Morphological characterization of sorghum lines with aluminium stress and phosphorus deficiency tolerance

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Abstract. Sorghum has a good adaptation in marginal land, such as acid soil. The development of acid soil tolerant variety was directed to combine with Al stress and P deficiency adaptation. Characterization of the qualitative and quantitative traits was necessary to obtain the line performance information. The traits were expected to have a distinct, uniform, and stable traits as a requirement for variety registration. The purpose of this research was to obtain information about the quantitative and qualitative character of F9 sorghum lines. The study was conducted from March to July 2019 at Cikahayan Bawah Experimental Field, Plant Breeding Laboratory, and Micro technical Laboratory, Department of Agronomy and Horticulture, IPB University. Randomized complete block design with genotype as a treatment factor was carried out in this experiment. Analysis of variance showed significant differences in quantitative and qualitative traits observed. Based on cluster analysis, Sorghum lines and check varieties formed three groups, group 1 (Super 2), group 2 (170-9, 151-8, dan 114-7) and group 3 (67-9, 104-7, 115-9, 286-6, 331-8, and Numbu). Three promising sorghum lines were identified for varietal release, i.e., 114-7, 115-9, and 331-8.

1. Introduction
Sorghum is one of the alternative food crops that have the potential to develop. People consume sorghum as food and drinks such as rice, cakes, and beverages. Sorghum has various nutrients to meet human nutritional needs. According to Etuk et al. [1], sorghum contains 8.9-10.48% protein, 2.5-3.7% fat, 1.4-3.01% fiber, and 61.24-76.6% sugar. FAO [2] stated that there are 4.5 mg niacin (vitamin B3), 0.13 mg riboflavin (vitamin B2), and 0.47 mg pyridoxine (vitamin B6) in every 100 grams of sorghum grains. Developing sorghum with acid soil, Aluminium (Al) stress, and Phosphor (P) deficiency tolerant is one of sorghum breeding goals.

Department of Agronomy and Horticulture IPB had produced several promising lines by crossing B69 and Numbu. B69 is a mutant strain from the Durra line that irradiated with 200 Gy gamma rays [3]; meanwhile, Numbu is a public variety. B69 line had a moderate tolerance level to acid soil based on biomass weight and grain weight per panicle. Numbu had the best performance in Al stress conditions and P deficiency [4]. The promising lines selected by the single seed descent (SSD) method (Momongan et al, 2019) [5]. This selection was able to get homozygotes in a short time [6]. We can obtain information about the performance of the lines by characterizing the qualitative and quantitative characters. The characterization information was a requirement for variety register at the Pusat Perlindungan Varietas Tanaman dan Perizinan Pertanian or Center for Plant Variety and Agricultural Licensing Protection (PPVTTPP). The new promising lines were expected to have a unique, uniform,
and stable character. The purpose of this research was to obtain information about the quantitative and qualitative character of F9 sorghum lines.

2. Methods
This research conducted in Cikabayan Bawah Experimental Field, Plant Breeding, and Micro technical Laboratory, Department Agronomy and Horticulture, IPB University, from March to July 2019. Randomized complete block design with ten genotypes and three replications carried out in this research. The material used in the research were eight F9 generations of sorghum lines (67-9, 104-7, 114-7, 115-9, 151-8, 170-9, 286-6, 331-8) and two check varieties (Numbu and Super 2). Standard sorghum cultivation (planting, thinning, fertilization, pests and diseases controlling, and harvesting) was carried out. Analysis of variance was used to analyzed quantitative data and continued with the t-Dunnett test if there were any significant differences. Qualitative data explained descriptively. The reference for qualitative observation was the form of a guide UPOV [7] number TG / 122/4 (proj.2) about guidelines for testing the performance, uniformity, and stability of sorghum plants referred as well by Elangovan et al. [8]. The observed characters included anthocyanin intensity and morphology of leaf, stem, flower, panicle, and seed.

