



Enhancing the Viability of Rosella Seeds (*Hibiscus sabdariffa* L.) through Tetrazolium and Paper Media Test

AUTHORS INFO

Taufiq Hidayat RS
Indonesian Sweetener and Fiber Crops
Research Institute (BALITTAS)
hidayat.taufiq87@gmail.com
+6285242550877

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Mayasari Yamin
University of Cokroaminoto Palopo
mayasariyamin@gmail.com
+6285282437565

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Abstract

This study was aimed to determine the viability rate of rosella seed, to obtain the best result of seed testing for enhancing rosella seed viability, to find the best seed invigoration method for enhancing rosella seed viability, to obtain staining pattern through tetrazolium test of rosella seed, and to determine viability and vigor of rosella seed to be further used as estimation indicator for rosella plant growth in the field. The study was conducted in the Seed Laboratory, Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI), Malang, East Java during July - August 2018. The material used included accessions of rosella seed (*Hibiscus sabdariffa* L.) ACC. 1148 from the year 2015 and collection of ISFCRI, 100 ml of Tetrazolium solution (40 ml KH_2PO_4 , 60 ml Na_2HPO_4 and 1 gr of Tetrazolium powder). This research applied Completely Randomized Design (CRD) consisted of seed treatments of control (no immersion/0 hour), immersion for 5 hours, immersion for 10 hours, and scarification, each with 4 replications. Result of this study showed that the use of tetrazolium salt was found to be better in enhancing the viability of rosella seeds. Viable seeds was found to have bright red embryonic axis and bright red cotyledon. Testing using paper media on several seed invigoration treatments resulted in significantly different effect on parameters of vigor index, germination capacity, and dry weight of normal seedling. The best parameter of germination capacity and dry weight of normal seedling was obtained by treatment immersed in water at temperature of 27°C for 10 hours

Keywords: germination, paper media, rosella, seed invigoration technic, tetrazolium, viability

A. Introduction

Indonesia is a tropical and agrarian country with rich biodiversity that can be utilized in all aspects of community life, particularly in agricultural sector. One of multifunction commodities that is able to be used both as fiber and medicine is rosella. This plant is within the genus *Hibiscus* that can produce natural fibers with species quite popular in Indonesia includes *Hibiscus cannabinus* L. and *Hibiscus sabdariffa* L. *Hibiscus sabdariffa* L. is rosella species that is potential to be utilized as the source of functional food, antioxidant, anti-bacteria, natural dyes, and also in health aspect (Abdallah 2015; Chang, H.C., Peng, C.H., Yeh, D.M., Kao, E.S. & Wang, C.J. (2014); Lin, H.H., Chen, J.H., Kuo, W.H. & Wang, C.J. (2007). In term of plant morphology, parts of this plant are able to be used, particularly the flower petals (Christian, K.R., Nair, M.G. & Jackson, J.C., 2006; Kao, E.S., Hsu, J.D., Wang, C.J., Yang, S.H., Cheng, S.Y. & Lee, H.J., 2009; Zhen, J, Villani, T.S., Guo, Y., Qi, Y., Chin, K., Hsiung Pan, M., Ho, C.T., Simon, J.E. & Wu, Q., 2016), due to the potential of natural phytochemical content includes phenolic compound, alkaloid, tannin, flavonoid, saponin, organic acid, anthocyanin, and polysaccharides in leaves, stems, and flowers of rosella (Mungole & Charturvedi 2011; Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischell & Heinrich, M., 2014).

Normally, this plant has multipurpose function. Besides being consumed, flower petal (calyx) of this plant is also able to be processed into domestic and industrial product that positively affects the country's economy. Dried calyx has a high market potential, both to be sold locally and exported. Moreover, the stem produces fiber that can be used as replacement for ramie. In addition to calyx and stem, the plant seed contains 17% oil and can be processed into flour. The demand for rosella product tends to increase due to increased interest in natural and caffeine- free herbal product such as rosella tea. However, the demand for rosella is not yet fulfilled because of low seed quality which has impact on production and prime quality. Seed quality includes physiological quality indicating seed viability that can be investigated through testing.

