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Nugraheni · Pujiasmanto B · Samanhudi · Amalia TS

Comparison between the electrical conductivity method and radicle emergence test as a rapid test of sorghum seed vigor

Abstract. Sorghum belongs to multipurpose crops. All parts of sorghum can be used both as main products and derivative products; some derivative products produced from the sorghum plant consist of sugar, bioethanol, biomass, handicraft raw materials and starch. This study aims to determine the time required for testing the vigor and viability of sorghum seeds using the electrical conductivity and radicle emergence methods. The study was conducted in two experimental stages using a completely randomized design. The first experiment consisted of two factors: the number of sorghum seeds (40, 70 and 100 grains) and the volume of soaking water (75, 100, 125, and 150 ml). The second experiment consisted of two factors: research method (germination/control method, electrical conduction method, and radicle emergence method) and varieties (consisted of Numbu, Kawali, Suri 3, and Suri 4). The first experiment's results showed that the best electrical conductivity method on Suri 4 varieties were 40 seeds and 150 ml water volume. The electrical conductivity value is negatively correlated with Germination capacity, vigor index, growth of speed, maximum growth potential, and sprout growth rate. Time needed for Electrical Conductivity method in this study was three days. The germination period of 96 hours gives the best results on the radicle emergence of sorghum seed varieties. Vigor index, growth of speed, germination capacity, and maximum growth potential are positively correlated with the value of radicula emergence.

Keywords : Electrical conductivity · Germination · Radicle emergence · Viability · Vigor

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Nugraheni[1]* · Pujiasmanto B[1] · Samanhudi[1,2] · Amalia TS[1]
[1] Master Program of Agronomy, Faculty of Agriculture, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia
[2] Center for Research and Development of Biotechnology and Biodiversity, Universitas Sebelas Maret (UNS), Jl. Ir. Sutami 36A, Surakarta 57126, Indonesia
*Correspondence: heni0336@gmail.com

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Introduction

Sorghum contains various vitamins, including 0.09 mg of vitamin B1, 0.14 mg of vitamin B2, vitamin B3 as much as 2.8 mg and minerals such as iron 4.4 mg, sodium 7 mg, potassium 249 mg, calcium 28 mg, phosphorus 287 mg and contains relatively high carbohydrates 73.10-79.12% and Protein of 7.98-11.77% (Widowati and Luna, 2022).

Sorghum belongs to multipurpose crops. All parts of sorghum can be used both as main products and derivative products. The main products of the sorghum plant are seeds, leaves and stems, while some derivative products produced from the sorghum plant consist of sugar, bioethanol, biomass, handicraft raw materials and starch. (Subagio and Suryawati, 2013).

Seeds are one of the most important production factors in agricultural cultivation because they can affect the level of production achieved so that quality seeds are needed in the cultivation of sorghum plants. Quality seeds guarantee physical, genetic, and physiological qualities (Ilyas, 2012). The estimation of the physiological quality of a seed is the high and low viability of seeds which is reflected in the germination value, growing speed, and cohesiveness of growth (Widajati et al., 2013).

One of the influencing limiting factors development and production sorghum in Indonesia is the provision of quality sorghum seed . The providing of quality sorghum seeds is influenced by seed storage. Duration of storage and room temperature are the main factors that cause seed deterioration and seed vigor (Pramono et al., 2019).

Sorghum seeds have a fairly high carbohydrate and protein content, which causes the rapid decline of sorghum quality (Susilowati and Saliem, 2013). The deterioration of sorghum seeds during storage signals that the vigor of the seeds is low.

Deterioration is a life process leading to the deterioration of seeds even irreversible death. Seed deterioration can be viewed biochemically and physiologically. Biochemical indications in deteriorated seeds can be characterized by the occurrence of a decrease in enzyme activity, decrease in feeding reserves and increase electrical conductivity value. While physiological indications can be seen from the presence of changes in seed color, delayed seed germination, decreased growth rate of sprouts, reduced germination, as well as increased abnormal sprouting (Hartati, 2019).

