Rapid generation advanced for breeding of sorghum lines tolerant to low phosphorous condition

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Abstract. Sorghum lines tolerant to low phosphorous soil condition is essential to sorghum production in acid soil of Indonesia. An indirect breeding program to develop sorghum lines tolerant to low phosphorous soil condition had been conducted at the IPB University with Single Seed Decent method, which allow for generation advanced without selection. The population was developed from a cross of B69 x Numbu that have difference in tolerance to low P soil condition. The F2 generation was started in 2012 and advanced to 201 F6 recombinant inbred lines in 2016. The F6 lines were selected for good agronomic characters and followed by selection under low P and optimum P soil conditions in 2017. The single seed decent method was able to maintain wide genetic variability in the advanced generation for plant height, number of leaves, panicle length, panicle weight, and seed yield/plant. Selection based on Low P sensitivity index resulted in 12 sorghum lines selected as tolerant to low P soil conditions. This study showed that indirect breeding through rapid generation advances without selection can be used to develop sorghum lines with adaptation to unfavorable environment conditions.

1. Introduction
Sorghum is being developed as an alternative source of carbohydrates and health food in Indonesia. The cultivation of sorghum in Indonesia is directed to marginal areas, one of them is rain-fed areas with acid soil in the western part of Indonesia. In acid soil, phosphor availability is limited and becomes of the major constraints of sorghum cultivation in such areas, in addition to Aluminum toxicity. In sorghum, Phosphorous deficiency could lead to a reduction in plant height, delay flowering and reduced yield [9]. A high input approach by phosphorous fertilizer application is not always possible due to the limited resources of farmers in many marginal areas. Therefore, the more sustainable approach is to develop sorghum variety with adaptation to low P environment. Studies of sorghum adaptation to low P condition have been done in various sub optimum environmental conditions [14, 2, 1, 10].

A program to develop sorghum variety with adaptation to acid soil with high aluminum and low P condition has been conducted at IPB University since 2009. A selection of sorghum genotypes in acid soil showed that there is a high variability in sorghum adaptation to acid soil [18]. Further investigation showed that the acid soil tolerant variety, Numbu, is also tolerant to low P condition. The study also showed a differential mechanism of adaptation to low P condition among sorghum lines [1]. A cross between lines with different mechanisms of adaptation to low P condition is expected to result in progenies with higher tolerance to low P condition.
Breeding for adaptation to adverse environmental conditions should be conducted in a direct breeding approach, where successive generations of segregating population are selected in target environment. However, selection in sub-optimum condition often faced with low genetic gain resulting in tolerant genotype with low yielding potential [3]. An indirect breeding approach was used in the breeding of sorghum varieties adapted to low P condition to develop sorghum lines tolerant to low P condition with high yield potential. In this approach, early generations are grown under optimum condition, and selection for adaptation to low P condition is conducted in advanced generation [23].

The single-seed descent (SSD) method is one of the methods of advancing generation without selection in self-pollinated crops. The SSD method develops superior pure lines rapidly and has been used successfully used in cereals [7] and soybean [11]. The selection in the SSD method is done in advanced generation (F5 / F6) when the proportion of homozygosity is high. The advantage of the SSD method is that it can maintain broad genetic variability as in the early generations and can produce a number of lines that are superior for yields of advanced generations compared to another method such as pedigree [8,15].

In this study, the SSD method is used to form recombinant inbred lines of sorghum from segregating population following a cross of a P-deficient tolerant variety, Numbu, with a mutant line, B69. Both lines have high general combining ability and special combining ability (Rini et al, 2017). This paper reviews the effectiveness of single-seed descent method to develop P deficiency tolerant lines in sorghum.

2. Methods

2.1. Population Development and Structure
The population of Recombinant inbred lines were derived from a cross of B69 x Numbu. The female parent, B69, is a mutant line and the male parent, Numbu, is a high yielding national variety released in 2004. Numbu has been identified as tolerant to acid soil [18,21] and tolerant to low soil P condition [1,16]. The crossing was conducted in 2011. The generation advance was conducted using a single seed descent method without selection, started from the F2 generation in 2012 to F6 lines in 2016. The crossing and generation advanced were conducted in the IPB University Experimental Farm or farmer field, in Bogor, Indonesia.