Seed viability is the ability of seed to grow that is shown in various physiological and biochemical phenomena (Sadjad, 1994). Seed viability testing is conducted through physiological and biochemical test. Physiological test can be done by performing germination test, yet this test is only able to determine the percentage of seed normally germinated in optimum germination media that is mainly used like paper or sand substrate. Papers normally used as media are filter, blotter and towel (ISTA, 2010) that are imported products and relatively expensive (Purbojati & Suwarno, 2006). Therefore, there is alternative paper substrate namely rice straw paper that has been recommended to be used as substrate for seed testing in Indonesia (Sadjad, 1994).

Biochemical testing is done by 2,3,5 triphenyl tetrazolium chloride solution (Eviliani, 2016) or commonly known as Quick test. This test is considered quick in process. By applying the method of topographic staining in order to test seed viability using tetrazolium salt, cells will be dyed red, yet this color has non-toxic effect (Subantoro & Prabowo, 2013). According to Bradford (2004), tetrazolium test can be used for vigor test by adding criteria in viability test assessment. Tetrazolium test could identify damage to embryo at earliest stage and show seed deterioration that is the indicator of vigor (McDonald, 1998). Based on survey in 1976, 1982, and 1990, tetrazolium test is predicted to be the method mostly applied as vigor test (Leist, 2004).

Tetrazolium test is a test of dehydrogenase enzyme activity functions as an index of respiration rate and viability of hydrogen ion that oxidizes colorless tetrazolium salt which changes following embryonic topographical staining pattern and its intensity (Sudikno, 1984 in Subantoro & Prabowo, 2013). Location and size of stained area as well as staining intensity (topography) determine the classification, whether seed is viable or not-viable (ISTA, 2004). The basic principle of this test is to differentiate between viable and non-

viable seed according to the relative respiration rate in wet condition, thus seed viability can be figured out.

Nugraha, U.S., Rasam, S. & Wahyuni. (2003), showed that the use of rice straw paper as substrate for seed germination test succeeded to significantly produce higher result compared to the use of CD paper for rice seed var. IR 64. Dina, Widajati, E., Wirawan, B. & Ilyas, S. (2007), conducted viability and vigor test in soybean seed (*Glycine max* L. Merr) using tetrazolium and found high correlation between plant growth and yield. Hapsari & Suwarno (2008), used stencil paper to substitute rice straw paper for seed viability test by applying the method of rolled paper wrapped by plastic in standing position (UKDdp). Eviliani (2016), conducted staining pattern test using tetrazolium in chili seed (*capsicum annum*) and resulted in 4 groups of staining standard used to differentiate between seed that will grow into strong normal seedling, abnormal seedling, and ungerminated seed (dead).

This study was aimed to determine the viability rate of rosella seed, to obtain the best result of seed testing for enhancing rosella seed viability, to find the best seed invigoration method for enhancing rosella seed viability, to obtain staining pattern through tetrazolium test of rosella seed, and to determine viability and vigor of rosella seed to be further used as estimation indicator for rosella plant growth in the field.

B. Methodology

The study was conducted in the Seed Laboratory, Indonesian Sweetener and Fiber Crops Research Institute (ISFCRI), Malang, East Java during July – August 2018. The material used were accessions of rosella seed (*Hibiscus sabdariffa* L.) ACC. 1148 from the year 2015 collection of ISFCRI, working sample of rosella seed (*H. sabdariffa* L.) (20 gram, $\pm$ 36 gram), water at normal pH (400 ml), 100 ml of Tetrazolium solution (40 ml KH_2PO_4 , 60 ml Na_2HPO_4 and 1 gr of Tetrazolium powder).

Experimental design applied in this study was Completely Randomized Design (CRD) consisted of seed treatments of control (no immersion/0 hour), immersion for 5 hours, immersion for 10 hours, and scarification, each with 4 replications, resulted in 24 units of treatment combination. Each test unit consisted of 50 rosella seeds (*Hibiscus sabdariffa* L.). Significantly different result obtained from the analysis of variance at confidence level of 95% will be further analyzed using Duncan's Multiple Range Test.

