Agritech, 39 (2) 2019, 136-142

Controlled Deterioration Test (CDT) to Estimate Storability of Soybean Seed (*Glycine max* (L.) *Merr.*)

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Submission: April 11, 2018; Acceptance: May 7, 2019

ABSTRACT

Deterioration of seeds during storage may reduce the supply of high-quality seeds which become the limiting factor of soybean production in tropical countries. Controlled Deterioration Test (CDT) is one of the fastest vigor testing methods. The objective of this study was to achieve the suitable moisture content and duration of CDT treatment in soybean seed, then establish a model that associated with the seed vigor in the field after a certain time of storage. Split-plot with 2 factors, namely seed varieties and the combination of moisture content and heat exposure duration were used to determine the influence of CDT treatment. Another split-plot for 2 factors, namely storage period and seed varieties were used to determine the effect of shelf life. Viability parameters were observed by germination strength (GS) and seedling vigor index (VI), using a rolled paper towel method. The results showed that there was an interaction between soybean seed varieties and the combination of seed moisture content treatment with duration of CDT exposure. Sindoro and Tanggamus varieties had higher viability and storability than other varieties. Combinations of 28% seed moisture content for 24 hours CDT duration were sensitive enough to evaluate the physiological potential of soybean seeds, providing information that was closely related to seed germination after stored for 1 month.

Keywords: germination; seedling vigor; shelf life; soya bean; viability

INTRODUCTION

Soybean is one of the important food crops commodities in Indonesia. The production of soybean requires the supply of high-quality seeds. Being rich in oil, like other oilseeds, soybean is highly prone to seed deterioration if improperly stored (Chandel, Khan, & Gandotra, 2015). The high-rate decline of seed viability during storage is one of the limiting factors for soybean's production in the tropics (Purwanti, 2004). The information about seed quality should obtain quickly, precisely, and practically by seed producers before the seeds ready to be marketed (Karuniasari, 2016). The susceptibility of seed deterioration varies between varieties of the same species (Jatoi, Afzal, Nasim, & Anwar, 2001), therefore the appropriate methods are required for each type of seed to be tested.The seed viability test includes germination and vigor test. The vigor test assesses the seed quality that germinated under suboptimum conditions, providing information about seed quality on the actual field crop (Ilyas, 2012). Development of the ideal seed testing method according to Copeland and McDonald (2001) should meet six criteria: cheap, fast, easy, objective, reproducible, and closely correlate with the plant growth in the field. Vigor test measured the plant's ability to grow under suboptimum condition, which is meet one of the criteria for ideal seed testing method (Sadjad *et al.*, 1999).

Vigor testing methods were validated by ISTA (2017) i.e.: electrical conductivity tests for peas, beans, and soybeans; radicle emergence (RE) test for maize, controlled deterioration test (CDT) for *Brassica* spp.; and tetrazolium test for soybean. One commodity may be subjected to several vigor test methods, such as electric conductivity test, accelerated aging test (Ekowahyuni *et al.*, 2012), and controlled deterioration test (Basak *et al.*, 2006) on chili seed.

Controlled deterioration test (CDT) is one of the fastest vigor seed tests. The principle of CDT is similar

DOI: http://doi.org/10.22146/agritech.34571
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

with the accelerated aging test (AAT) method, in term exposing the seeds to high-temperature conditions, but different in the moisture content which is constant over a period of deterioration. This study aims to obtain the value of seed moisture content and the duration of CDT treatment for soybean seeds. The degree of correlation on viability after CDT treatment with viability after the storage may predict optimum seed shelf life.

MATERIALS AND METHODS

The study took place at IPB Seed Laboratory, Bogor, West Java. Six soybean varieties/lines, namely Gepak Kuning, Ijen, Kaba, Sindoro, Tanggamus, Wilis were used in this study. The seed were obtained from BB Biogen genbank, Bogor, West Java after we formally requested the seed.

Selection of CDT Method on Soybean Seed

The experimental design was split-plot RCBD (Randomized Complete Block Design) in two factors (Budiman, 2012; Ekowahyuni *et al*. 2012; Nizaruddin *et al*., 2014). Powell and Matthews (2005) performs CDT on small seeded vegetables in 20% SMC + 24 hours. We expand the treatment using three level of SMC; 20%, 24%, 28%.