3. Results and Discussion
3.1 Quantitative Character Performance
The analysis of variance revealed highly significant differences among genotypes on the plant height, flowering time, panicle branch length, and 1000 grains weight characters. Genotypes had a significant difference in the character of leaf length; meanwhile genotypes did not have a significant difference in the character of leaf width, stem diameter, and panicle length. The value of the coefficient of variance (CV) in the observed characters ranged from 3.917% - 13.090%. According to Gomez and Gomez [9], the coefficient variance showed the experiment diversity. The higher level of variable accuracy reflected by the lower CV value. CV value tolerance for field research was 20%.

Table 1. Analysis of variance for various quantitative characters in sorghum lines and check varieties

| No. | Characters               | MS Genotype | Pr>F  | CV (%) |
|-----|-------------------------|-------------|-------|--------|
| 1   | Leaf length             | 67.069*     | 0.0132| 5.676  |
| 2   | Leaf width              | 1.505<sub>tn</sub> | 0.0658 | 10.386 |
| 3   | Stem diameter           | 0.042<sub>tn</sub> | 0.1498 | 11.589 |
| 4   | Plant height            | 2620.171** | <.0001 | 6.050  |
| 5   | Days to flowering       | 39.836**    | 0.0020 | 3.917  |
| 6   | Panicle length          | 4.431<sup>tn</sup> | 0.0945 | 7.667  |
| 8   | 1000 grain weight       | 31.449**    | 0.0011 | 13.090 |

* = significantly different, ** = highly significant, ns = non-significant, MS: Mean Square. CV: coefficient of variation.

Table 2 showed the leaf performance variation in the observed lines. Lines 115-9 and 170-9 had broadleaf, and line 115-9 had very longleaf. Leaf width character controlled by the gene on chromosomes 1, 4, and 6; meanwhile, chromosome number 10 controlled leaf length characters [10]. Leaf width and length were related to the leaf area. The leaf area determined the ability of plants to carry out photosynthesis. An increase in leaf area can increase the interception of solar radiation for photosynthesis [11]. The observation result showed that line 115-9 had significantly higher leaf length compared to Super 2 variety. Also, lines 67-9 and 104-7 had significantly shorter leaf length compared to leaves of the Numbu variety.
Table 2. Characteristics of leaf width, leaf length, days to flowering, panicles length of sorghum lines and check varieties

| Lines  | Leaf width (cm) | Categories | Leaf length (cm) | Categories | Days to flowering (DAT) | Categories | Panicle length (cm) | Categories |
|--------|-----------------|------------|------------------|------------|------------------------|------------|---------------------|------------|
| 67-9   | 6.550           | Broad      | 72.41a           | Long       | 72b                    | Medium     | 4.74                | Short      |
| 104-7  | 7.039           | Broad      | 73.59a           | Long       | 68b                    | Medium     | 5.10                | Medium     |
| 114-7  | 7.967           | Broad      | 78.49            | Long       | 69b                    | Medium     | 6.22ab              | Medium     |
| 115-9  | 8.633           | Very broad | 81.04b           | Very long  | 70b                    | Medium     | 6.51ab              | Medium     |
| 151-8  | 7.794           | Broad      | 75.78            | Long       | 71b                    | Medium     | 6.35ab              | Medium     |
| 170-9  | 8.800           | Very broad | 78.64            | Long       | 69b                    | Medium     | 6.86ab              | Medium     |
| 286-6  | 7.944           | Broad      | 79.49            | Long       | 70b                    | Medium     | 5.91ab              | Medium     |
| 331-8  | 7.667           | Broad      | 79.24            | Long       | 70b                    | Medium     | 6.36ab              | Medium     |
| Numbu  | 7.889           | Broad      | 85.41            | Very Long  | 74                     | Medium     | 4.19                | Short      |
| Super 2 | 6.550           | Broad      | 69.09            | Long       | 81                     | Late       | 4.12                | Short      |

Note: the number followed by the letter is significantly different from the comparative variety at the 5% level. a = significantly different than the Numbu variety, b = significantly different than the Super 2 variety, DAT = Days after transplant.

