13].

In spite of its importance, the natural population of *T. tetraptera* is declining at an alarming rate due to its over- exploitation [14]. This calls thus for effective strategies to sustainably manage and use this prestigious tree species. Meanwhile, like any other forest tree, *T. tetraptera* regeneration cycle is long and the natural germination of the seeds is still a challenge to its establishment as a result of seed dormancy effects [15]. *T. tetraptera* exhibits poor germination when freshly collected [14]. These intrinsic factors make the species not quite adequate for use in a good and rapid reforestation programme. There is, therefore, a need to establish sustained conservation measures and explore of seed testing methods on the species to facilitate its use in forest rehabilitation programmes.

Seed testing is the cornerstone of any seed programme. It is an analysis of physical parameters and the physiological quality of a seed lot and it is used for controlling quality parameters during seed handling [7]. Seed testing includes a number of parameters such as seed weight, purity, moisture content and germination. Although germination testing is the primary and worldwide accepted criterion for seed viability, its results alone could not provide sufficient information about the potential performance of seeds lot [16]. For more accurate information, seed quality is assessed using vigour tests which provide information on the potential behaviour of a seed lot under storage and field conditions [17]. Seed vigour is a concept describing several characteristics associated with seed performance [7]. However, previous studies on seed quality testing Other authors demonstrated that a single test may be less effective to efficiently assess the physiological quality of different seed lots ([18]; [19]). Several tests are then used to increase the accuracy of the resultant information. This research therefore aimed at investigating rapid vigour testing methods for efficiently estimating the physiological potential of *T. tetraptera*, and thereby helping to guide its domestication and *ex-situ* conservation.

2. MATERIALS AND METHODS

2.1 Experimental Laboratories

This study was conducted in the laboratory and plant house of the National Tree Seed Centre located at the CSIR-Forestry Research Institute of Ghana in Kumasi and the Soil Science Laboratory of the Faculty of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology, Kumasi.

2.2 Source of Experimental Materials

The experimental materials include eight seed lots of *T. tetraptera* obtained from the National Tree Seed Centre of the Forestry Research Institute of Ghana in October 2015. The seeds were cleaned, kept for a month at ambient temperature (24-28°C) and subsequently used for the accelerated ageing and electrical conductivity experiments.

2.3 Experimental Design

The experiment was conducted in a completely randomized design with the seed leachate electrical conductivity tests being an 8 x 7 factorial (lots x soaking period) and the accelerated ageing an 8 x 2 x 3 factorial (lots x temperature x exposure time).

2.4 Initial Characterisation of the Eight Seed Lots

Initial characterisation of the seed lots involved the assessment of the following parameters: Moisture content, 1000 seed weight (g), seed germination and seedling growth test.

2.4.1 Moisture test

Seed moisture content was determined before and after the ageing test to assess moisture variations using the constant temperature oven method at 103±2 °C for 17h±1 [20]. The test was carried out in duplicate samples of 5 grams each and the results expressed as a mean percentage (wet basis) using the formula: $MC = 100(M1 - M2)/M1$, where M1 is the initial weight of seed sample and M2 is the final weight of seed sample.

2.4.2 1000 Seed weight (g)

One thousand seed weight was determined by counting at random 8 replicates of 100 seeds. Each replicate was weighed and the weight recorded in grams. The mean weight of the 8 replicates was calculated, and multiplied by ten as per the procedure given under ISTA Rules [21].

2.4.3 Seed germination and seedling growth

Tetrapleura tetraptera seeds were mechanically scarified by rubbing seeds between two rough surfaces of sand paper for three minutes prior to sowing as described by [22]. Germination was assessed before and after the ageing test by sowing 100 seeds in four (4) replicates of 25 seeds each in plastic bowls filled with sterilised river sand in a greenhouse. Watering was done daily in the morning. Germination data were recorded at two days interval for four weeks period from the first day of germination. Seeds were counted as germinated when the plumule emerged from the seed coat and was free from visual fungal infection or deformation. Results were expressed as mean percentage of normal seedlings, for each lot using the formula:

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds in all replicates}} \times 100$$

Seedlings were allowed to grow for two weeks for the final germination scoring (six weeks from first germination count). Five normal seedlings were selected at random from each replicate and seedling root length and shoot length were measured using a ruler after which the seedlings fresh weights were taken while the mean seedling length (cm) was calculated. The five seedlings were placed in a paper envelope and put in the oven at 80°C for 24 h, after which they were weighed to determine mean seedling dry weight (g).