Germination testing for sorghum seeds is 10 days. This test is considered inefficient for quick and continuous provision of sorghum seeds, so it is necessary to increase the efficiency of the seed vigor test in order to shorten the testing time through a fast, cheap and applicable method. Innovation in the development of seed quality testing methods needs to continue to be carried out to obtain test results in a short time/ efficiently (Suhartanto, 2021).

Vigor testing of seeds that have been validated by International Seed Testing Association (ISTA) to obtain results in a relatively short time is radicle emergence method and electrical conductivity method. The principle of the electrical conductivity method is a physical seed test that reflects the leakage rate of the cell membrane (ISTA, 2021). The principle of the radicle emergence method is when the radicles have appeared at least 2 mm long (Astuti et al., 2020). The Radicle emergence method is characterized by the rate of radicle emergence at the beginning of germination, which is an indication of the vigor of a seed (Yukti et al., 2018). Radicle emergence (RE) is a rapid vigor test recommended by International Seed Testing Association (ISTA) in 2021 for corn seeds at 20 $\pm$ 1^0C for 66 hours $\pm$ 15 minutes or at a temperature of 13 $\pm$ 1^0 C after 144 hours $\pm$ 1 hours, radish seed (*Raphanus sativus*) at 20 $\pm$ 1^0C for 48 hours $\pm$ 15 minutes, *Brassica napus* (oil seed rape, *Argentine canola*) at 20 $\pm$ 1^0C temperature for 30 hours $\pm$ 15 minutes and *Triticum aestivum L. Subsp aestivum* at 15 $\pm$ 1^0C temperature for 48 hours $\pm$ 15 minutes (ISTA, 2021).

Vigor testing is accepted as an official method in ISTA regulation of 2011 is conductivity testing for *Pisum sativum, Phaseolus vulgaris* and *Glycine max* (ISTA, 2011). Conductivity test can predict the field emergence and standard germination. Electrical conductivity test has been proved as indicator of seed vigor in wide range of crop species and is related to field emergence and stand establishment (Fatonah et al., 2017). Radicle emergence test is considered as a quick test to predict varying vigor level and field performance of seed lots than the standard germination test in several crops (Matthews and Powell, 2011).

The purpose of the study was to obtain the fastest level of time efficiency in Radicle emergence method compared to germination

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method and electrical conductivity method in vigor testing and viability of sorghum seeds.

Materials and Methods

Research Materials and Tools. This research was carried out in June – October 2022 at the Laboratory of the Center for Supervision and Certification of Horticultural Food Plant Seeds in Central Java Province. In addition, potassium, magnesium and sodium leakage was conducted at Testing Laboratory of the Department of Agricultural Industrial Technology, Bogor Agriculture University. The materials used include sorghum seeds four varieties, aquadest, frosted paper, aluminum foil, label paper, tissue, plastic, cow manure, soil, polybags, ultraviolet plastic thickness 200 microns, and bamboo.

The tools used in the study consisted of glass jar cups, Eutech Instrument Conductivity meter Con 110, measuring cups, plastic tubs, germinators temperature 25 $\pm$ 2^0C, divider, analytical scales, grinders, ovens, desicators, alumunium dishes, tweezers and rulers.

Methods. The study was conducted in two experimental stages consist of:

