Using Ethanol Degradation as Accelerated Chemical Seed Ageing Indicator for Different cultivars of Soybean

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Abstract: Indonesia has a wide range of swampy lands that need to be recovered for safe agriculture. The swampy lands and vegetation are a source of soil contamination through root exudates that need serious improvement. The plant exudates in soil could disturb seed germination. This study aimed to determine the effect of ethanol vapor treatment on the decrease in the vigor of four soybean seed cultivars. This study used a randomized block design with two treatment factors, namely ethanol-based ageing treatments as the main factor and cultivars as subfactor. The observation variables consisted of the percentage of germination, growth rate, vigor index, sprout length and sprout dry weight. The results showed that there was an interaction effect between fast ageing time and ethanol treatments and soybean cultivars which caused a decrease in the seed vigor. The soybean seeds of the cv. Gema was able to maintain more vigor compared to the cv. Grobogan which experienced more decline in germination. The 5 minutes of ageing time induced fewer damages with the highest vigor while 20 minutes of ageing the seeds induced maximum damage with the lowest seed vigor.

Keywords: Ethanol degradation, germination, growth factor, seed ageing, soybean

Farklı Soya Fasulyesi Çeşitleri İçin Hızlandırılmış Kimyasal Tohum Yaşlanma Göstergesi Olarak Etanol Bozunmasının Kullanılması

Öz: *Endonezya, sürdürülebilir tarımsal üretim için kurtarılması gereken çok çeşitli bataklık arazilere sahiptir. Bataklık araziler ve vejetasyon kök salgıları yoluyla toprak kirliliği kaynağı olup üzerinde durulması gereken önemli bir konudur. Topraktaki bu denli bitki kök salgıları çimlenmeyi etkilemektedir. Bu çalışmada, etanol buharı uygulaması ile dört soya fasulyesi çeşidinde tohum gücünün azalmasına etkisinin belirlenmesi amaçlamıştır. Tesadüf blokları deneme desenine uygun olarak iki faktörlü kurulan çalışmada ana faktör olarak etanol bazlı yaşlandırma uygulamaları alt faktör olarak ise çeşitler olacak şekilde çalışma yürütülmüştür. Çalışmada çimlenme değeri, büyüme oranı, vigor indeksi, fide uzunluğu ve fide kuru ağırlığı parametreleri incelenmiştir. Elde edilen sonuçlara göre; hızlandırılmış yaşlanma zamanı ve etanol uygulamaları arasında önemli düzeyde interaksiyon bulunmuştur ve soya fasulyesi çeşitlerinde tohum gücünde azalma tespit edilmiştir. Gema soya fasulyesi çeşidi çimlenme değerinde daha fazla düşüş gösteren Grobogan çeşidine kıyasla daha yüksek tohum vigoru değeri ile ön plana çıkmıştır. Tohum yaşlandırma süresi 5 dakika olan uygulama ile tohum canlılığında daha az hasara yol açarak daha yüksek tohum gücü değerine ulaşılırken 20 dakika uygulaması ile en düşük vigor değeri elde edilerek tohum canlılığı bakımından en yüksek düzeyde hasara yol açtığı sonucuna ulaşılmıştır.*

Anahtar kelimeler: Etanol degredasyonu, çimlenme, büyüme faktörü, tohum yaşlılığı, soya fasulyesi

INTRODUCTION

Indonesian islands are inhabited by more than 28.000 species of flowering plants and swamps over an area of 23.000 square km^2. More than 20% or 40 million ha^{-1} of the Indonesian land is classified as wetland and about 50% of it is peat swamp (Morley 1981, Lakitan et al. 2018). Most of these swamps are found in Papua, Sumatra, and Kalimantan. All this makes swampy lands not suitable for cultivation or make them marginal lands (Margono et al. 2014). The degradation or throwing of a large number of metabolites volatile organic compounds, acids phenols, alcohols, and metabolites contaminate these soils (Atagana et al. 2003, Levén et al. 2006) having deep chemical soil erosion and low fertility (Agus et al.1998). Halim et al.2007) with vast marginal lands (Hanson 2019). The recovery of these lands for agriculture is a difficult and time-consuming process. Seeds of many plant species degrade during sowing on these lands show poor growth or no growth at all due to rapid degradation and ageing ending up with non-uniform stand of crops (Shen-Miller et al. 1995) during sowing. Mangrove swamps are extensively developed along the shallow seas on eastern Sumatra, Southern Kalimantan (Southern Borneo), and the southeastern segment of western New Guinea. Anaerobic metabolism in trees is more widespread than previously realized (Harry and Kimmerer 1991). All living tissue in trees can synthesize ethanol with limited oxygen supply (Kimmerer and MacDonald 1987; Kimmerer and Stringer 1988), but the quantities synthesized by a specific tissue vary considerably among species (Kimmerer and MacDonald 1987; MacDonald and Kimmerer 1993).