2.5 Electrical Conductivity Test (EC)

The EC test was carried out to determine the appropriate soaking period to estimate the physiological potential of the seed lots in comparison with ageing and germination results. Four replications of twenty seeds were taken from each sample and weighed in grams to two decimal places prior to testing. Each replicate was rinsed once with 20 ml of de-ionized water and placed in 150 ml beaker, with 50 ml of de-ionized water, and stirred. Two beakers with the same quantity of de-ionized water were set up as blank. All beakers were kept on a laboratory bench at room temperature (24-28°C) at which the cell was calibrated and covered to avoid pollution and evaporation of water. The samples were stirred and the specific electrical conductivity were measured and recorded after 1, 2, 3, 4, 5, 6 and 24 h. The conductivity dip cell was rinsed twice between each sample measurement. The conductivity of the samples in the control beakers (background reading) were measured and the mean value was subtracted from the readings of the seed samples [21]. Conductivity was calculated using the formula,

$$\text{Conductivity } (\mu\text{S cm}{-1}g{-1}) = \frac{\text{Conductivity reading} - \text{background reading}}{\text{Weight (g) of replicate}}$$

2.6 Accelerated Ageing Test

For this experiment, a pilot experiment was conducted on a single seed lot under four (4) temperature levels: 38°C, 41°C, 43°C and 45°C (data not shown). 38 and 41°C levels gave consistent results and were therefore selected and applied on the eight seed lots. For the ageing experiment, two different tests were conducted namely: the traditional accelerated ageing (ageing at 100% relative humidity (RH)) and salt-saturated accelerated aging (ageing at 76% relative humidity (RH)).

2.6.1 Traditional accelerated ageing (TAA)

For the accelerated ageing (AA) test, the aspect studied was the interaction between temperatures/exposure periods. Seeds were aged at 100% RH using distilled water at 38 and 41°C for 48, 72 and 96 h. Six (6) conditions of ageing differing in ageing temperature (38 and 41°C) and ageing periods (48h, 72h and 96h) were studied. The tests were conducted in clear plastic containers (boxes) having suspended wire mesh screen inside, in which 80 g of seeds were spread to form a single layer. The screens were positioned in the plastic containers 6 cm above the water level [23]. The boxes were covered with lids and the samples were placed in an incubator at the various temperatures. After ageing, seeds of each treatment were subjected to moisture and germination tests as described earlier.

2.6.2 Salt-saturated accelerated ageing (SSAA)

The salt-saturated accelerated ageing (SSAA) test was carried out in a similar way as described for the traditional test, except that it was done using the procedure proposed by Jianhua and McDonald [24], by replacing the water added to each individual compartment with the same amount of a saturated NaCl solution. This solution was obtained by dissolving 40 g of NaCl in 100 ml of water to establish an environment of 76% relative humidity.

2.7 Data Analysis

All data were subjected to analysis of variance (ANOVA) in Genstat (11th edition) Statistical Package. Tukey test was used to assess the difference in means within each column at 5% probability. Correlation analysis was done to assess the relationship between ageing procedure, seed germination characteristics; seedling vigour parameters and electrolyte leachate conductivity.

3. RESULTS AND DISCUSSION

3.1 Initial Characterisation of the Eight Seed Lots

The characterisation of the eight *T. tetraptera* seed lots, seed moisture content, 1000 seed weight, seed germination (emergence) percentage, seedling root and shoot length, seedling total length and seedling dry weight, is shown in Table 1. Significant differences ($p < 0.01$) were observed among means of the seed lots for all parameters assessed, except seedling root length and seedling dry weight. Seed moisture content ranged from 8.32 to 9.09% for the eight lots with an average of 8.66%. The moisture variation among the samples was 0.77%, which was less than 2%, and required limits to obtain consistent results for accelerated ageing tests [25]. One thousand seed weight ranged from 132.2 to 141.8 and seed lot 7 was the heaviest followed by lot 2 and the lowest value was recorded in lot 5. As emphasized by Elliott et al. [26], seed size and weight play major roles in the process of germination and are usually associated with seedling vigour. Based on the result of the present study, seed lot 7 could be considered as having the highest performance and lot 5 the lowest. However, only one test cannot identify all possible aspects of seed performance, since most accepted concepts of seed vigour suggest a "sum of all those properties" and not a unique characteristic [19].