a. Experiment I aim to determine the right combination of treatment for the number of seeds and the volume of soaking water in the electrical conductivity test of sorghum seeds which is arranged in completely randomized design consisting of 2 factors repeated 4 times. The first factor is the number of sorghum seeds consisting of 3 levels (40, 70 and 100 seeds), the second factor is the volume of soaking water consisting of 4 levels (75, 100, 125 and 150 ml of aquadest) so that 12 treatments combinations are obtained.
b. Experiment II, i.e., seed vigor test, was carried out using the completely randomized design which was factorially arranged with 2 factors and repeated 4 times. First factor was test method consisting of three levels (seed germination method (Control/M_0), electrical conductivity method (M_1) and radicle emergence method (M_2), the second factor is sorghum seed varieties consisting of 4 levels (V_1 : Numbu, V_2 : Kawali, V_3 : Suri 3 and V_4 : Suri 4) repeated 4 times, resulting in 48 treatment combinations. Vigor and viability testing of sorghum seeds using test media between paper rolls as many as 50 sorghum seeds were repeated 8 times with 3 sheets of planting media, germinated at a standard temperature germinator of 25 $\pm$ 2^0C (Pujiasmanto, 2017).

Observational modifiers, including:

a) Vigor index (VI), percentage between the number of normal seedling at first count (four days after seedling) and the total number of seeds planted
b) Growth speed (GS), the calculation of the percentage number of normal sprouts on the first day until the last observation of testing
c) Electrical conductivity test (EC).

EC value is calculated using the following formula:

$$EC = \frac{(EC\ seed - EC\ blank\ value)}{(Weight\ (gram))}$$

d) Leakage analysis, this analysis is performed by soaking 50 sorghum seed in 250 ml of aquadest for 24 hours; further, leakage of ion potassium, magnesium and sodium ions present in water are measured using tool named *DHL Horiba.*
e) Radicle emergence test, the calculation of the radicle emergence test is carried out every 24 hours, then the percentage of the number of seeds that have appeared radicules with the criterion of the minimum length of the radicle that grows is 2 mm.
f) Mean germination time (MGT)
MGT testing is carried out to determine the average germination time of seeds starting from imbibition to the emergence of radicles of at least 2 mm (Matthews and Khajeh, 2006). MGT testing is performed to determine the average germination time of seeds. The average germination time is calculated by the formula:

$$MGT = \frac{(\Sigma\ (nx\ t))}{total\ germinated\ seeds}$$

Note :
n = the number of germinated seeds at time t
t = hour after germination
(Astuti et al., 2020)

g) Germination capacity, the ability of seeds to germinate normally at the first count (4 days after planting) and the final observation of testing (10 days after planting) divided by the total number of seeds planted
h) Maximum growth potential, the overall percentage of sprouts that grew both normal and abnormal sprouts until the end of the observation.

i) Sprout growth rate, the dry weight of seedling divided by the number of normal seedling in the final calculation of germination observation.

Data Analysis. The data obtained were analyzed using analysis of variance (α = 5%) and continued with Duncan Multiple Range Test (α = 5%). Coefficient correlation is performed to calculate the closeness of the relationship between EC and RE values with various observed vigor test benchmarks.

Results and Discussion

Based on Figure 1 of the electric conductivity test results showed that the treatment 40 seed, 75 ml aquadest in Kawali variety has the highest electrical conductivity value of 58.1 µS/cm.g, while the lowest electrical conductivity value was obtained from the treatment 40 seed, 150 ml aquadest in Suri 4 variety was 11.4 µS/cm.g.

The electrical conductivity is a vigor test whose principally based on integrity of cell membranes. Electrical conductivity done by measuring electrolyte leaking from seed tissue is dissolved into seed soaking water due to leakage of cell membranes (ISTA, 2014). Low seed vigor will indicate a high electrical conductivity value, otherwise high seed vigor will indicate the membrane leakage value or electrical conductivity value was low (Widajati et al., 2013).In Figure 2 showed that the Kawali variety has the highest Potassium (K) value 3.31 mg/L, and Sodium (Na) ion leakage value 0.303 compared to other varieties, and the Suri 4 variety in a addition to having the lowest electrical conductivity value in figure 1, also has the lowest ion membrane leakage ions compared to other sorghum varieties. According Andini et al. (2021) damage to cell membrane in seed result in leakage of sugars and electrolytes which has an impact on decreasing metabolic and transportation efficiency. The higher the electrical conductivity value, the higher the membrane leakage. Changes in membrane integrity are early symptoms of the seed deterioration process resulting in the release of compounds from the seed which are observed based on electrical conductivity and concentration of metabolite compounds (sugars, amino acids, fatty acids, enzymes, inorganic ions such as K+, Na^+, Ca^{2+} and Mg^{2+} (Vieira et al., 2008).