Testing method used to enhance rosella seed viability included tetrazolium test and paper media test (Rolled Paper Wrapped by Plastic in Standing Position/UKDdp). Seed viability test was done using tetrazolium solution, namely KH_2PO_4 weighed 9 g which further dissolved in 1 liter of aquades) and Na_2HPO_4 weighed 11 g that was dissolved in 1 liter of aquades. Later, 40 ml of KH_2PO_4 and 60 ml of Na_2HPO_4 were homogenized and added with 1 g of tetrazolium powder. Seeds to be used were peeled, put into glass cup, and added with tetrazolium solution until all seeds were completely immersed. Moreover, seeds were dried in oven for 2 hours at temperature of 35° C.

Concerning UKDdp test, rice straw papers were soaked in water. Later, 50 rosella seeds (*H. sabdariffa* L.) were selected randomly and grown on rice straw paper substrate. Seeds were arranged in 5 rows where each row consisted of 10 seeds. Rice straw papers planted by seeds were covered by 2 sheets of rice straw papers that have been soaked in water. Then, rolled the paper well. Rolled paper was put inside germinator at temperature of 20-30°C in standing position where the row of seed arrangement was placed on top position. Observation was done once in 4 days to calculate the number of normal germinated seed, abnormal germinated seed, and ungerminated seed (seed was fresh, hard, attacked by fungi or decayed).

C. Result and Discussion

1. *Tetrazolium Test of Rosella Seeds*

Enhancing rosella seed viability from 2015 collection through tetrazolium test with application of several treatments presented in Table 1.

Table 1. Rosella seed viability through tetrazolium test

Treatment of seed invigoration	Number of seed tested		Percentage of seed tested (%)	
	Viable	Non-Viable	Viable	Non-Viable
Control	4	46	8	92
Immersion in water at temperature of 27°C for 5 hours	1	49	2	98
Immersion in water at temperature of 27°C for 10 hours	1	49	2	98
Seed cutting	0	50	0	100

Table 1 shows that control treatment (no immersion) produced the best number of viable seed for number of viable seeds and seed viability percentage. This finding indicates that pretreatment prior to tetrazolium test reduce rosella seed and viability seed was totally lose viability after cutting treatment prior to tetrazolium test.

Tetrazolium test, without any seed treatments following the test, can determine staining pattern that is able to indicate whether seed is viable or not viable. It is due to the reason that immersion in tetrazolium solution may stimulate imbibition by meristematic tissue in embryo, thus enhancing seed viability. According to Copeland & Mcdonald (1976) *in* Hasrawati, Mustari, K., & Dachlan, A. (2015), process of seed immersion in tetrazolium salt solution will lead to water imbibition by meristematic tissue in the embryo and reduction of H^+ released from respiration process. Coker & Barton (1957), *in* Hasrawati *et al.*, (2015), mentioned that respiration in seed could increase water content when seed is immersed in tetrazolium solution.

Tetrazolium test in rosella seed resulted in embryonic staining with color intensity of bright red, pink, and, colorless (white) as presented in Figure 2. This staining pattern provides indication and can be used as a benchmark whether seed will grow into strong normal seedling or has high viability (Figure A), weak normal seedling or has low viability (Figure B), abnormal seedling, or dead seed (Figure C).



Figure 1. Tetrazolium staining pattern in rosella seed (A). Normal seedling; (B). Abnormal seedling; and (C). Dead seed.

Figure A shows high viability seed and is classified into the criteria of strong normal seedling as indicated by maximum tetrazolium staining pattern of bright red. It is because tetrazolium is chemical substance that shows both oxidation and reduction properties. In term of oxidation, tetrazolium is soluble in water and easy to enter and exit

plant cells. Reduction of tetrazolium may occur for its insolubility property, hence this chemical substance still remains within the cell and provides red staining in plant cell (Subantoro & Prabowo, 2013).