The result in Table 2 showed that promising lines had an average flowering age compared to the Super 2 variety. Days to flowering information can be used to breeding selection for early harvest variety and prevent long anthesis silking intervals (ASI) [12]. Days to flowering in this research was one to two weeks delayed or around 68-81 days after transplant (DAT). However, the promising lines had an early flowering age compared to check variety. The delayed flowering time in this research, presumably because of high humidity pressure because of the rainy season during the research. High humidity had a negative correlation with temperature and day length, meanwhile sorghum need a high temperature and a long day period to hit the cumulative degree unit for the growth stage [13, 14].

The promising lines produced by IPB breeding program had medium panicle lengths except for lines 67-9. Lines 114-7, 115-9, 151-8, 170-9, 286-6, and 331-8 had significantly longer panicle branch length compared to Numbu and Super 2 varieties. Numbu and Super 2 varieties had short panicle branch lengths. Rakshit et al. [15] explained that panicle branch length had a strong and positive correlation with panicle length and panicle width. Kaitaniemi [16] also found that there was a positive correlation between panicle branch length and the number of seeds per panicle.

The promising line had medium to high plant height categories. The result was due to the selection process in F4 lines conducted by Sulistyowati [17]. The selection criteria based on relatively medium plant height. Plant with high height performance usually used as a criteria selection for the sugar production and animal feed breeding, while short crop performance was a criteria selection for early harvesting age. Plant height character was a vegetative character that shows plant growth because good plant growth will provide good yields [18].

All of the promising lines and check varieties had small stem diameters. The low result of stem diameter caused by various environmental conditions, especially in block 1, which had the least optimum conditions. However, there were some sorghum lines (115-9, 170-9, and 331-8) that had relatively larger stem diameters (Table 3). Tsuchihashi and Goto [19] explained that large stem diameters could reduce the risk of falling stems, and have good ratability.
Table 3. Performance of plant height, stem diameter, panicle length, and weight of 1000 grains of sorghum lines and check varieties

| Lines   | Plant height (cm) | Categories | Stem diameter (cm) | Categories | Panicle length (cm) | Categories | 1000 grain weight (g) | Categories |
|---------|-------------------|------------|-------------------|------------|---------------------|------------|-----------------------|------------|
| 67-9    | 198.00b           | Medium     | 1.158             | Small      | 16.86               | Short      | 21.043                | Low        |
| 104-7   | 227.89b           | Tall       | 1.212             | Small      | 18.64               | Short      | 14.358b               | Very Low   |
| 114-7   | 209.31b           | Medium     | 1.292             | Small      | 20.20               | Medium     | 14.036b               | Very Low   |
| 115-9   | 267.46ab          | Tall       | 1.467             | Small      | 19.58               | Short      | 18.723                | Low        |
| 151-8   | 220.06b           | Medium     | 1.324             | Small      | 20.08               | Medium     | 14.944b               | Very Low   |
| 170-9   | 219.00b           | Medium     | 1.451             | Small      | 18.69               | Short      | 13.871b               | Very Low   |
| 286-6   | 231.06b           | Tall       | 1.361             | Small      | 20.17               | Medium     | 20.328                | Low        |
| 331-8   | 234.67ab          | Tall       | 1.436             | Small      | 18.14               | Short      | 22.569                | Low        |
| Numbu   | 199.36            | Medium     | 1.432             | Small      | 17.12               | Short      | 19.507                | Low        |
| Super 2 | 290.51            | Tall       | 1.204             | Small      | 20.44               | Medium     | 21.050                | Low        |

Note: the number followed by the letter is significantly different from the comparative variety at the 5% level. a = significantly different than the Numbu variety, b = significantly different than the Super 2 variety.