Based on Table 1 it showed that Suri 4 variety sorghum seeds have significantly different effects on vigor parameters and seed viability but have average electrical conductivity value did not significantly different in other variety. Khairani et al. (2022) reported that a high indicates that seed vigor is low; this is the opposite way around that a low electrical conductivity value has a high vigor and viability value. This is indicated by the high germination value. Seeds germinate faster, classified in seeds with strong vigor. Growing speed testing is one of the seed vigor tests. Seeds with high vigor are able to grow faster than seeds that are less vigorous (Widajati et al., 2013).

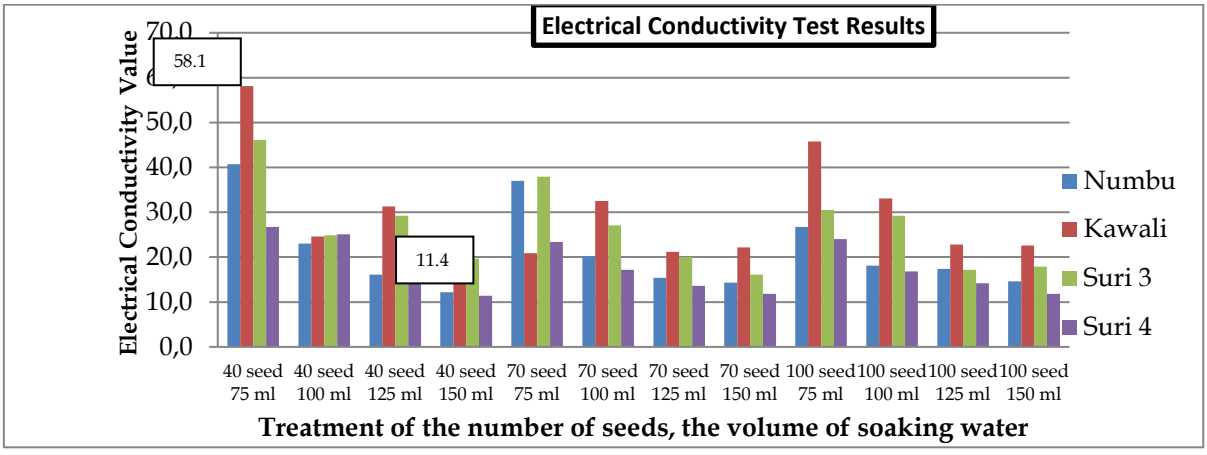

Figure 1. Electrical Conductivity Test Results Consisting of Twelve Treatment Combinations of Number of Seeds and Volume of Soaking Water

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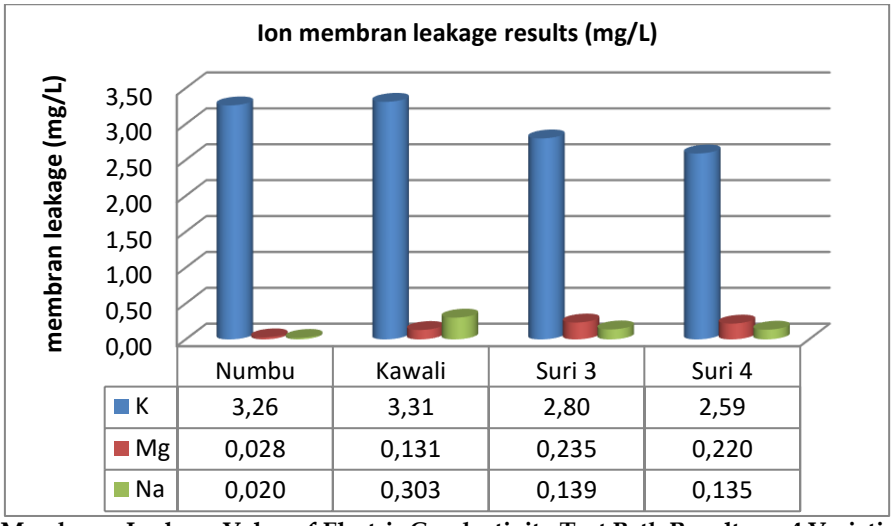