The seeds viability is affected by genetic variations (Walters et al, 2005, Sasaki et al. 2015; Nagel et al. 2016; the effects of the environment and (Ellis et al. (1982) or treatments with chemicals from degraded plants during sowing (Li & Pritchard 2009) germination percentage, vigor detection,

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and sprout length are the most common and visible features that are noticed after the ageing of the seeds. Ethanol degradation as an accelerated based chemical seed ageing indicator was tested for different cultivars of soybean (seed water content <10%).

Therefore the study aimed to estimate the degradation or ageing of soybean seeds belonging to different cultivars after treatment with ethanol for different durations of time.

MATERIAL AND METHOD

The materials used were four cultivars of soybean (Burangrang, Demas I, Grobogan, and Gema) obtained from the Indonesian Research Institute for Legumes and Tubers Plants, Malang, East Java. The seeds belonged to the crop of the 2016-17 season. A total number of 75 seeds divided equally into 5 replications containing 15 seeds per replicate were used in each seed treatment. The other materials used in the study were 96% ethanol, 20 × 30 cm jagged paper sheet, gauze, plastic wire sheets (streaming), label paper, distilled water, plastic sheets measuring 20 × 30 cm, and aluminum foil. The other equipment used was quick-wear tools, trays, scissors, erlenmeyer flasks, volume pipettes, petri dishes, germination, electric scales, ovens, rulers, and stationery.

The research activities were carried out from June to August 2017 in Lampung State Polytechnic Food Plant Laboratory.

Pre-Treatment and Accelerated Ageing Test on *Glycine max* L. Merrill

The seeds were selected manually screening pithy, defective, and wrinkled, seeds were discarded and not used in the study (Cargil et al. (2014). The initial viability of the soybean seeds was tested using 1 mg per ml tetrazolium chloride, (Tetrazolium test) following the procedure of das Virgens et al. (2019) to evaluate seed viability. Random seed samples were taken from different seed lots and moistened for 3-4 hours. The moist seeds were left in the solution for 12 h to check the viability of the seeds. The seed samples turning red due to the formation of formazan were accepted viable. The results of this study were validated by checking the viability of seed lots that showed > 80% viability. These were selected to check the effects of ethanol evaporation. The results of this study were validated by carrying out their germination in rolled jagged papers using 75 seeds equally divided into five replications containing 15 seeds per bag (Figure 1 a, b, c).

Thereafter, the seeds of four cultivars Burangrang, Demas I, Grobogan, and Gema were treated for 0 (control treatment), 10, 15, 20, and 25 min with 96% ethanol at room temperature (24±1°C). Each of these were air-dried for 1 hour. These seeds were moistened and wetted with 3.5 ml of water daily and incubated in a Seed Germination Tool type 72-A/B at 25± 2°C with a 16h/8h light/dark photoperiod until they began to germinate. When 2mm of radicle were visible, they were counted germinated. Counting of the germination was stopped when all the seeds germinated or the seeds failed to germinate in the last 3 days.

This experiment used a randomized complete block design with two factors, using treatment duration as the main factor and soybeans cultivars as subfactor.

Germination Percentage

Germination is the percentage of total healthy seedlings after treatment. The observations were made twice, during seed germination. The germination percentage was calculated using the following formula based on Sadjad (1994):

$$\text{Percentage germination} \frac{\text{Total number of germinated seeds}}{\text{total number of seeds}} \times 100$$

Growth Rate

Growth rate = $\Sigma N/\Sigma(n \times g)$

Where GR = Growth rate

n = n is the number of germinated seeds on a specific day

g = number of total germinated seeds (Ellis and Robert 1981).

Vigor Index

VI = $S \times \Sigma$ (Gt/Dt)

was calculated following Zhu and Hong (2008), where

S = seedling height on the 7th day,

Gt is the number of germinated seeds in the “n th” day,

Dt is the number of days from the first day to the “n th” day).

The percentage of vigor index was observed in seedlings on the 7th day.

Sprout Dry Weight (g). The incubator temperature was set within 25± 1°C and the duration sprout dry weight was arranged at 3 × 24 hours.