The first factor were 6 levels of varieties as main-plot: Gepak Kuning, Ijen, Kaba, Sindoro, Tanggamus, Wilis. The second factor as sub-plot were a combination of CDT treatment at ± 40 °C (SMC/seed moisture content and incubation time) consisting of 9 levels: E1: 20% SMC + 0 hour; E2: 20% SMC + 24 hours; E3: 20% SMC + 48 hours; E4: 24% SMC + 0 hour; E5: 24% SMC + 24 hours; E6: 24% SMC + 48 hours; E7: 28% SMC + 0 hour; E8: 28% SMC + 24 hours; E9: 28% SMC + 48 hours. The combination of these two factors was performed in three replications, so there were 162 experimental units.

The adjustment of SMC was performed by measure the initial moisture content of the seeds by constant low-temperature oven method. Approximately 100 seeds of each variety were weighed and two replicates were placed in the oven at 103 ± 2 °C for 17 hours; results are expressed as percentages.

The moisture content of the seeds was adjusted to the desired value due to CDT treatment: 20%, 24%, and 28%, based on the ISTA (2017) formula.

$$W2 = \frac{100 - A \times W1}{100 - B} \qquad (1)$$

Remarks:

A = initial seed moisture content (%)
B = desired seed moisture content (%)
W1 = initial weight of the seeds (g)
W2 = final weight of the seeds with desired moisture content (g)

The moisture contents of seeds were raised by adding the calculated amounts of distilled-water over the scales. Each sample was sealed in an aluminum foil packed and equilibrated at 4 °C overnight to ensure an even distribution of moisture. The seeds package was then placed in a plastic envelope, allowing no ingression of water, and kept in a water bath at ±40 °C for up to 0, 24, and 48 hours. Following this period, seed were germinated in a moistened rolled paper towel which placed vertically in a beaker at 30 °C for five days. Samples were drawn at 2 days interval, 3 and 5 days after planting (DAP). Only normal seedlings were counted and expressed as the percentage of germination (%GS) and vigor index (%VI).

Effect of storage duration on the viability of soybean seeds

The experimental design was split-plot design with the main plot arranged completely randomly (Budiman, 2012; Ekowahyuni *et al*. 2012; Nizaruddin *et al*., 2014). The experiment consisted of two factors, the first factor was the shelf period as the main plot with 3 levels: m1: 1 month of storage, m2: 2 months of storage, m3: 3 months of storage in laboratory room conditions. The second factor is varieties as sub plot with 6 levels: Gepak Kuning, Ijen, Kaba, Sindoro, Tanggamus, Wilis. Each combination between the shelf period and the varieties was repeated 2 times, so there were 36 experimental units.

A total of 25 seed grains were placed in a paper bag wrapped in a plastic package. Packaging was prepared to be stored in a laboratory room for up to 3 months. Each end of the month the sample was drawn for moisture content measurement by constant low-temperature oven method (103 ± 2 ºC for 17 ± 1 hour). The samples were germinated in a moistened rolled paper towel which placed vertically in a beaker at 30 °C for five days. Samples were drawn at 2 days interval, 3 and 5 days after planting (DAP). Only normal seedlings were counted and expressed as the percentage of germination (%GS) and vigor index (%VI).

Data analysis

All data were subjected to analysis of variance based on Split Plot Design with variety as the main plot and CDT treatment and storage period as a subplot. A 2-way ANOVA was used to test for variety, CDT treatment, and storage period effects, as well as their interactions. F-test at 5% probability level was carried out to evaluate the significance of each source of variation. The significance variables will be tested further by using Duncan Multiple Range Test at 5% probability level. Anova analysis was performed with DSAASTAT ver. 1514 (add-ins for Office Excel 2016). A homogeneity test was performed with SPSS v.20. Correlation analysis was established among viability test after storage and controlled deterioration results.

RESULTS AND DISCUSSION

Three stages of the analysis were statistically performed in obtaining at least one CDT method to estimate the storage ability of soybean seeds. The first phase of analysis is F-test (variance analysis) from the viability test results, which expressed as a percentage of germination (GS) and vigor index (VI) (Table 1). The result of two-way variance analysis showed that there is an interaction between varieties and CDT treatment on GS and VI parameters. Ekowahyuni *et al*. (2012) decided the coefficient of variation (COV) <10% as criteria on the chili seed accelerated aging method. The high value of COV in this study showed a high degree of response to the CDT treatment on the observed parameters (Table 1).