Moreover, Figure C shows low viability and classified into the criteria of dead seed. It is because the unstained part of seed is actually dead tissue or necrotic. Widajati, E., murniati, E., palupi, E.R., Kartika, T., Suhartanto, M.R. & Qadir, A. (2013), said that the area stained red is living tissue, while unstained area is dead tissue or necrotic tissue and the area where necrotic tissue is located provides information that determine the category whether seed is alive or dead.

2. Test of Paper Media

Analysis of variance presented in Table 1 shows that enhancing rosella seed viability through test of paper media for several seed invigoration treatments significantly affected the parameter of vigor index, germination capacity, and dry weight of normal seedling. Moreover, concerning the parameter of speed of germination and maximum growth potential, it is expected that several seed invigoration treatments were not able to show significantly different effect concerning the viability of rosella seed from the year 2015.

Different finding was shown by the indicator of coefficient of variance (CV) in which CV is a coefficient reflecting the precision degree of result obtained in an experiment. Parameter of maximum growth potential showed low CV of 1.47%, while parameter of dry weight of normal seedling generated higher CV of 6.86% compared to other parameter. It is expected that parameter with low CV value obtained homogeneity of observation data and higher degree of precision. Hanafiah (2010), mentioned that lower CV value indicates higher degree of precision and validity of conclusion resulted from the experiment.

Table 2. Recapitulation of analysis of variance of rosella seed (*Hibiscus sabdariffa* L.) invigoration

Parameter of observation	Treatment of seed invigoration	Coefficient of Variance (%)
Speed of germination (%KN/etmal)	0.2476 tn	2.76
Vigor index (%)	<.0001**	3.29
Germination capacity (%)	<.0001**	2.29
Maximum growth potential (%)	0.0541 tn	1.47
Dry weight of normal seedling (g)	<.0001**	6.86

Description: tn = not significant; * = significant at $\alpha=5\%$; ** = significant at $\alpha= 1\%$

Based on the result of analysis of variance in Table 1, parameter of vigor index, germination capacity, and dry weight of normal seedling had significantly different effect. Therefore, DMRT at α 5% was applied to three parameters as shown in Table 2. The best parameter of vigor index was obtained by treatment of seed cutting with average of 99.5%, while significantly different result was found in treatment of immersion in water at temperature of 27°C for 5 hours with average of 62.0%.

Mechanical scarification, namely seed cutting that enables water to enter the seed, thus increasing vigor index of rosella seed. Widajati, *et al* (2013), in Noflindawati (2014); Sadjad (1994), mentioned that common technic applied in treatment of mechanical scarification is seed cutting to ease water entering the embryo as well as increasing vigor index both in optimal and sub-optimal condition.

The best parameter of germination capacity and dry weight of normal seedling was obtained in seed invigoration treatment through immersion in water at temperature of 27°C for 10 hours with average of 99.5% and 0.4849 g, respectively. Seed immersion at optimum temperature in such a long period can soften seed coat and accelerate cell division and elongation due to the existence of process where water and oxygen enter the

seed, thus increasing radical cells and further growth. Fitri (2015), mentioned that water in seed may stimulate cell division and elongation and quicken the growth of root cells since water and oxygen that enter the seed will wet the protein and colloid within the seed, hence enzyme formation and activation will lead to increasing metabolic activity, elongation of radical cell and further growth.

Table 3. Effect of invigoration treatment of rosella seed (*Hibiscus sabdariffa* L.)