Figure 2. Ion Membrane Leakage Value of Electric Conductivity Test Bath Results on 4 Varieties of Sorghum Seeds

Source: Analysis of the Testing Laboratory of the Department of Agricultural Industrial Technology IPB

Table 1. Value of Electrical Conductivity, Germination Capacity, Vigor Index, Growth of Speed, Maximum Growth Potential and Sprout Growth Rate of 4 sorghum varieties.

Varities	Average					
	Germination Power (%)	Vigor Index (%)	Growth of Speed (% Normal Seedling/etmal)	Electrical Conductivity (µS/cm.g)	Maximum Growth Potential (%)	Sprout Growth Rate (mg/Normal Seedling)
Numbu	84.50b	25.75a	19.02a	21.30 a	89.25b	11.58b
Kawali	82.75b	20.50a	17.26a	29.40 a	90.75b	9.8a
Suri 3	76.00a	30.0ab	17.43a	26.37 a	82.75a	9.24a
Suri 4	91.75c	39.25b	21.63b	17.65 a	97.25c	11.72b

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

Table 2. The Results of the Correlation Analysis between Electrical Conductivity EC) with Germination Power (GP), Vigor Index (VI), Growth of Speed (GS), Maximum Growth Potential (MGP) and Sprout Growth Rate (SGR) and Growth Test (GT).

	GP	VI	GS	MGP	SGR	GT	EC
GP	1						
VI	0.353	1					
GS	0.678**	0.755**					
MGP	0.880**	0.262	0.557*	1			
SGR	0.621*	0.495	0.635**	0.444	1		
GT	ns	ns	Ns	ns	ns	ns	
EC	-0.179	-0.360	-0.530*	-0.108	-0.397	ns	1

Description: r = correlation coefficient, * = real effect on 5% test, **= very real effect on 1% test, ns = not real significant 5% level

The correlation coefficient (r) of each test in Table 2 is obtained from the results of the correlation test between the value of electrical conductivity to vigor modifiers and seed viability and gives negative or negatively correlated results. The value of Electrical Conductivity correlates very low with the germination power variable with the value of the correlation coefficient (r) -0.179, low correlated with the vigor index variable with the value of the correlation coefficient (r) -0.360, correlated quite strongly with the variable growth speed with the value of

the correlation coefficient (r) -0.530, very low correlated with the maximum growth potential variable with the value of the correlation coefficient (r) -0.108 and low correlated with the variable sprout growth rate with the value of The correlation coefficient (r) is -0.397. The negative correlation value is shown between the value of electrical conductivity with vigor modifiers and seed viability. In accordance with the opinion Chhetri (2009) that seeds with low germination and vigor provide high electrolyte leakage, in contrast to high-vigor seeds with low electrolyte leakage. The correlation relationship between the electrical conductivity test and the germination value, growth power, vigor index and tetrazolium of wheat seeds in the study Murwantini (2013) provides negative correlation results which show that the higher the value of electrical conductivity, the lower the value of germination power, growing power, vigor index and tetrazolium.