Uniform seedling emergence and seedling growth (length or dry weight) are also important components of seed vigour [19]. Initial germination (emergence) percentages of the eight seed lots ranged from 49 to 65% with an average of 56.50%. The germination percentages recorded for all the eight lots of the species tested were relatively low (below 70%) even after some level of mechanical scarification was carried out on the seed lots. This may probably be due to the problem of dormancy in *T. tetraptera* seed as reported by [15] and [14]. Nevertheless, the results permitted the classification of the seed lots into different vigour levels, in which lots 7 and 8 were of superior performance, and lot 1 showed the poorest performance. Lots 2, 3, 4 and 6 were of intermediate quality (performance). Seed lots significantly differed ($p < 0.01$) for seedling shoot length and total seedling length means while no significant differences were observed for seedling root length and seedling dry weight. Seedling shoot length and total seedling length allowed thus a satisfactory differentiation among seed lots, in that seed lots 7 and 8 scored the best performance for these two parameters. The evaluation of seedling length and seedling emergence constitutes important vigour parameters and the fact that these tests usually do not require special equipment besides those used for germination makes them excellent options to evaluate seed vigour in species such as forest, ornamental, native and recalcitrant seeds [19,27] and [27].

In addition, significant and positive association was observed between seed emergence and seedling shoot length ($r = 0.70$), total seedling length ($r = 0.72$), seedling root length ($r = 0.48$), and seedling dry weight ($r = 0.44$) (Table 2). Seedling total length showed highly significant correlation with seedling shoot length ($r = 0.83$) and root length ($r = 0.81$). Seedling dry weight was significantly correlated with seedling root length ($r = 0.50$), but was not significantly correlated with seedling shoot length (0.39). It appears therefore from these results that, the germination (emergence) tests together with the early seedling growth parameters could be used in the classification of *Tetrapleura tetraptera* seed lots at different vigour levels.

3.2 Electrical Conductivity (EC) for *T. tetraptera* Seed Vigour Test

Table 3 shows changes that occurred in the leachates conductivity from the seed lots when they were soaked for different periods (h) of time. The results show significant differences in conductivity readings for soaking periods 1, 2 and 24 h ($p < 0.05$) but not for 3, 4, 5 and 6 h. This could be attributed to the ability of the seed coats of all the seed lots being able to reorganize and reinstate their original structures to prevent more leakages of solutes from them. The long period of soaking (24 h) may have disrupted the integrity of the seed coats from less vigour seed lots to allow further solute leakages. According to [28], water uptake by desiccation tolerant seeds reinstates their original structure of cellular membrane, whereas the membranes of desiccation-sensitive seeds and low vigour seeds that have been dehydrated are not able to reform completely allowing more solutes leakages. These results are divergent from those reported by Ramos et al. [29], where different soaking times were not associated with any significant differences in conductivity in *Kielmeyera coriacea* seeds.

Table 1. Characterisation of eight *Tetrapleura tetraptera* seed lots

Seed lot	MC%	1000SW (g)	Vigour indicators				
			SE%	RL(cm)	SL(cm)	TL(cm)	DW(g)
1	8.48 cd	135 bc[1]	50 b	6.15	12.66 c	19.17 c	0.156
2	8.55 cd	138.2 ab	58 ab	7.50	13.72 ab	21.21 a	0.188
3	8.47 cd	136.6 bc	54 ab	6.85	12.53 c	19.38 c	0.188
4	8.66 bc	135 bc	59 ab	7.60	13.11 c	20.71 ab	0.257
5	8.32 d	132.2 c	49 b	7.15	13.00 bc	20.15 abc	0.193
6	8.90 ab	134.7 bc	53 ab	6.26	13.20 abc	19.47 bc	0.178
7	8.76 bc	141.8 a	65 a	7.10	13.70 ab	20.80 a	0.208
8	9.09 a	137.6 ab	64 a	7.40	14.07 a	21.47 a	0.193
Mean	8.66	136.39	56.50	7.05	13.25	20.30	0.20
CV%	0.1	0.3	2.5	1.8	0.7	0.1	2.1
$Lsd_{(5\%)}$	0.18**	2.94**	7.99*	0.80 ns	0.54**	0.76**	0.04 ns

ns = not significant; (, **) = significant at 5% and 1%probability respectively; 1000SW = one thousand seed weight; CV (%): coefficient of variation; MC% = moisture content; SE = Seed emergence; RL (cm) = root length; SL (cm) = Shoot length; TL (cm) = seedling total length; DW (g) = seedling dry weight.*
[1]Mean comparisons within each column by Tukey test, 5%.

