

Short Communication: Tetrazolium test for evaluating viability of *Capsicum annum* seeds

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Abstract. Kusumawardana A, Pujiasmanto B, Pardono. 2018. Tetrazolium test for evaluating viability of *Capsicum annum* seeds. *Nusantara Bioscience* 10: 142-145. Seed quality is important in seeds production. This research was conducted to obtain information of topographical tetrazolium staining pattern of pepper (*Capsicum annum*) seeds. Tetrazolium test was conducted to determine the seed viability and plant growth vigor. Laboratory test for standard germination and field performances were performed on four lots (A, B, C, D) of pepper seeds. The viability categories pattern were determined by Root Mean Square (RMS), regression, and correlation analyses. Nine topographical patterns were recognized. The laboratory test results and field performances were compared with the topographical pattern. Combination of patterns 1,2 (embryonic axis and cotyledon completely stained) selected as viable category as it gave the least RMS value, the highest determination (R^2) and correlation (r) coefficient with standard germination (RMS = 4,06; R^2 = 0,761; r = 0,872). Combination of patterns 1,2 also gave the highest determination (R^2) and correlation (r) coefficient with field stand (R^2 = 0,921; r = 0,959). The combination of patterns 1,2 is recommended for estimating plant growth performance in the field.

Keywords: Pepper seed, tetrazolium, topographical pattern, viability

INTRODUCTION

The quality of chili pepper seeds (*Capsicum annum*) must be known before they can be released to the market-the sooner the quality identification, the better. The initial assay of chili pepper quality is the germination assay. However, through this assay, it takes at least 14 days to know the viability of seeds. Thus, a convenient method is necessary to quickly assess the quality of seeds and their germination.

The majority of seed technologist defines seed viability as the capacity of a seed to germinate and form a normal seedling. Therefore, the seed viability is tantamount to the germination capacity (Copeland and McDonald, 1995). Deminiciis et al. (2014) suggest that tetrazolium test can be chosen as an alternative to the conventional viability assay in *Stizolobium aterrimum* seeds. Tetrazolium test is a biochemical approach to assay seed viability based on the color change of plant life tissue upon treatment with tetrazolium salts (Dina 2006). Upon staining with tetrazolium salts, living cells will be dyed red whereas dead cell will be colored white. The red color of the staining is produced as the result of dehydrogenase enzyme activity that releases H^+ ions reacting with the tetrazolium salts and forms stable, insoluble red formazan precipitate. The color intensity and the extent of the dyed area, also known collectively as the topography, indicate the seeds viability (ISTA, 2016). The advantage of this staining method is that it only requires 2-3 days to perform.

Our study aimed to classify the staining topography of tetrazolium assay on chili pepper seeds, as the basis to determine the seed viability for future application in the field.

MATERIALS AND METHODS

Time and place

The study was conducted from October 2017 to February 2018 at the laboratory and greenhouse of Research Center for Development of Food Crop and Horticulture Seed Quality Assessment (BB PPMB-TPH), Ministry of Agriculture, Depok, West Java, Indonesia.

Experiments

Determination of seed quality

Chili pepper seeds were obtained from several seed companies. There were four lots of seeds analyzed in this experiment, chili pepper variety Laskar (Lot A), variety Sret (Lot B), variety Serambi (Lot C), and variety Madun (Lot D). Lot B chili seeds have been stored for 12 months at 25°C.

Viability assay

Seed viability assay was done by observing the germination capacity of seeds and by tetrazolium-based method. For the germination assay, seeds were grown on a filter paper in an electrical germination chamber at 20°-30°C. Observation and recording of normal seedlings were done at day 7 and day 14 after seed placement (ISTA, 2016). For tetrazolium staining, seeds were put in a wet paper at 20°C for 18 hours. Seeds were then wounded by cutting the testa between the radicle and the cotyledon. Wounded seeds were immersed in 1% tetrazolium chloride solution (in phosphate buffer) for 6 hours at 30°C, at dark condition (ISTA, 2016). Seeds were cut in half, observed, and grouped based on their staining topographical pattern.

The percentage of each topographical pattern group was calculated. Each experiment used 50 seeds with 8 replicates.