2.2. Estimation of Genetic Variances
Estimation of genetic variances was conducted at every generation up to F5 generation to maintain genetic variances of the population. The genetic materials used were the populations of F2-F5 from the crossing of B69 x Numbu developed by a single seed descent method. One genotype is represented by one individual plant. The control varieties used were the two parental lines. The experiment was carried out by planting one genotype represented by one individual with a spacing of 70 cm x 10 cm. Urea fertilizer application was carried out twice, at sowing time, 50 kg ha\(^{-1}\) together with SP-36 fertilizer and KCl 100 kg ha\(^{-1}\). Second urea fertilization is carried out when the plants were 4 weeks after sowing at a dose of 100 kg ha\(^{-1}\). Observations were conducted for agronomic characters and yield components. Estimation of genetic variances, broad sense heritability and coefficient of Genetic Variances were done according to [19,20].

2.3. Selection for Tolerance to Low Soil-Phosphorus Condition
The selection for sorghum inbred lines tolerant to low soil P condition was conducted from May-September 2017 in Bogor, West Java. The genetic materials used were 19 inbred lines resulting from SSD from a population of B69 x Numbu. This experiment was conducted in a randomized complete block design with 3 replications in each environment differing in level of P fertilizer. The seeds were planted with a spacing of 10 cm in rows and 75 cm between spacing. Doses of urea and KCl during planting were 50 kg ha\(^{-1}\), and 100 kg ha\(^{-1}\), respectively. The second urea fertilizer was applied four weeks after showing at a dose of 100 kg ha\(^{-1}\). The SP-36 fertilizer was administered at a dose of 161 kg ha\(^{-1}\) under adequate P conditions and 80.5 kg ha\(^{-1}\) at low P conditions based on [22]. Observations
were made on plant height of the final vegetative phase, panicle weight, and seed weight per panicle. Data were analyzed by analysis of variance followed by Dunnet test, and t-test. Selection was based on the tolerance index according to [5].

3. Result and discussion
Line development following hybridization in the self-pollinated crops can be achieved through pedigree method, bulk population method and single seed decent. The single seed decent method was chosen for this breeding program because it can drastically reduce the size of the field required for advancing generations. In SSD method was done at an advanced generation when the homozygosity is high. The main constraint of the SSD method is the lost of genotypes due to seed viability. In this study the size of the population differed from generation to generation. There were 600 individuals in the F2 generation, 450 individuals in the F3 generation and 225 individuals in the F4 generation. The seeds of the F5 generation were planted head to row to develop 201 recombinant inbred lines (RILs).

The availability of diverse genetic material is very much needed in the selection activities in any breeding program. The success of selection is largely determined by genetic variability as estimated by the value of broad-sense heritability [19]. The estimated value of broad-sense heritability (Table 1) for agronomic characters of the F2 population derived from a cross of B69 x Numbu are high, except for stem diameter which has medium heritability [17]. A high value of heritability in the early generation of the segregating population is important for single seed descent methods to be effective.

Table 1. Components of variances, estimates of broad-sense heritability and coefficient of genetic variability of some agronomic characters in F2 population from B69 x Numbu

| Characters     | \((\sigma^2_p)\) | \((\sigma^2_g)\) | \((\sigma^2_e)\) | \(h^2_{bs}\) (%) | CGV (%) |
|----------------|------------------|------------------|------------------|------------------|---------|
| Plant height   | 698.96           | 162.32           | 536.64           | 76.77            | 10.79   |
| Stem diameter  | 0.07             | 0.04             | 0.03             | 42.86            | 17.25   |
| Panicle length | 15.40            | 6.96             | 8.44             | 54.81            | 13.58   |
| Panicle weight | 1269.31          | 498.04           | 771.27           | 60.76            | 34.43   |

In the F4 generation, the genotypes were still planted as a single plant representing all the genotypes of the previous generation. The data in Table 2 showed that the genetic variances and estimate of heritability of some agronomic characters and yield components of sorghum genotypes were still high [6].

Table 2. Components of variances, estimates of broad-sense heritability and coefficient of genetic variability of some agronomic characters in F4 population from B69 x Numbu

| Characters     | \((\sigma^2_g)\) | \((\sigma^2_p)\) | \((\sigma^2_e)\) | CGV (%) | \(h^2_{bs}\) (%) |
|----------------|------------------|------------------|------------------|---------|------------------|
| Stem diameter  | 2.2              | 11.0             | 13.1             | 20.1    | 84.0             |
| Plant height   | 87.1             | 799.4            | 886.5            | 12.6    | 90.0             |
| Panicle length | 2.3              | 9.9              | 12.2             | 14.9    | 81.0             |
| Panicle weight | 117.3            | 971.9            | 1089.2           | 39.9    | 89.0             |