Based on Table 3 and Table 4 shows that the treatment of the Radicle emergence method has a significantly different influence on the germination method (control) and the electric conductivity method. The radicle emergence ($M_{2)}$ method gives the highest vigor index result of 45.06%, germination method 34.75% and electric conductivity method 28.88%. High radicle emergence calculations at the beginning of germination indicate high seed vigor, while low radicle emergence indicates low seed vigor. The radicle emergence test time is faster than other vigor methods because the calculation of radicle emergence is done earlier, which is when the radicles have appeared at least 2 mm long. Calculation of high radicle emergence indicated high seed Vigor (ISTA, 2014). The results of Table 4 showed that Vigor Index of Suri 4 varieties have a significantly different influence on Suri 3, Kawali and Numbu varieties. The vigor index value in the Suri 4 variety is 47.17%. According to Dwipa and Saswita (2017) the initial growth of seeds is more influenced by the genetic ability of seeds, so vigor and viability are strongly influenced by genetic factors. The Suri 4 variety is able to germinate faster compared to other varieties. A high vigor index value indicates that the seeds germinate faster, so the Suri 4 variety is classified under strong vigor.

Table 3. Mean of Vigor Index Values on different test method treatments.

Test Method	Vigor Index
Germination Method (M_0)	34.75 a
Electrical Conductivity Method (M_1)	28.88 a
Radicle Emergence (M_2)	45.06 c

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

Table 4. Mean of Vigor Index Value in different varieties.

Varieties	Vigor Index
Numbu (V_1)	29.67 a
Kawali (V_2)	31.25 ab
Suri 3 (V_3)	36.83 b
Suri 4 (V_4)	47.17 c

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

Table 5. Mean of Radicle Emergence (RE) at each observation period.

Varieties	Mean of radicle emergence (%)				
	24 hours	48 hours	72 hours	96 hours	120 hours
Numbu	66.75 a	82.25 a	82.75 a	86.00 a	88.00 a
Kawali	78.25 b	89.50 b	92.75 b	95.00 b	95.00 b
Suri 3	65.00 a	80.00 a	80.50 a	83.50 a	83.50 a
Suri 4	81.25 b	92.25 b	92.50 b	95.25 b	95.25 b

Note : Numbers followed by the same lowercase alphabet in the same column in not significantly different based on Duncan's Multiple Range Test at the level of 5%

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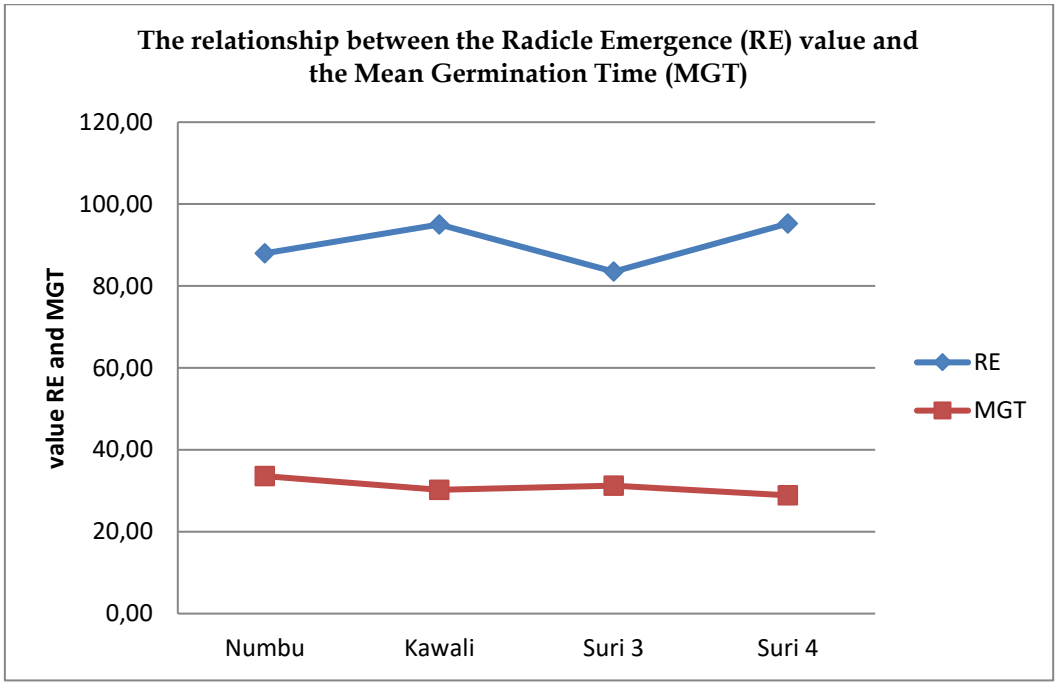