Growth performance test

To assess the growth performance of chili peppers varieties, seeds were grown uniformly in polybags in a controlled condition in a greenhouse. One seed was sown in each polybag at 2-3 cm depth. During the experiment, no refilling done on any dead plants. The observation of plant height was done at week 2 until week 6. This experiment aimed to verify the correspondence between the staining topographical pattern with the plant viability standard.

Data analyses

All experiments used a completely randomized design with one factor, the seed lot. Data were analyzed using analysis of variance and Duncan Multiple Range Test at 5% significance level. Determination of topographical pattern of tetrazolium staining as the standard test for seed viability is based on the root mean square (RMS) value between the seedling viability test and tetrazolium staining (Kuo et al. 1996; Pant et al. 1999). RMS was calculated using the following formula:

$$RMS = \sqrt{\frac{(G_1 - P_1)^2 + (G_2 - P_2)^2 + \dots + (G_n - P_n)^2}{n}}$$

Where, G is the percentage of germinated seedlings; P is the percentage of each topographical pattern group or a combination of multiple patterns; n is the number of seed lots (n = 4). G and P are obtained from the mean of values from eight replicates.

RESULTS AND DISCUSSION

Seed quality test results showed moisture value, weight of 1000 grains and germination of consecutive lots A was 7.5%, 4.34 g and 80%, lot B 9.4%, 3.41 g and 64%, C lots 7.4%, 4.66 g and 98% and lot D 5.5%, 4.49 g and 85% (testing in October 2017). Tetrazolium assay can clearly indicate the structure and condition of a chili pepper. The staining topography of the embryo upon 1% tetrazolium treatment can be observed under a microscope at 40x magnification.

Viability assay by tetrazolium staining

Our tetrazolium staining experiment revealed at least 9 topographical patterns in tested seeds. The patterns were sorted from the pattern that indicates the highest viability (pattern 1) to the lowest (pattern 9) (Figure 1).



Figure 1. Topographical pattern of tetrazolium

The percentage of each topographical pattern of tetrazolium staining is shown in Table 1. Pattern 1 and 2, collectively, are the predominant patterns observed on each seed tested: lot A 82%, lot B 69%, lot C 92%, and lot D 86%. Meanwhile, the percentage of pattern 3, 4, 5 occurrences was 5-6%; pattern 6 and 7 2-10%, pattern 8 and 9 0-17%.

Our result indicates that there are 8 viable staining patterns (Table 2) with an RMS value less than 10. RMS value greater than 10 shows that the representative pattern is considered as non-viable (not included in Table 2). Wibawa (2015) reported the RMS values obtained from the analysis of tetrazolium staining in viable papaya seeds are below 10.

Regression and correlation analyses between the tetrazolium staining test and germination test are shown in Table 3. Regression analysis can predict the dependent variable (Y) if the independent variable is known. The regression line equation describes the relationship between tetrazolium test (X axis) and the germination test (Y axis). The contribution of X variable to the Y value depends on the determination coefficient (R^2) x 100%. The higher the R^2 , the higher the effect of the X variable to the Y variable. Table 3 shows that the determination coefficient for pattern 1,2 is 76,1% suggesting that the germination capacity is 76% determined by the pattern and 23.9% by other variables.

Plant growth performance assay

Plant height is an indicator of plant growth performance in the field (Figure 2). The plant height of the four seed lots corresponds to their viability upon laboratory testing.

Table 1. Percentage of each topographical pattern of tetrazolium staining in each seed lot

Topographical pattern	Lot A (%)	Lot B (%)	Lot C (%)	Lot D (%)
1	42	36	54	46
2	40	33	38	40
3	3	1	3	2
4	1	2	1	2
5	1	3	2	1
6	4	2	1	2
7	6	6	1	2
8	3	9	0	3
9	0	8	0	2

Table 2. Root mean square value of tetrazolium staining pattern of viable seeds

Combination pattern	Root mean square
1, 2	4.06
1, 2, 3	4.44
1, 2, 4	4.79
1, 2, 5	4.82
1, 2, 3, 4	5.67
1, 2, 3, 5	5.78
1, 2, 4, 5	5.93
1, 2, 3, 4, 5	7.17

Table 4 shows that lot C is the lot with the best growth performance, followed by lot D, A, and B, consecutively. Within 2 to 6 weeks after planting, lot C clearly produce the tallest plants

Analysis of variance showed that the plant height of 4 seed lots from 2 to 6 WAP is significantly different (Table 4). Lot C and D is not significantly different from each other, suggesting good vigor of the two plant groups (Table 4). Analysis of regression and correlation between staining topology (patterns 1,2; 1,2,3; and 1,2,4) and plant height was done. Correlation coefficient of each pattern combination is shown in Table 5. Our result indicates a positive correlation between the tetrazolium staining pattern and the plant height. Pattern combination 1,2 shows the highest correlation among other combination (1,2,3 and 1,2,4). The correlation coefficient of pattern 1,2 is 0.959.