Panicles length of promising lines had a short to moderate size in the range of 16.86-20.20 cm (Table 3). Line 114-7 had longer panicle length compared to other lines. Momongan [20] selected sorghum lines based on the largest stem diameter and panicle length. The result showed that promising lines and check varieties had very low and low categories of 1000 grains weight character. Lines 104-7, 114-7, 151-8, and 170-9 were significantly lower than Super 2 variety. The low weight of 1000 grain weight in this research occurred due to the low stay green character at the time of the research. The leaves and stems of the plant began to turn brown before the harvest time and caused the photosynthesis process is less efficient in filling seeds. 1000 grain weight indicates the efficiency of photosynthate accumulation in seeds [21].

3.2 Qualitative Character Performance

3.2.1 Anthocyanin intensity in Coleoptoptiles, Leaves, Pistils, and Husk. Anthocyanin intensity observations carried out on coleoptiles, leaves, pistils, and husks. The results showed that the sorghum promising lines did not have anthocyanin content in coleoptiles, leaves, and pistils. Anthocyanins found in Numbu flower husks with moderate anthocyanin intensity. Anthocyanins are flavonoid compounds that produce orange, orange, red, purple, and blue colors in some parts of the plant [22]. This compound was polar, so it dissolves quickly in polar compounds such as water [23]. The type of anthocyanin found in sorghum plants is 3-deoxyanthocyanidin. These compounds found in many parts of the pericarp [24]. Sukartini and Syah [25] explain that the synthesis of anthocyanin occurring during leaf growth, senescence period, and response to abiotic stress.

3.2.2 Performance of Leaves and Flowers. The observation result showed the diverse intensity of green leaves ranging from weak to strong intensities. Check varieties had medium leaf green intensity. The promising lines had a yellowish-white leaf bone color except for the 170-9 line, which has a white leaf bone color. All of the breeding lines and check varieties do not have gradations of leaf bone color to leaf strands. The green leaf intensity character is related to chlorophyll content in the leaves [26]. Chlorophyll is a green pigment in the plants. This pigment functions in the process of plant photosynthesis by absorbing and converting light energy into chemical energy. Higher chlorophyll content indicates the process of photosynthesis in plants more efficiently. Leaf bone color has a close relationship with sugar levels. Sweet sorghum has leaf bone color from white to green. The color of green leaf bones has a strong correlation with sugar content and water content [27].
The green leaf intensity character is related to chlorophyll content in the leaves [28]. Chlorophyll is a green pigment in the plants. This pigment functions in the process of plant photosynthesis by absorbing and converting light energy into chemical energy. Higher chlorophyll content indicates the process of photosynthesis in plants more efficiently. Leaf bone color has a close relationship with sugar levels. Sweet sorghum has leaf bone color from white to green. The color of green leaf bones has a strong correlation with sugar content and water content [29].

The pistil colors of the lines were bright white, yellow, and yellow. Numbu and Super 2 varieties had white pistil color. The pistil color controlled by the locus y-cs (the candystripe locus). The locus controls the yellow pigment in the pistil flower [30]. The stamens of promising lines had reddish-orange and orange colors. Numbu varieties had reddish-orange dry stamen color, and Super 2 was yellow. There were various length pistil in IPB breeding lines from very short, short, medium, and long. Lines 104-7, 114-7, and 286-6 had very short pistils. Lines 170-9 and 331-8 had short pistils. Lines 67-9 and 115-9 had medium-sized pistils. Line 151-8 had long sized pistils. Pistil length has little correlation to pollen germination and pollen tube growth [31].