Figure 4. The Relationship between the Radicle Emergence and Mean of Germination Time

The mean germination time is the time needed to germinate from imbibition to the emergence of radicles at least 2 mm long. Roots with a length of ≥ 2 mm can already be called germinated (Luo et al., 2015). Observations of radicle emergence (RE) were carried out at a temperature of 25 ± 2^0C with germination times ranging from 24 hours to 120 hours. The determination of the observation time was carried out to see the difference in radicle emergence values for each observation period in each sorghum variety. Based on Table 3 shows that the most radicles appear in the first 24 hours followed by the next 48 hours. Determination of the observation time of radicle emergence can be done by correlating the radicle emergence in each observation period with the mean of germination time. According to research Noeryanti et al., (2022) the mean of germination time describes the average time for seeds to germinate from the beginning of imbibition to the emergence of radicules. The lower the average value of germination time, the faster the seeds germinate.

Table 6 shows that using the Radicle Emergence method the Suri 4 (M_2V_4) variety provides the highest percentage of growth in the variables of growing speed, germination capacity and maximum growth potential with a growth speed value of 26.32% Normal Seedling/ etmal, germination value: 97.50%, maximum growth potential: 98.50%. According to Kartasapoetra (2003) there is a close relationship between the speed of growing and the vigor of seeds. Seeds that have a high growth rate will be more resistant to conditions or environments that are not suitable (suboptimum). Measurement of growing speed is carried out by summing the normal growth of sprouts daily or ethmal during the germination period. The value of growing speed indicates the vigor of seeds under sub-optimal environmental conditions, assuming fast-growing seeds are able to overcome sub-optimal conditions (Sadjad, 1993). The difference in germination value of each variety is strongly influenced by genetic factors of each sorghum variety, this as stated by Justice and Bass (1990) interspecies variation affects the shelf life of seeds. Calculation of the appearance of high radicles indicates high seed vigor. Some of the advantages of the Radicle Emergence method are that the testing method is fast, the procedure is simple and can predict seed vigor and obtain preliminary information in predicting normal germination of seeds compared to the germination method (Matthews and Powell, 2011). Seed lots that need a longer time between imbibition and Radicle emergende, as seen in low vigor lots, are necessary to allow for metabolic repair. In high quality seeds, Radicle emergence earlier since there is much less ageing induced

damage need DNA repair (Demir et al., 2022). The Suri 4 variety is classified as a seed that has high vigor and viability. This is in accordance with the opinion Ilyas (2012) seeds that have high viability are characterized by maximum growing potential, high germination and dry weight, while seeds with high vigor are characterized by high growing speed and high growing ability in a suboptimal environment. The maximum growth potential is one of the determinants of seed viability which is greatly influenced by seed quality and maximum seed growth limits. Seed quality is largely determined by environmental and genetic factors (Fitriani et al., 2021). Maximum Growth Potential is the percentage of all seeds that live or show symptoms of life both producing normal and abnormal sprouts, namely the potential for a seed lot where seeds can have maximum germination (Hartati, 2019). The sprout growth rate is the dry weight of the sprout divided by the number of normal sprouts until the end of the observation. Control treatment (germination method), Suri 3 variety gave the highest sprout growth rate yield of 15.48% compared to other treatments. Normal sprout dry weight is an indicator of seed vigor, a high normal sprout dry weight value indicates a high vigor value.

The highest normal sprout value, which is an indicator that affects the total dry weight of sprouts. This is in consistent with the opinion Dehnavi et al. (2020) that differences in germination parameters in sorghum seeds are influenced by genetic variation between sorghum genotypes.