Figure 2. Plant growth in polybag

Table 3. Regression and correlation analyses of germination assay and tetrazolium staining of three staining topography combinations with least root mean square

Combination pattern	a	b	R^2	r
1, 2	-8.872	1.026	0.761	0.872
1, 2, 3	-13.63	1.105	0.755	0.869
1, 2, 4	-4.137	0.990	0.756	0.869

Note: a: interception, b: regression coefficient, R^2 : coefficient of determination, r: correlation coefficient

Table 4. Height of plants grown in polybag

Seed lot	Plant height (cm)				
	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
Lot A	2.4b	4.0b	6.2b	10.7b	15.8b
Lot B	0.8c	1.7c	2.8c	4.1c	5.5c
Lot C	3.8a	6.3a	8.6a	13.7a	18.7a
Lot D	3.7a	5.4a	8.1a	13.a	17.6a

Note: Different letters indicate significant difference according to Duncan's Multiple Range Test at 5% significance level; WAP: week after planting.

Table 5. Analysis of regression and correlation between tetrazolium staining assay and plant height

Combination pattern	a	b	R ²	r
1, 2	60.21	1.527	0.921	0.959
1, 2, 3	60.59	1.657	0.927	0.963
1, 2, 4	62.44	1.476	0.919	0.958

Note: a: interception, b: regression coefficient, R²: coefficient of determination, r: correlation coefficient

Discussion

Combination of tetrazolium staining pattern to determine seed viability level uses correlation coefficient (r). In this analysis, the tetrazolium staining pattern is the X-axis variable, and the germination capacity is the Y-axis variable. High correlation coefficient is demonstrated by pattern 1 and 2. The value of this correlation coefficient shows a value of 0.872 (close to 1). This result indicates a strong correlation between the tetrazolium staining result and the germination assay result. Correlation coefficient $r = -1$ suggests a perfect negative correlation, $r = 0$ no correlation, and $r = 1$ a perfect positive correlation (Mattjik and Sumertajaya 2002).

Seed vigor is indicated by the plant growth performance in polybag. Our analysis showed that tetrazolium staining and the seed vigor assay in the field are highly correlated with an r value of 0,0959 (Table 5). According to tetrazolium staining assay, seeds from lot C exhibit highly viable staining pattern (pattern 1,2) with a percentage of 92% (Tabel 1). Meanwhile, the lot C plants grow tall with a height of up to 18,7 cm (Table 5). Viable seeds as determined by tetrazolium staining are highly likely to grow into plant with good growth performance in the field. Kulik and Yaklich (1982) reported that tetrazolium staining can estimate the germination rate of soybean seeds in the field. Pattern 1 and 2, characterized by the uniform red coloring in the embryonic axis and cotyledon, are sensitive enough to indicate seeds that will grow into normal and healthy seedlings. In line with our report in this current study, previously, Leist (2004) stated that seeds with good vigor are stained bright red uniformly upon tetrazolium assay.

Poor plant vigor in the field can be caused by the physiological deterioration of the seeds (Basak et al., 2006). This may be true for lot B seeds that had been in long storage for about 12 months. Plants grown from lot B seeds exhibited the shortest stature among all group tested.

This poor growth performance of lot B corresponds to the result of the lot's tetrazolium staining which indicates low seed viability.

In conclusion, 1% tetrazolium staining assay revealed 9 topographical patterns on chili pepper seeds. These patterns can be used as a reference to determine viable seeds from non-viable ones. Staining patterns 1 and 2 show that the whole cotyledon and embryonic axis are stained red evenly with or without dark red coloration at the tip of the radicle. Staining patterns 1 and 2 is highly correlated with seed's germination capacity and plant height. Thus, staining patterns 1 and 2 can be utilized to estimate plant growth performance. Future studies using more seed lots from different varieties need to be done.

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