Treatment of seed invigoration	Parameter of observation				
	Speed of germination (%KN/etmal)	Vigor Index (%)	Germination Capacity (%)	Maximum Growth Potential (%)	Dry weight of normal seedling (g)
Control	13.8	96.0 ^a	96.0 ^b	97.0	0.4353 ^b
Immersion in water at temperature of 27°C for 5 hours	13.5	62.0 ^b	69.5 ^c	100.0	0.3139 ^d
Immersion in water at temperature of 27°C for 10 hours	14.0	99.0 ^a	99.5 ^a	99.5	0.4849 ^a
Seed cutting	14.0	99.5 ^a	99.0 ^{ab}	99.5	0.3836 ^c

Description: Values within the same column following by different subscript letters show significantly different result in DMRT at level of 0.05

Isnaeni & Habibah (2014), found that temperature above 60°C led to ungerminated kepel seed and further dead. Lima (2012), mentioned that immersion in hot water 60°C for 10 minutes resulted in the highest percentage of germination in centro and siratro seeds. This proved that temperature may facilitate germination of hard coated seeds in a relatively short period of time.

3. Comparison between tetrazolium test and paper test

Enhancing the viability of rosella seed from the year 2015 was done through two test, namely test using tetrazolium salt solution and test using paper media as shown in Figure 2. Figure 2 presents the comparison between the two seed testing which further followed by seed invigoration treatment. According to the figure above, significantly different result was not obtained in general. However, there is only one treatment that obtained significantly different result, that is treatment of immersion in water at temperature of 27°C for 5 hours that produced average percentage of 70 % and 98 % or about 1 : 1.4 %.

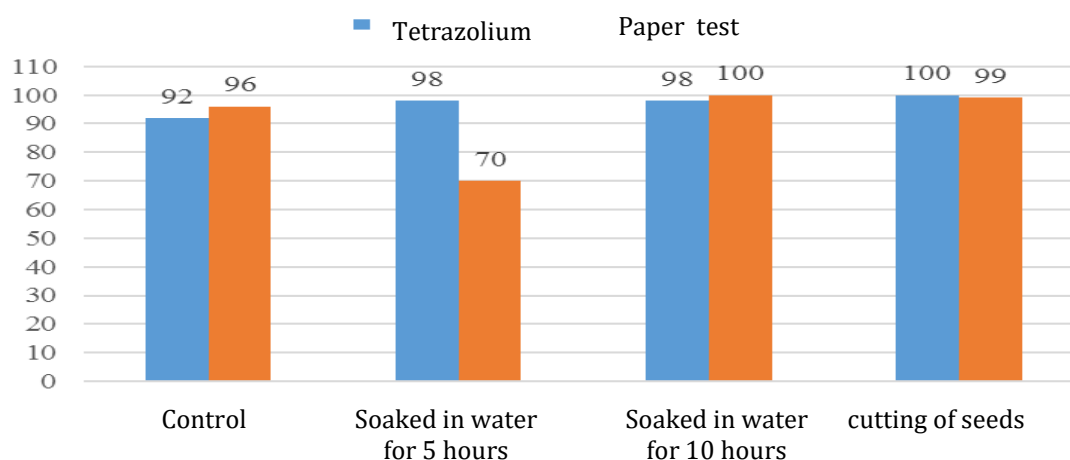


Figure 2. Comparison of rosella seed viability between tetrazolium and paper test

Seed vigor test using tetrazolium in embryonic structure may provide complete information. Seed vigor obtained from tetrazolium staining showed criteria of seed with high, moderate, and low vigor according to the indicator whether staining process of formazan precipitate was thoroughly applied or not. Budiarti (2002), performed seed testing by tetrazolium test and found that electrical conductivity and respiration could be developed to estimate seed viability and vigor.

D. Conclusion

Seed testing using tetrazolium salt solution was found to be able to enhance the viability of rosella seed from the year 2015 relatively faster compared to seed testing using paper media. Viable seeds were found to have bright red embryonic axis and bright red cotyledon. This staining provides indication or becomes a benchmark that seeds will grow into strong normal seedling or have high viability. Testing using paper media on several seed invigoration treatments resulted in significantly different effect on parameters of vigor index, germination capacity, and dry weight of normal seedling. The best seed invigoration treatment was obtained through seed cutting related to the parameter of vigor index with average of 99.5%. The best parameter of germination capacity and dry weight of normal seedling was obtained by treatment immersed in water at temperature of 27°C for 10 hours.

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