Table 1. Middle square and percentage of COV

Source of variance	GS (%)	VI (%)
Varieties	7389,664**	6135,178**
Seed moisture content	2579,254**	1033,127**
CDT	25460,319**	7728,147**
Interactions (varieties x SMC /CDT)	363,440 **	75,475**
COV (%)	22,308	43,699

Remarks: **) Mean values is significantly different at <0.05 probability level according to F-test; GS = germination strength test; VI = vigor index; COV = coefficient of variance

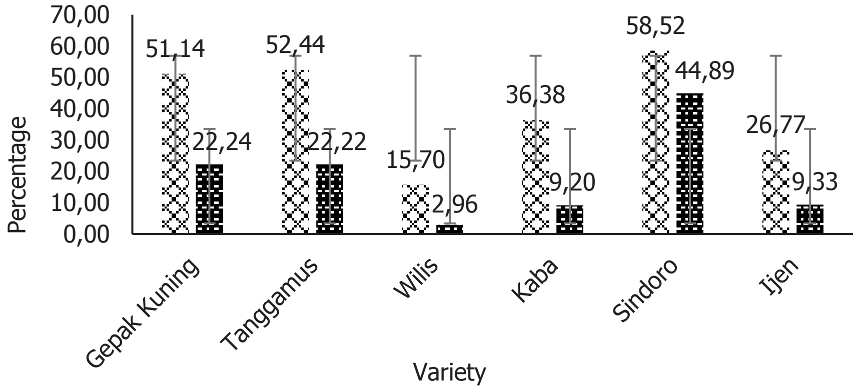

Figure 1. Mean value of germination strength (GS) and vigor index (VI) in 6 soybean varieties

Table 2. The interaction between CDT treatment on germination strength (GS/%)

CDT treatment		Varieties						
seed moisture content (%)	CDT duration (hour)	Gepak kuning	Ijen	Kaba	Sindoro	Tanggamus	Wilis	Mean value
20	0	69.33 mn	73.33 no	22.67 de	60.00 nop	78.67 op	53.33 mn	59.56
20	24	68.00 m	66.67 lm	29.33 g	50.67 m	81.33 p	53.33 mn	58.22
20	48	68.00 m	64.00 l	28.00 fg	48.00 j	65.33 l	49.33 j	53.78
24	0	70.67 n	69.33 mn	18.67 cd	54.67 k	77.33 o	32.00 h	53.78
24	24	69.58 mn	66.67 lm	14.67 bc	48.78 j	73.33 no	30.67 gh	50.61
24	48	61.33 kl	52.00 j	20.00 d	34.67 hi	65.33 l	5.33 a	39.78
28	0	21.33 d	49.33 j	5.33 a	28.00 fg	38.67 i	24.00 de	27.78
28	24	26.67 f	16.00 C	1.33 a	1.33 a	33.33 h	2.67 a	13.56
28	48	5.33 a	14.67 bc	1.33 a	1.33 a	13.33 b	1.33 a	6.22
Mean value	51.14	52.44	15.70	36.38	58.52	28.00	40.36	

Remarks: Different superscript letters denote significant differences between groups at the different column according to 5% DMRT.

Table 3. The interaction between CDT treatment on vigor index (VI/ %)

CDT treatment		Varieties						
seed moisture content (%)	CDT duration (hour)	Gepak kuning	Ijen	Kaba	Sindoro	Tanggamus	Wilis	Mean value
20	0	45.33 lm	37.33 l	4.00 bc	17.33 gh	68.00 o	16.00 f	31.33
20	24	38.67 l	28.00 jk	5.33 C	13.33	66.67 o	24.00 ij	29.33
20	48	34.67 kl	26.67 j	5.33 C	12.00 e	54.67 n	22.67 i	26.00
24	0	29.33 k	28.00 jk	2.67 bc	18.67 h	72.00 o	0.00 a	25.11
24	24	26.79 j	40.00 l	4.00 bc	5.44 C	52.00 n	8.00 d	22.71
24	48	16.00 f	14.67 ef	5.33 C	10.67 de	45.33 lm	1.33 b	15.56
28	0	6.67 cd	21.33 hi	0.00 a	5.33 a	25.33 j	12.00 e	11.78
28	24	2.67 bc	4.00 bc	0.00 a	0.00 a	17.33 gh	0.00 a	4.00
28	48	0.00 a	0.00 a	0.00 a	0.00 a	2.67 bc	0.00 a	0.44
Mean value	22.24	22.22	2.96	9.20	44.89	9.33	18.47	

Remarks: Different superscript letters denote significant differences between groups at the different column according to 5% DMRT.