Statistical Analysis

The data obtained were analyzed by Analysis of Variance (ANOVA). If the results showed a significant difference, then the analysis was proceeded with the Duncan’s Multiple Range Test. The level of statistical significance was all set at 5%.

RESULTS AND DISCUSSION

Initial Viability Seed Testing

The research was conducted to determine the viability of seeds in each soybean cultivar. Tetrazolium test showed seed viability of 86%, 89%, 83%, and 93%. These results showed a confirmed high viability of the seeds. The seed germination tests validated the seed viability tests and ended up with an mean germination percentage of 88%, 90%, 84%, and 96% seed germination. Seed viability and seed germination tests showed that both tests supported each other. Therefore, it was decided to use all of them for ethanol treatment tests.

Effects of Ageing with 96% Ethanol Treatment on Soybean Seeds Germination

The best seed germination was noted on control treatments (Figure 1d). The germination percentage of cultivars varied significantly under the influence of ethanol treatment durations. cv. Burangrang , Demas I, Grobogan and Gema had germination percentage in range of 76.67 -86.00%, 77.33-92.67%, 64.86% and 86-96.67% (Table 1) confirming ageing of seeds with any ethnol treatment. Germination is the ability of seeds to grow into seedlings in an optimum

growing environment. Germination is a measure of the potential viability of seeds. The reaction between time duration and soybean cultivars had a significant effect on germination, growth rate, and vigor index. All cultivars showed maximum germination percentage without using ethanol. Each increase in the treatment period of ethanol-induced consistent decrease in the germination percentage. The minimum seed germination percentage was noted on 20 minutes treatment with ethanol. The least maximum and minimum germination percentage was noted on cv. Grobogan. The results confirm the findings of Widajati et al. (2013), which indicated that suboptimum conditions reduce the ability of seeds to grow and germinate.

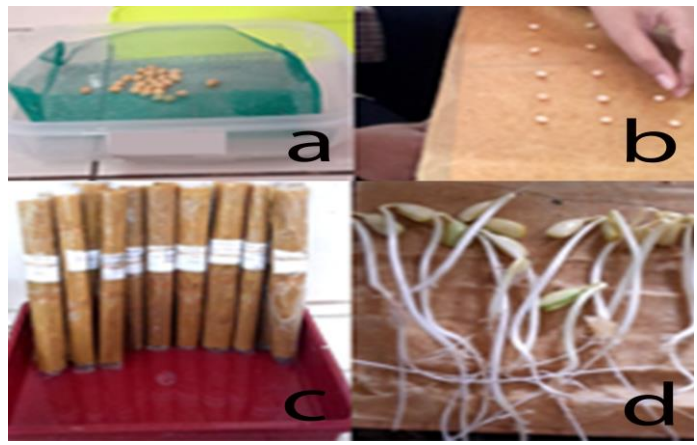

Figure 1. Seed ageing (a) treatment with alcohol (b) arranging seeds on jagged paper (c) role of seeds placed in jagged paper in the laboratory for germination (d) germinated seedlings on the control treatment

Table **1.** The effect between time duration and soybean cultivars on germination, growth rate, and vigor index

Duration of treatment in minutes with ethanol vapors (96%)	Soybean cultivars			
	Cv. Burangrang	Cv. Demas I	Cv. Grobogan	Cv. Gema
Percentage of germination (%)				
Treatment Duration (min)				
0	86.00 bcd	92.67a	86.00 bcd	96.67 a
5	84.00 bcde	84.00 bcde	79.33 ef	94.67 a
10	81.33 def	82.67 cde	77.33 f	88.00 b
15	79.33 ef	79.33 ef	64.00 g	86.67 bc
20	76.67 f	77.33 f	64.00 g	86.00 bcd
Growth rate (% etmal^{-1})				
0	48.80 b	48.40 b	45.80 bc	54.20 a
5	47.20 bc	46.70 bc	45.03 bc	48.63 b
10	47.73 bc	45.60 bc	44.57 bc	48.80 b
15	46.97 bc	43.63 cd	38.53 ef	48.33 b
20	46.17 bc	40.57 de	35.73 f	47.17 bc
Vigor index (%)				
0	9.53 bcde	5.53 h	6.83 fg	6.37 g
5	10.17 abc	10.10 abc	10.37 ab	10.60a
10	9.77 abcde	9.70 abcde	9.53 bcde	8.97 e
15	9.93 abcd	9.40 cde	10.43 ab	9.10 de
20	7.20 fg	9.07 de	6.50 g	7.53 f

Note: means followed by the different letter within a column differ significantly at a 5% probability level of significance.