Table 2. Correlation analysis (r) between seed emergence and the other vigour indicators tested

Test	SE	SL	RL	TL
SL	0.70**			
RL	0.48*	0.35ns		
TL	0.72**	0.83**	0.81**	
DW	0.44*	0.15ns	0.50*	0.39ns

*SE= Seed emergence, SL= Shoot length, RL= Root length, TL= Total length, DW= Dry weight. ns= non-significant *, ** = significant difference at p < 0.05 and p < 0.01, respectively*

For the soaking periods of 1, 2, 3, 4 and 24 h, lot 7 showed the lowest conductivity reading (3.70 $\mu Scm^{-1}g^{-1}$; 3.70 $\mu Scm^{-1}g^{-1}$; 5.54 $\mu Scm^{-1}g^{-1}$; 5.54 $\mu Scm^{-1}g^{-1}$ and 11.1 $\mu Scm^{-1}g^{-1}$, respectively) followed by lot 8 (5.08 $\mu Scm^{-1}g^{-1}$; 5.08 $\mu Scm^{-1}g^{-1}$; 7.15 $\mu Scm^{-1}g^{-1}$; 7.15 $\mu Scm^{-1}g^{-1}$ and 12.5 μScm−1g^{-1}, respectively). Based on the principle that vigorous seeds release lower amounts of solutes when soaked for a specific time [23], the seed lots 7 and 8 can therefore be characterised as lots of superior quality. The soaking period of 24 h permitted the segregation of lots 1 (35.7 $\mu Scm^{-1}g^{-1}$, highest value) as inferior to the other ones, but 1,2,3 and 4 h of soaking did not allow such separation. In addition, 24 h of soaking permitted the classification of lots 2, 3, 4, 5 and 6 as of intermediate quality. The long period of 24 h soaking needed to show highly significant differences in conductivity of leachate from seed lots and segregate them into vigour levels may be as a result of the hard seed coat which made it difficult for water to penetrate into the seed to cause leakages of solutes from the soaked seed lot.

The correlation analysis (Table 4) between leachate conductivity and the other vigour indicators tested above showed a negative association of conductivity values with all the other vigour indicators (seed emergence, seedling root length, seedling shoot length, seedling total length and seedling dry weight). Soaking periods of 1, 2, 3, 4, 6 and 24 h showed highly significant negative correlation (-0.69; -0.69; -0.55; -0.60; -0.57; -0.76, respectively) with seed emergence while 5 h soaking was significantly correlated (-0.49). The 1 and 2 h of soaking were significantly correlated with seedling shoot length and seedling total length, but were no correlated with seedling root length and seedling dry weight. Soaking for 3, 4, 5 and 6 h were not significantly correlated with seedling root length, seedling shoot length, seedling total length and seedling dry weight. Concerning the soaking period of 24 h, the correlation analysis showed the highest coefficient (r = -0.76) with seed emergence. In addition, 24 h soaking was highly correlated with all the other vigour indicators: seedling root length (r = -0.58), seedling shoot length (r = -0.66), seedling total length (r = -0.75) and seedling dry weight (r = -0.53). Therefore, it could be inferred that for *T. tetraptera* seed, a 24 h soaking time may be used to effectively discriminate among seeds lots for their physiological potentials.

Table 3. Conductivity ranges ($\mu Scm^{-1}g^{-1}$) of the eight *Tetrapleura tetraptera* seed lots after soaking for different periods

Seed lot	Soaking periods						
	1h	2h	3h	4h	5h	6h	24h
1	7.52 a[1]	7.52 a	7.52 a	9.34 a	9.34	9.34	35.7 a
2	7.67 a	7.67 a	7.66 a	8.88 a	8.88	10.18	15.3 ab
3	7.71 a	7.71 a	7.70 a	7.70 a	7.70	11.56	27.0 ab
4	7.21 a	7.21 a	7.20 a	7.20 a	8.96	8.96	14.4 ab
5	7.88 a	7.88 a	9.93 a	9.93 a	13.88	13.87	30.0 ab
6	7.25 a	7.25 a	9.06 a	9.06 a	12.69	12.69	27.2 ab
7	3.70 b	3.70 b	5.54 a	5.54 a	7.40	7.40	11.1 b
8	5.08 ab	5.08 ab	7.15 a	7.15 a	7.15	7.15	12.5 ab
Mean	6.75	6.75	7.72	8.10	9.50	10.15	21.7
CV%	4.2	4.2	12.7	15.0	6.3	7.8	8.5
Lsd (5%)	1.99*	1.99*	3.68ns	3.78ns	4.60 ns	4.63 ns	13.74*