Table 3. Components of variances, estimates of broad-sense heritability and coefficient of genetic variability of some agronomic characters in F6 population from B69 x Numbu

| Characters     | \(\sigma^2_g\)  | \(\sigma^2_p\)  | \(\sigma^2_e\)  | \(h^2_{bs}\) (%) | CGV (%) |
|----------------|-----------------|-----------------|-----------------|------------------|---------|
| Plant height   | 1020.24         | 399.95          | 655.01          | 61.06            | 8.54    |
| Stem diameter  | 0.02            | 0.02            | 0.03            | 66.67            | 6.74    |
| Panicle length | 1.81            | 5.31            | 5.76            | 92.18            | 9.14    |
| Panicle weight | 149.88          | 152.04          | 301.92          | 50.35            | 3.67    |
The advanced generation (F7) inbred lines derived from the SSD method that was previously selected for high yield potential in F6, were grown under two levels of P nutrient conditions. Low soil P nutrient conditions can cause a decrease in sorghum production by up to 59% [9]. The B69 mutant line, which is sensitive to low soil P nutrient, showed a marked decrease in yield from 44.77 g/ plant to only 16.18 g (62.5%), while the P-deficient tolerant line, Numbu, the decrease was only by 26.9%. The sorghum inbred lines in this study did not show a significant decrease in grain weight/panicle under low P soil condition, except for line 226.3. The sorghum inbred lines 114-7, 170-9, 115-9, 286.6 showed higher grain weight per panicle than the tolerant parent line, Numbu [12].

Table 4. Stress tolerance index of sorghum inbred lines based on grain yield/plant

| Inbred Lines | Grain yield/plant | YN | YS | YS/YN | STI |
|--------------|-------------------|----|----|--------|-----|
| 104-7        |                   | 46.10 | 36.56 | 0.79 | 1.05 |
| 110-6        |                   | 21.50 | 25.73 | 1.20 | 0.35 |
| 114-7        |                   | 46.53 | 45.80 | 0.98 | 1.33 |
| 115-9        |                   | 51.79 | 45.97 | 0.89 | 1.49 |
| 151-8        |                   | 48.76 | 38.11 | 0.78 | 1.16 |
| 170-9        |                   | 47.14 | 40.56 | 0.86 | 1.20 |
| 177-4        |                   | 30.83 | 21.40 | 0.69 | 0.41 |
| 221-8        |                   | 35.43 | 28.43 | 0.80 | 0.63 |
| 226-3        |                   | 43.59 | 32.99 | 0.76 | 0.90 |
| 286-6        |                   | 60.60 | 42.42 | 0.70 | 1.61 |
| 315-6        |                   | 40.92 | 35.13 | 0.86 | 0.90 |
| 331-8        |                   | 43.26 | 44.58 | 1.03 | 1.21 |
| 341-7        |                   | 38.07 | 37.21 | 0.98 | 0.89 |
| 377-9        |                   | 25.90 | 19.85 | 0.77 | 0.32 |
| 413-7        |                   | 44.43 | 35.38 | 0.80 | 0.98 |
| 418-4        |                   | 42.43 | 28.77 | 0.68 | 0.76 |
| 48-4         |                   | 23.55 | 18.05 | 0.77 | 0.27 |
| 68-5         |                   | 42.39 | 36.78 | 0.87 | 0.98 |
| 93-5         |                   | 25.49 | 19.31 | 0.76 | 0.31 |
| B69          |                   | 44.77 | 16.18* | 0.36 | 0.45 |
| NUMBU        |                   | 50.78 | 37.08* | 0.73 | 1.18 |

STI = Stress Tolerance Index, YS = mean yield of a line at low P, YN = mean yield of a line under optimum P, YN^2 = squared mean yield of a line under optimum P.

Tolerant lines are lines that are able to maintain growth and yield under stress conditions. Selection for tolerant lines was performed using the Stress Tolerance Index criteria that can compare the differences in the mean values of the inbred lines tested with their parents insufficient P and low P conditions. The tolerant line, Numbu, was able to maintain both growth and yields at low P conditions, with STI value greater than 1. Sorghum inbred lines 286-6, 115-9, 114-7, 331-8, 170-9, 151-8, 104 -7, 331-8 have STI values greater than 1 and were selected as tolerant to low soil P conditions [12]. Inbred lines 114-7, 170-9, 115-9, 286.6, in addition to being tolerant lines they are also adaptive lines with high grain weight/panicle under stress of low soil-P condition.

This breeding program has successfully selected inbred lines that are tolerant of low soil P nutrient conditions. This shows that the SSD method used to develop lines from the segregating population following hybridization can maintain genetic variability for generations. Inbred lines developed from the SSD method can be selected for yield potential in F6 generation and selected for tolerance to low soil P nutrient conditions in F7 generation. The SSD method can be recommended for sorghum
breeding with the aim of improving yield and improving adaptation to abiotic stress with an indirect breeding approach.

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