Growth Rate (% etmal^{-1})

Growth rate had range of 46.17-48.80, 40.57-48.80, 35.73-48.50, 47.17-54.20 % using cv. Burangrang, Demas I, Grobogan and Gema in the same order. The minimum growth rate was noted for cv. Grobogan irrespective of the treatment period including control treatment. The growth rate percentage did not show significant differences between the duration of 5 and 10 minutes duration and non treated seeds (control treatment).

Germination Vigor

Excluding control treatment germination index ranged 7.20-10.17, 9.07-10.10, 6.50-10.37, and 7.53-10.60 using cv. Burangrang, Demas I, Grobogan and Gema in the same sequence.

Vigor index of cv. Burangrang in control treatment was lower compared to the other vigor indice treatments. Vigor index of cv. Demas I, and Gema in control treatments was lower compared to the vigor index for any duration of treatment.

Vigor index of cv. Grobogan was lower compared to the vigor index of 5-15 minutes treated seeds.

Germinated vigor confirms that ethanol is a weak acid disinfectant that damages and bleach cells of seed embryos. If they remain in contact for a longer time, ethanol damages metabolic activity in cell walls, cytoplasm, and nucleoplasm in direct proportion to the time of treatment (Maesaroh 2012).

A vigor index is the number of seedlings calculated in the first viability test. The vigor index would increase with increased germination of seedlings and vice versa (Copeland and McDonald 2001). According to the results, the highest and the minimum vigor index was noted on non treated seeds of cv. Gema, and cv. Gema is in the same order. If soybean seeds have high germination and vigor index they have high storage capacity. The high levels of protein and lipids also affect seed damage, especially if storage and sowing conditions are unfavorable (Tatipata 2008). This is with the characteristics of the cv. Grobogan, that has higher protein and fat content compared to 39.07% and 19.11% in cv. Gema.

Sprout Length (cm)

The analysis of variance results indicated (table 2) that the ageing time had a significant and variable effect on the sprout length. It was further noted that the 5 minute treatment had the highest sprout length value of 9.72 cm, which was significantly different between 15 minutes and 20 minutes of treatments. The minimum sprout length of 7.07 cm was found after treating the seeds for 20 minutes.

Table 2. Effect of ageing duration on sprout length

Treatment Duration (min)	Sprout Length (cm)	Critical Range
0	10.30 a	-
5	9.72 b	0.39
10	9.49 b	0.42
15	7.57 c	0.43
20	7.07 d	0.44

Note: The mean value marked with the same letter is not significantly different according to Duncan's multiple-distance test at the 5% level of significance.

Sprout length is the length of the sprout from the tip of the shoot crown to the tip of the sprouting roots (Sari et al. 2018). It can be seen (Table 2.) that the ageing time treatment affects the variable length of sprouts. In the 5 minute treatments with ethanol, the cultivars showed variable, values for each cultivar and the cultivar had the highest value (Kim et al. 1987; Sako et al. 2001). Hypocotyl length and root length are indicators of vigor because they are influenced by water content. Therefore, when afflicted with ethanol that enters cell walls along with water, it cause accumulation of ethanol in the cytoplasm and intra spaces in between seed cells that inhibit sprout growth and reduces root lengths.

Table 3. Effect of cultivars on sprout length

Treatment Duration (min)	Sprout length (cm)	Critical Range
0	8.76 b	0.37
5	8.94 b	0.36
10	8.22 c	0.39
15	9.41 a	-

Note: The mean value marked with the same letter is not significantly different according to Duncan's multiple-distance test at the 5% level of significance.

The analysis of variance results (Table 3) showed that the genotypes affected and influenced the induction of variable sprout length. It was noticed that the cultivars can withstand chemical obsolescence of 95% ethanol vapor. This can be seen from the ability of seeds dependent on genotypes to conserve their food reserves. Induction of sprout length with the highest value of 9.41 cm was noted on cv. Gema cultivar. The minimum sprout length value (8.22 cm) was observed on cv. Grobogan.