*ns = not significant, (**, *) = significant at 1% and 5% probability, respectively*
[1]Mean comparisons within each column by Tukey test, 5%

Table 4. Correlation analysis (r) of electrical conductivity tests and the other vigour indicators

Test	SE	SL	RL	TL	DW
1h soak	-0.69**	-0.51*	-0.27ns	-0.48*	-0.15ns
2h soak	-0.69**	-0.51*	-0.27ns	-0.48*	-0.15ns
3h soak	-0.55**	-0.24ns	-0.16ns	-0.24ns	-0.08ns
4h soak	-0.60**	-0.24ns	-0.24ns	-0.29ns	-0.22ns
5h soak	-0.49*	-0.22ns	-0.26ns	-0.29ns	-0.06ns
6h soak	-0.57**	-0.42ns	-0.26ns	-0.42ns	-0.12ns
24h soak	-0.76**	-0.66**	-0.58**	-0.75**	-0.53*

*SE= Seed emergence, SL= Shoot length, RL= Root length, TL= Total length, DW= Dry weight. ns= non-significant *, ** = significant difference at p < 0.05 and p < 0.01, respectively*

3.3 Accelerated Ageing for *Tetrapleura tetraptera* Seed Vigour Test

In this study, the initial seed moisture content was almost identical. This fact is important in order to carry out the tests, since, within certain limits, seeds with a higher moisture content are more affected by the accelerated ageing conditions [25].

In general, germination of seeds after being exposed to accelerated ageing conditions is used in the classification of seed lots into vigour levels [23]. Tables 5 and 6 show the separation of seed lots into different vigour levels based on the traditional and salt-saturated accelerated ageing tests, respectively. It appears that none of the ageing conditions tested in this study showed a classification similar to those of seed emergence, electrical conductivity tests or any of the other vigour indicators. However, in the traditional method seeds lot 1 had a lower physiological potential.

The correlation analysis between the results obtained from the traditional ageing test and the others vigour tests conducted in this study showed no significant relationship (Table 7). A significant positive correlation was found between 38°C/96 h (traditional method) and seedling total length (r = 0.50), 41°C/72 h (traditional method) and seedling dry weight (r = 0.59) and 41°C/96 h (traditional method) and seedling dry weight (r = 0.55). However, no significant relationship was found between the ageing conditions, seedling root length and shoot length. The salt-saturated accelerated ageing conditions showed a negative correlation with seed emergence but only 41°C/96 h was significant (r = -0.52) (Table 8).

From the result of the traditional accelerated ageing test, the inferiority of seed lot 1 is evident as detected from the seed emergence, seedling vigour and conductivity test. However, the traditional accelerated conditions tested in this trial showed low sensitivity for differentiation of the other seed lots into vigour levels. In the same way, the salt-saturated method showed no sensitivity for differentiation of the other seed lots into vigour levels. This may explain the absence of linear relationship observed between the results of the accelerated ageing test and those of vigour test assessed in this experiment. So, the accelerated ageing conditions used in this study were not sensitive for differentiation of *T. tetraptera* seed lots into different vigour levels. Seed dormancy in *T. tetraptera* might have influenced the results of the tests. According to Bonner (1998), seed dormancy has an influence on the interpretation of accelerated ageing results. In the current study seeds of *T. tetraptera* were rubbed with sandpaper as a form of mechanical scarification to break the hard and impermeable seed coat prior to germination test.