The Sindoro variety showed the best GS and VI percentage, while the Wilis variety has the lowest mean value (Figure 1). This is due to differences in initial viability and genetic factors of seed. The response of varieties expressed in phenotype is influenced by genetic and environmental factors (Rao *et al.*, 2002). Varietal response to CDT treatment showed a constant pattern, i.e. the longer the CDT duration and the higher moisture content will decrease the percentage of GS and VI (Tables 2 & 3).

The second stage statistical analysis was based on the 5% Duncan multiple range test (DMRT) to see how much the CDT treatment affects the seed viability (Tables 2 & 3). The higher SMC and the longer CDT treatment may lead accumulation of heat in seed, resulted in the degradation of seed viability as indicated by significant reductions of GS and VI (Table 2 & 3). The high temperature may trigger lipid peroxidation which brings damage to cell membrane (Chandel *et al.*, 2015).

Seeds with higher fatty acid tend to decline faster compared with lower composition. Wilis varieties which contain the highest levels of fatty acid among other varieties in this study (Balitkabi, 2016), showed the lowest percentage of GS and VI (Table 4).

Table 4. The viability test on 6 soybean varieties

Varieties	GS (%)*	VI (%)*
Gepak Kuning	78.00 b	46.67 bc
Ijen	79.33 b	43.33 b
Kaba	69.33 b	39.33 ab
Sindoro	80.67 b	60.00 d
Tanggamus	74.67 b	57.33 cd
Wilis	50.00 a	30.00 a

Remarks: *) Different superscript letters denote significant differences between groups at the same column according to 5% DMRT; GS = germination strength test; VI = vigor index.

Effect of Storage Period on Vulnerability of Soybean Seeds

The storage period has significantly different effects on VI and GS, and maximum seedling growth potential (MGP) of soybean seed at the same moisture

Table 5. Effect of storage period on germination, vigor index, maximum growth potential, and moisture content of soybean seed

Storage period	VI (%)*	GS (%)*	MGP (%)*	SMC (%)NS
1 month	73,00±12,66 a	82,67±9,7 a	92,00±8,18 a	12,28±0,4
2 months	43,33±15,05 b	73,33±15,85 b	85,33±12,22 b	12,27±0,67
3 months	22±15,77 c	60,00±16,45 b	83,67±12,59 b	12,13±0,38

Remarks: *) Different superscript letters denote significant differences between groups at the same column according to 5% DMRT; NS: Not-significant; VI: vigor index; GS: germination strength; MGP: Maximum growing potential; SMC: seed moisture content.

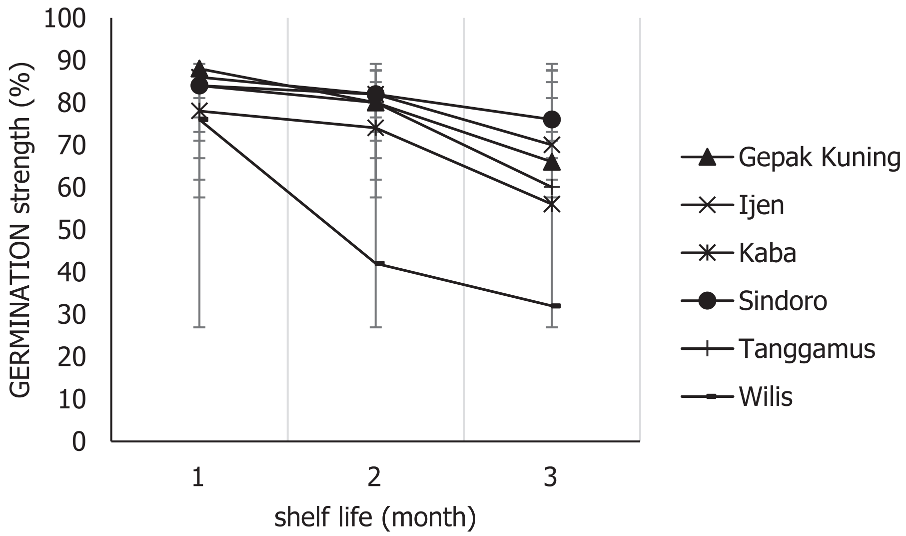

Figure 2. Effect of storage period on germination strength 6 varieties of soybean seeds