### 3.2.3 Performance of Leaves and Flowers

Panicle length of breeding lines were broad panicles in the middle and width at the top. Lines 67-9, 115-9, and 170-9 had a broad panicle shape at the top. The panicles of Numbu and Super 2 varieties have a broad middle shape (Table 4). According to Menkir et al. [32], panicle forms have a weak correlation with grain mold attack. Rao et al. [33] also explained that the panicle form was an essential character in determining grain yield, also variety identification and classification. The density of sorghum panicles observed after the pollination phase. Promising lines had sparse to dense panicles (Table 4). Numbu variety had medium panicle density, and Super 2 variety had sparse panicle density. Brown et al. [34] explained that panicle density was affected by branching and elongation of the inflorescence. The aborted spikelet also influences panicle density.

The ability to self-pollinate observed in covered panicles before the flowers bloomed to avoid cross-pollination. The percentage of successful pollination itself assessed from the number of flowers that succeed in forming seeds. The results showed that line 104-7 could self-pollinate as a whole. Lines 67-9, 114-7, and 170-9 had low self-pollinating ability. Lines 115-9, 151-8, 286-6, and 331-8 can self-pollinate on a partial level. Check varieties had a moderate level of ability to self-pollinate. The low percentage of self-pollination thought to be due to the panicle condition, which is covered, is less than optimal for pollination. According to Major [35], temperature and relative humidity can influence the pollination process. House [36] also explains that panicle forms play a role in sorghum pollination. Self-pollinated sorghum had a closed and compact panicle, while sorghum with open panicle form had a 30-60% chance to cross-pollinate.

### Table 4. Performance of morphological characters of leaves and flowers of sorghum lines and check varieties

| Lines  | Green leaves intensity | Leaf bone color   | Pistil color     | Pistil length | Dry stamen color |
|--------|-----------------------|-------------------|------------------|---------------|------------------|
| 67-9   | Weak                  | Yellowish white   | Bright yellow    | Medium        | Reddish orange   |
| 104-7  | Medium                | Yellowish white   | White            | Very short    | Orange           |
| 114-7  | Weak                  | Yellowish white   | White            | Very short    | Orange           |
| 115-9  | Medium                | Yellowish white   | Yellow           | Medium        | Reddish orange   |
| 151-8  | Strong                | Yellowish white   | Bright yellow    | Long          | Reddish orange   |
| 170-9  | Strong                | White             | Yellow           | Short         | Reddish orange   |
| 286-6  | Medium                | Yellowish white   | White            | Very short    | Reddish orange   |
| 331-8  | Strong                | Yellowish white   | Bright yellow    | Short         | Orange           |
| Numbu  | Medium                | Yellowish white   | White            | Medium        | Reddish orange   |
| Super 2| Medium                | White             | White            | Short         | Yellow           |

The ability to self-pollinate observed in covered panicles before the flowers bloomed to avoid cross-pollination. The percentage of successful pollination itself assessed from the number of flowers that succeed in forming seeds. The results showed that line 104-7 could self-pollinate as a whole. Lines 67-9, 114-7, and 170-9 had low self-pollinating ability. Lines 115-9, 151-8, 286-6, and 331-8 can self-pollinate on a partial level. Check varieties had a moderate level of ability to self-pollinate. The low percentage of self-pollination thought to be due to the panicle condition, which is covered, is less than optimal for pollination. According to Major [35], temperature and relative humidity can influence the pollination process. House [36] also explains that panicle forms play a role in sorghum pollination. Self-pollinated sorghum had a closed and compact panicle, while sorghum with open panicle form had a 30-60% chance to cross-pollinate.
Husk color observed after the harvesting process. The promising lines had yellow and brown husks. Lines 67-9, 104-7, 115-9, and 331-8 had yellow husks. Lines 114-7, 151-8, 170-9, and 286-6 had brown husk color. The difference color suspected from lignin content in the husk. Sorghum husk had high lignin and cellulose content [37]. The husk will turn dark when entering the ripening phase. The husk color controlled by the same two loci (P and Q). Dominant gene action will make the black and red color appear, while the brownish-red color appears by the recessive gene [38]. Sorghum husk lines had short to long size. The comparative variety has a short husk size. The short husk gene (Sg) controls the size of the husk. The gene has an epistatic gene action [39, 40].