Table 5. Classification of seed lots using the traditional accelerated ageing method

Seed lot	38°C			41°C		
	48 h	72 h	96 h	48 h	72 h	96 h
	Germination (%)					
1	48 ab	32 b	18 d	10 b	18c	12 d
2	24 c	42 ab	41 ab	21 ab	21 bc	17 cd
3	31 c	42 ab	32 c	53 a	36 b	22 bc
4	32 c	42 ab	36 bc	46 a	56 a	34 a
5	32 c	50 a	45 a	38 ab	55 a	33 a
6	38 cb	44 ab	37 bc	42 ab	34 bc	24 bc
7	32 c	50 a	41 ab	32 ab	28 bc	16 cd
8	59 a	40 ab	34 bc	32 ab	36 b	29 ab

Mean comparisons within each column by Tukey test, 5%

Table 6. Classification of seed lots using the salt-saturated method

Seed lot	38°C			41°C		
	48 h	72 h	96 h	48 h	72 h	96 h
	Germination (%)					
1	22.5 b	30b	26 cd	32 b	39 abc	42 b
2	38 ab	14d	10 e	24 b	48 ab	40 b
3	40 a	28bc	17 de	26 b	32 bc	46 ab
4	32 ab	32ab	24 cd	30 b	30 c	52 ab
5	34 ab	38a	38 ab	54 a	50 a	60 a
6	34 ab	28bc	30 bc	26 b	48 ab	58 a
7	23 b	28bc	42 a	26 b	49 ab	38 b
8	26 ab	36a	30 bc	32 b	26 c	48 ab

Mean comparisons within each column by Tukey test, 5%.

Table 7. Correlation analysis (r) of electrical conductivity, traditional accelerated ageing test and five other vigour indicators

TEST	SE	SL	RL	TL	DW	EC (24 h soak)
TAA38/48	0.09ns	0.20ns	-0.12ns	0.05ns	-0.33ns	0.06ns
TAA38/72	0.24ns	0.20ns	0.22ns	0.26ns	0.41ns	-0.23ns
TAA38/96	0.27ns	0.43ns	0.38ns	0.50*	0.42ns	-0.44ns
TAA41/48	0.04ns	-0.14ns	0.13ns	-0.02ns	0.31ns	-0.08ns
TAA41/72	-0.08ns	-0.12ns	0.28ns	0.09ns	0.59**	-0.11ns
TAA41/96	0.02ns	0.07ns	0.42ns	0.29ns	0.55**	-0.11ns

*SE= Seed emergence; SL= Shoot length; RL= Root length; TL= Total length; DW= Dry weight; TAA= Traditional Accelerated Ageing; ns= non-significant; *, ** = significant difference at $p < 0.05$ and $p < 0.01$, respectively*

Table 8. Correlation analysis (r) of electrical conductivity, salt-saturated accelerated ageing test and the other vigour indicators

TEST	SE	SL	RL	TL	DW	EC (24 h soak)
SSAA38/48	-0.29ns	-0.22ns	-0.03ns	-0.16ns	0.04ns	0.05ns
SSAA38/72	-0.10ns	-0.12ns	-0.07ns	-0.12ns	0.07ns	0.20ns
SSAA38/96	0.11ns	0.17ns	-0.17ns	0.01ns	0.09ns	0.02ns
SSAA41/48	-0.37ns	-0.17ns	0.08ns	-0.06ns	-0.04ns	0.30ns
SSAA41/72	-0.28ns	0.06ns	-0.21ns	-0.09ns	-0.32ns	0.20ns
SSAA41/96	-0.52*	-0.22ns	-0.10ns	-0.20ns	0.08ns	0.28ns

*SE= Seed emergence; SL= Shoot length; RL= Root length; TL= Total length; DW= Dry weight; SSAA= Salt-Saturated Accelerated Ageing; ns= non-significant; *, ** = significant difference at $p < 0.05$ and $p < 0.01$, respectively.*

4. CONCLUSION

This study has shown that seed emergence as well as early growth of seedlings are good vigour indicators for *Tetrapleura tetraptera* seeds as the results showed high significant, positive correlations. However, they are time consuming methods. This study evidenced that Electrical conductivity test has the advantages of providing rapid and reliable results and the technique is not destructive. Besides, it was observed that different soaking times had significant effects on the leachates conductivity of *T. tetraptera* seeds with 24 h being the most efficient soaking period to discriminate seed lot for seed quality. Electrical conductivity test, therefore, proved to be a feasible option for vigour testing of *T. tetraptera* seeds, as the results obtained were consistent and highly correlated with seed emergence and seedling growth.

With regard to the accelerated ageing test, the results showed a significant effect of the ageing conditions tested on seed proximate composition, germination and seedling vigour of *T. tetraptera* seeds. However, despite the traditional method pointing out the lot with the lowest physiological potential, none of the conditions tested was sensitive enough to classify the seed lots into different vigour levels.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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