Table 6. Mean of interaction of test method and sorghum seed varieties on Growing Speed, Germination, Maximum Growth Potential, and Sprout Growth Rate of 4 sorghum varieties.

Treatment	Mean			
	Growth of Speed (%Normal Seedling/etmal)	Germination Capacity (%)	Maximum Growth Potential (%)	Sprout Growth Rate (mg/ Normal Seedling)
M_0V_1	15.38 a	80.00 abc	90.25 cde	12.63 de
M_0V_2	19.60 cd	84.75 bcd	87.25 bc	10.60 bc
M_0V_3	16.44 ab	74.75 a	77.00 a	15.48 g
M_0V_4	22.77 e	95.00 fg	96.25 efg	14.32 fg
M_1V_1	18.18 bc	84.50 bcd	89.25 cd	11.58 cd
M_1V_2	17.26 abc	82.75 bcd	90.75 cde	9.8 ab
M_1V_3	17.43 abc	76.00 a	82.75 b	9.24 a
M_1V_4	21.63 de	91.75 efg	97.25 fg	11.72 cd
M_2V_1	18.71 bc	78.75 ab	91.75 cdef	13.68 ef
M_2V_2	19.78 cd	88.50 def	95,25 defg	10.84 bc
M_2V_3	21.27 de	86.75 cde	91.25 cdef	13.43 ef
M_2V_4	26.32 f	97.50 g	98.50 g	14.59 fg

Note: M_0: Control (Germination Method), EC : Electrical Conductivity Method and RE : Radicle Emergence Method. V1 : Numbu, V2 : Kawali, V3 : Suri 3 dan V4 : Suri 4. Numbers followed by the same letters in the same column are not significantly different based on the 5% DMRT test

Table 7. Results of correlation analysis between Radicle Emergence (RE) value with variables VI, GS, G, MGP, SGR and MGT.

	RE	VI	GS	GC	MGP	SGR	MGT
RE	1						
VI	0,505*	1					
GS	0,439	0,878**	1				
GP	0,336	0,548*	0,831**	1			
MGP	0,361	0,336	0,599*	0,824**	1		
SGR	-0,062	0,646**	0,625**	0,230	0,70	1	
MGT	-0,202	-0,188	-0,476	-0,726**	-0,519*	0,068	1

Description: r = correlation coefficient, *= real effect on 5% test, **= very real effect on 1% test, tn = no real at 5% level, VI = vigor index, GS= growth speed, MGT (mean germination time), GC= germination capacity, MGP= maximum growth potential and SGR = sprout growth rate

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Based on Table 7 shows that the variables of vigor index, growth of speed, germination power and maximum growth potential have a positive correlation with the value of radicle emergence, while the variables of mean germination time and sprout growth rate show a negative correlation with the value of radicle emergence. According Kusumawardana (2019) the highest of radicle emergence had positive correlation with germination, vigor index, speed of growth, and field emergence. Research by Noeryanti et al (2022) reported that radicle emergence test had a high positive correlation with seed viability test for germination, seed vigor index, growth of speed, dry weight of germination normal and germination growth rate, and also give high negative correlation with mean germination time.

Conclusion

a. The time needed to determine the value of electrical conductivity is three days. The value of electrical conductivity is negatively correlated with vigor and viability parameters. The higher the germination power, vigor index, growth of speed, maximum growth potential and sprout growth rate, the lower the Electrical conductivity value
b. The observation time for radicle emergence (RE) testing is four days. The most radicles emergence in the first 24 hours, followed by the next 48 hours until the end of observation, which is 96 hours (4 days). The Suri 4 variety has the smallest average germination time of 28.87 hours, having the highest radicle emergence rate of 95.25%.
c. The variables of vigor index, growh of speed, germination power and maximum growth potential are positively correlated with the value of radicula emergence, while mean germination time and sprout growth rate are negatively correlated with the radicle emergence value.

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