The promising lines had various seed colors. Lines 114-7 and 151-8 had white grain color, and lines 104-7 and 115-9 had a yellowish-white grain color. Grain color of lines 286-6 and 331-8 were yellow, lines 67-9, and 170-9 had brown grain color. Numbu variety had yellow grain color, and Super 2 had dark brown grain color. The vary grain color caused by pericarp color differences. The pericarp color influenced by the combination of anthocyanin and anthocyanidin pigments and the presence of flavonoid compounds [41]. Grain color played an important role in determining the choice of sorghum as food. Sorghum with white grain color commonly used as food because of containing little tannin compounds [42].

### Table 5. Performance of panicles of sorghum lines

| Lines | Panicle shape | Panicle density | Self-pollinated rated |
|-------|---------------|-----------------|-----------------------|
| 67-9  | Broad at the top | Medium          | (0-10%) not self-pollinated |
| 104-7 | Broad at the middle | Medium          | (71-100%) self-pollinated |
| 114-7 | Broad at the middle | Sparse         | (0-10%) not self-pollinated |
| 115-9 | Broad at the top | Dense           | (11-70%) partial self-pollinated |
| 151-8 | Broad at the middle | Dense         | (11-70%) partial self-pollinated |
| 170-9 | Broad at the top | Medium          | (0-10%) not self-pollinated |
| 286-6 | Broad at the middle | Sparse         | (11-70%) partial self-pollinated |
| 331-8 | Broad at the middle | Medium         | (11-70%) partial self-pollinated |
| Numbu | Broad at the middle | Medium         | (11-70%) partial self-pollinated |
| Super 2 | Broad at the middle | Very sparse    | (11-70%) partial self-pollinated |

The promising lines had oval and circular grain shape. Check varieties had an oval seed shape (Table 5). The locus that controls seed shape found on chromosome 7. Seed shape controlled by major genes along with other characters such as grain size and grain weight [43]. Audilakshmi and Aruna [44] also explained that dominant genes controlled the grain shape. The dominant gene action was advantageous for the formation and development of sorghum, which has the desired seed shape by using one of the elders who possesses these characteristics.

The promising lines had medium embryo size and white endosperm. The Super 2 variety has a relatively smaller embryo size than other lines. All promising lines and comparative varieties had white endosperms. Embryo size had a positive correlation with seed quality. The greater the embryo,
the higher the seed quality [45]. Endosperm color influenced by the content of compounds contained in the endosperm. Yellow color in endosperm correlates to the presence of carotenoids, β-carotene, and zeaxanthin [46].

The endosperm of promising lines had various textures. Lines that had a starchy endosperm texture were 67-9, 104-7, 114-7, 115-9, 151-8, and 170-9, while lines 286-6 and 331-8 had a relatively harder endosperm texture. The Numbu and Super 2 varieties have relatively harder endosperm texture. The texture of the endosperm determines the quality of the seeds. Seeds that have a hard endosperm texture have relatively smooth seeds, which make it easier to thresh. Endosperm texture also has a relationship to seed weight. Seeds that have harder endosperms have higher seed weights, while seeds with starchy texture have relatively lower yield potential [47].

4. Conclusion
The promising lines produced by IPB breeding had varied quantitative and qualitative characters. The characters that had significantly different in quantitative characters were leaf length, leaf width, panicle length, plant height, and 1000 grain weight. Leaf green intensity, leaf bone color, pistil color, pistil length, dry stamens color, panicle shape, panicle density after pollination, self-pollination, husk color, husk length, seed color, seed shape, and endosperm texture had various qualitative characters. All sorghum lines showed no diversity in the characteristics of anthocyanin intensity, gradation of leaf bone color, embryo size, and endosperm color.

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