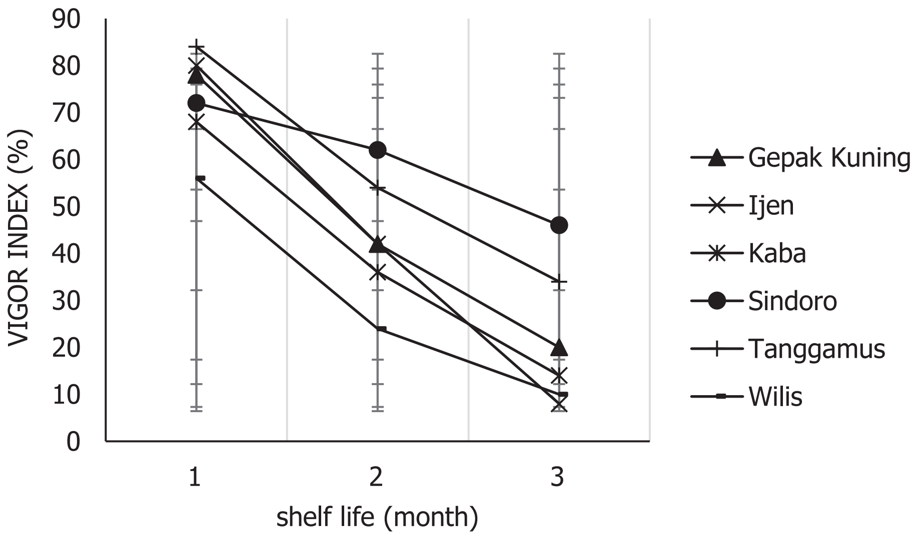

Figure 3. Effect of storage period on vigor index of 6 varieties of soybean seeds

content. The longer the seeds were stored, the lower percentages of VI, GS and MGP (Table 2, Figure 2 & 3). This describes the decline of the viability of soybean seed which naturally happens during storage.

Sindoro, Tanggamus, Ijen, and Gepak Kuning varieties have higher GS values for one month of storage and remain high up to 2 months of storage compared to Kaba and Wilis (Figure 2). Sindoro and Tanggamus varieties have higher VI than Gepak Kuning, Ijen, Kaba and Wilis (Figure 3). Seeds with high initial vigor will have a better vigor at the end of storage than seeds with low initial vigor.

The viability of seeds was naturally decreased during storage. The rate of seed deterioration varies greatly between species, between varieties, between lots, even between individuals in the same lot (Jatoi *et al.*, 2001).

The correlation between CDT treatment and soybean seed vigor

The final stage of statistical analysis was done by simple linear regression analysis on germination strength variable. Regression analysis showed that there was a strong correlation on CDT treatment (28% SMC, 40 °C, 24 hours) with 1-month storage, with the coefficient of correlation (r) 0.90122 and coefficient of determination (R2) 0.8122. Regression function drawn from devigoration and deterioration formulated by the

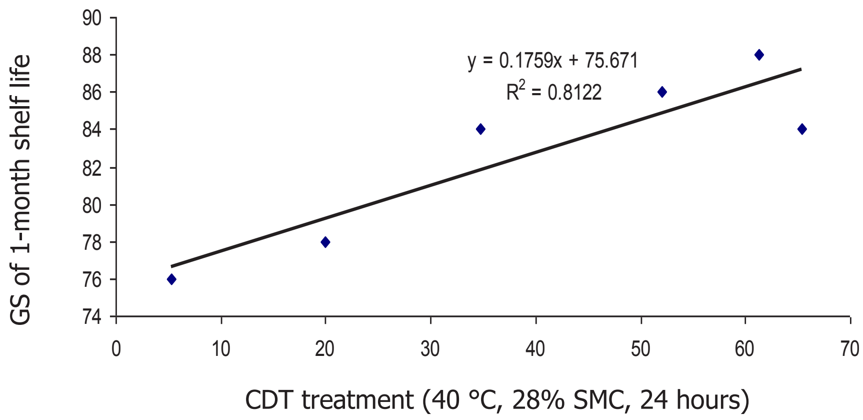

Figure 4. Regression model describing correlation of germination strength for 1-month storage