Chemical rapid ageing using 96% ethanol vapor is thought to damage functional proteins including enzymes and reduce seed germination (Dalapati 2012) by membrane leakage affecting the condition of the embryos and cotyledons of soybean seeds. The amount of organic and inorganic solutions that leave the cell induce a decrease in membrane integrity as a result of denaturation of the membrane of proteins (Sadiman et al. 2003). Denaturation of membrane proteins affects membrane permeability negatively ending up in reduced cellular activity causing the seedlings to slow down in their growth. Reduction or cessation of cellular activity reduces the observed number of viable seedlings; thereby, affecting germination, vigor index, and growth rate (Maesaroh 2012). In this study, seed agitation using 96% ethanol steam caused seed deterioration. The deterioration in the activity of cv. Grobogan was more prominent. This is presumably because of the initial viability of the cv. Grobogan was the lowest, while the cv. Gema had the highest germination percentage, accelerating growth and vigor index. Highly vigored seeds survive in extreme conditions compared to low vigored seeds, therefore. Dina-Hartati et al. (2006) indicated that the parameters that show an early decline in seed viability had a more sensitive and lower vigor index.

The results of the analysis of variance (Table 4) on the treatment of ethanol steam drying time and soybean cultivars on the observed variable length of sprouts showed that the these treatments significantly influenced seed germination. The 5 minutes treatment showed significantly variable differences among cultivars. The cv. Gema showed the highest (10.60 cm sprout length after 5 minutes ethanol treatment) and the cv. Grobogan showed the lowest sprout length value of 6.50 cm, after 20 minutes ethanol treatment.

Sprout Dry Weight (g)

Sprout dry weight can be seen in (table 4) and (table 5) showing that the treatment of ageing time and cultivar treatment had an effect on the sprout dry weight variably.

Table 4. Mean sprout length based on the interaction of ageing time with soybean cultivars

Treatment Duration (min)	Sprout Length (cm)			
	V1 (Burangrang)	V2 (Demas 1)	V3 (Grobogan)	V4 (Gema)
0	5.53 h	6.83 fg	6.37 g	9.53 bcde
5	10.10 abc	10.37 ab	10.17 abc	10.60 a
10	9.70 abcde	9.53 bcde	8.97 e	9.77 abcde
15	9.40 cde	10.43 ab	9.10 de	9.93 abcd
20	9.07 de	7.53 f	6.50 g	7.20 fg

Note: The mean value marked with the same letter is not significantly different according to Duncan's multiple-distance test at the 5% level of significance

The results of the analysis of variance (Table 5) showed that the ageing time greatly influenced the sprout dry weight of genotypes used in the study. The minimum dry weight of 1.14 g was noted on seeds treated with ethanol for 20 minutes, which was statistically similar to the other treatments excluding control treatment that was not treated with ethanol.

Table 5. Effect of ageing time on the dry weight of sprouts

Treatment Duration (min)	Dry Weight of Sprouts (g)	Critical Range
0	1.43 a	-
5	1.30 ab	0.15
10	1.25 b	0.16
15	1.16 b	0.17
20	1.14 b	0.17

Note: The mean value marked with the same letter is not significantly different according to Duncan's multiple-distance test at the 5% level of significance.

The results of the analysis of variance (table 6) showed that the cultivar affected the sprout dry weight variably. Based on the results of the DMRT, it was found that the Gema cultivar had the highest value of 1.38 g, approximately equivalent to the value obtained from cv. Demas and significantly different from the values obtained from cv. Grobogan. The cv. Grobogan had the minimum sprout dry weight.

Table 6. Effect of cultivar on sprout dry weight of sprouts

Treatment Duration (min)	Sprout Dry Weight (g)	Critical Range
0	1.30 cb	0.14
5	1.21 ab	0.14
10	1.13 c	0.15
15	1.38 a	-

Note: The mean value marked with the same letter is not significantly different according to Duncan's multiple-distance test at the 5% level of significance.

Sprout Dry Weight. The dry weight of sprouts of a plant reflects the accumulation of organic compounds which are the result of plant synthesis from organic compounds derived from the reshuffle of food reserves which are then rearranged into new cell constituents so that they contribute to the dry weight of the plant (Wijiono 2016). It can be seen in (table 4) and (table 5) that the treatment of ageing time on treated cultivars influenced the sprout dry weight variably depending on the corresponding genotypes. Grobogan had the lowest sprout dry weight. This is because the ethanol vapor absorbed by the seeds could have reduced the length of the hypocotyl and primary root length (Nugroho et al. 2013) thus affecting the sprout dry weights.

CONCLUSION

The results showed that the interaction effect between treatment duration (96% ethanol vapor) and soybean cultivars caused a decrease in the vigor of different soybean genotype seeds, as indicated by the variability in germination, accelerating growth, and vigor index. The results of the study meet the objectives of the study and indicate that alcohol can be successfully used to induce rapid chemical ageing in soybean seeds.

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