regression equation y = 0.1759x + 75.671 (Figure 4). The closer the relationship between two functions, the higher the validity of the simulation model (Sadjad *et al*., 1999).

In addition to the combined moisture content of 28%, 40 °C, and 24 hours, other CDT treatments did not indicate a close relationship with seed GS on shared storage period. Most analysis provide correlation coefficient values and small determinant coefficients. The smaller the correlation coefficient the further the connection. The magnitude of the correlation coefficient does not describe a causal relationship between two or more variables, merely describing linear interrelations between variables (Mattjik and Sumertajaya, 2006).

CDT treatment with 24% SMC, 45 °C, for 24 hours is the best method to estimate the physiological quality of onion seeds (Rodo & Filho, 2003) and beetroot (Silva *et al*., 2006)seeking to associate these results with seedling emergence in the field. Consequently, five seed lots of Top Tall Early Wonder cultivar were submitted to the tests of germination, seedling emergence in the field, accelerated ageing (using periods of 24, 48 and 72 hours at 42\u00b0C. Wafiroh *et al*. (2010) confirmed the CDT on sesame seeds with 20% SMC for 24 hours as a suitable condition to test the seed vigor.

Determination of CDT method on soybean seeds has been done by Nizaruddin *et al*. (2014). The results showed that the appropriate CDT method was 17.5% SMC for Wilis variety and 15% for Detam-1 variety, with the same storage period, i.e. 16 hours. A close correlation was demonstrated between the variables in controlled deterioration with the variables in the actual conditions by the 16-week storage period, which indicated that the duration of the exposure represents the shelf period.

In addition to temperature and moisture content, the physical parameters of seeds that may be factors affecting shelf life are seed coat color and seed size. The interaction between soybean coat color and room temperature was reported by Sukarman and Raharjo (2000), that small seed varieties and darker coats were more tolerant of physical stress (42 °C and 100% moisture) than large and light-coated varieties. The similar result also reported by (Purwanti, 2004). Seeds of yellow soybean that stored for six months at low temperatures of 21 °C - 23 °C still have high germination (> 80%), whereas at high temperatures 27 °C - 29 °C seed germination decreases to 41% the second month until the end of storage period. Black coated soybean seeds stored at low temperatures and high temperatures for six months are still able to maintain germination (> 90%), seed vigor, and growth rate compared to yellow soybeans. Based on the results of this study, it is recommended to validate CDT method on black soybean, because all the soybean seeds used in this study have a yellow coat.

The effect of soybean seed size with seed quality during storage was presented through Yulyatin & Diratmaja (2015)especially if stored in conditions that are less optimum savings. Soybean seed size can affect the quality of the seed. Seed quality is characterized by germination of seeds. Grain size effect on soybean utilization. Large seed size tends to be used as an industrial raw material utilization while small seed size as a seed planted back. Purpose of this study was to determine whether soybean seed size can affect the quality of the seeds while in storage. The experimental design used a Completely Randomized Design (CRD research. Large seeds (Grobogan), have a lower amount of normal seeds and lower germination than medium seeds (Kaba) and small (Wilis). The seeds used in this study include small seed size (Gepak Kuning & Wilis) and medium (Tanggamus, Kaba, Sindoro, Ijen), so it can give different results if used for large soybean seed.

CONCLUSIONS AND SUGGESTION

Combination of 28% seed moisture content at 40 °C for 24 hours in the controlled deterioration test was sensitive enough to evaluate the physiological potential of soybean seeds, providing information that was closely related to seed germination after stored for 1 month. Sindoro and Tanggamus varieties were shown to have higher viability and longer shelf life than other varieties.

CONFLICT OF INTEREST

There was no conflict interest of the article publication.

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