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Characteristics contribution and similarity of F5 soybean lines resistant to cowpea mild mottle virus derived from different crossing

SITI ZUBAIDAH[1,♥], HERU KUSWANTORO[2,♥♥], EMERENSIANA UGE[2], KENNIS ROZANA[1], PURWANTORO[2]

[1]Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang. Jl. Semarang No. 5, Malang 64145, East Java, Indonesia. Tel.: +62-341-562180, ♥email: siti.zubaidah.fmipa@um.ac.id

[2]Research Center for Food Crops - Research Organization for Agriculture and Food, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km. 46 Cibinong, Bogor 16911, West Java, Indonesia. Tel.: +62811-1933-3639, ♥♥email: heru032@brin.go.id

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Abstract. *Zubaidah S, Kuswantoro H, Uge E, Rozana K, Purwantoro. 2023. Characteristics contribution and similarity of F5 soybean lines resistant to cowpea mild mottle virus derived from different crossing. Biodiversitas 24: 5399-5408.* Virus is one of the pathogens that can infect soybeans. One of the crucial viruses is Cowpea Mild Mottle Virus (CpMMV), which attacks many soybean plants in Indonesia. The objective of this study was to explore the character's contribution and similarity to identify F5 CpMMV-resistant soybean lines. The plant materials were F5 CpMMV-resistant soybean lines from 26 different crosses. CpMMV inoculation was performed naturally by placing the experiment at the site previously infected with *Bemisia tabaci* as the CpMMV virus vector. Observations were made on agronomic characters and disease scores. The results showed that there was a variation in the observed agronomic characters, but disease scores were generally at a score of 2. Seed yield, number of filled pods, and number of seeds showed strong character contributions. Disease scores generally had the opposite direction to seed size. There was a significant correlation between agronomic characters, but disease scores had no correlation with agronomic characters. M and N crosses showed the highest seed yields. The disease score for these two crosses was 2. These two crosses also formed a separate group. There were four groups of crosses with different numbers of members. The groups formed were more influenced by the variability of the number of seeds, filled pods, and plant height. Based on seed yield and disease score, M and N crosses should be further developed to obtain high-yielding CpMMV-resistant soybean varieties.

Keywords: Agronomic characters, *Bemisia tabaci*, cluster, CpMMV, resistant lines, soybean

INTRODUCTION

Soybean is an important food crop and the number one commodity of various legumes in Indonesia. Approximately 3.09 million tons of soybeans are consumed nationally every year. However, 2.67 million tons (86.4%) are imported (Triyanti 2020). One of the production constraints is the attack of diseases due to soybean plants' susceptibility to many pathogens, such as fungi, and viruses. Cowpea mild mottle virus (CpMMV) is a carlavirus group known as a pathogenic virus in soybean. Symptoms of CpMMV infection in soybean plants vary widely, including stem necrosis, systemic mottling, mosaic on shoots, and stunting (Zubaidah and Kuswantoro 2016; Wei et al. 2021). CpMMV can be transmitted by whitefly (*Bemisia tabaci*) and carried by seeds (Barreto da Silva et al. 2020; Sutrawati et al. 2021). This virus is also reported to have a broad host range (Lamas et al. 2017). The wide range of hosts and the mechanisms of transmission through seeds and vectors lead to the spread in the field can take place quickly. CpMMV has been reported to cause yield loss in quantity and quality (Yadav et al. 2013). Virus infection in soybeans can cause yield loss of 50-100% and failure to harvest (Song et al. 2016).

The use of varieties is one of the techniques recommended in the principle of integrated pest management because it is effective, inexpensive, and sustainable (Yadav et al. 2023). The development of disease-resistant varieties can be done by selecting resistant lines by looking at the response of plants after infection, which can be seen in the form of infection rates. Resistance to pathogen infection is one of the focuses of global breeding programs (Ross et al. 2021). In Indonesia, information on soybean varieties resistant to CpMMV is not yet available. The availability of information on CpMMV-resistant genotypes can provide a more incredible opportunity for the success of a CpMMV-resistant soybean breeding program (Barreto da Silva et al. 2020). Sutrisno and Kuswantoro (2016) tested ten soybean varieties from South Korea against CpMMV infection and found that two varieties were recommended as sources of resistance and tolerance to CpMMV. Two genotypes from India were also identified as sources of soybean resistance to CpMMV (Cheruku et al. 2017).

Using host plants and resistant vectors is the most effective control option against the transmission of seed-borne viruses (Sastry 2013). CpMMV is known to be a seed-borne virus and vector. Therefore, resistant and tolerant varieties are very supportive for preventing yield loss due to infection with this virus (Nurrohman et al. 2019). The CpMMV-resistant soybean variety breeding program is a crucial activity to produce new high-yielding soybean varieties with the objectives and characteristics of

CpMMV resistance. The success of breeding programs depends on the availability of genetic resources that have high diversity. Crossing soybean genotypes with high genetic diversity and distant kinship can speed up breeding program (Liu et al. 2017; Gwinner et al. 2017). Plant genetic diversity can be identified using various methods, including studying phenotypic characters and enzymatic markers (Zambiazzi et al. 2017), agromorphology, phytochemical profiles, and antioxidant activity (Bouargalne et al. 2022). The phenotypic characteristics of each genotype with diverse agronomic traits can refer to differences in geographic environment (Cuevas and Prom 2020).

Variability in breeding materials can improve the chance to expedite the breeding process for cultivars with desired characteristics. Many breeding materials with various characteristics require qualified tools to select materials for later use. Various selection methods have been developed to select breeding materials so that the selected lines match the desirable characteristics. Character contribution from breeding materials plays an important role in selecting these lines. The lines can be grouped using the contribution of the characters (Badiaraja et al. 2021a; Kuswantoro et al. 2020; Maranna et al. 2021) so that the character possessed and its similarity becomes clear. Besides, the similarity or cluster can describe the population structure (Jeong et al. 2019; Tiwari et al. 2019). In this study, the contribution of each character from several crosses was examined to determine the main character of the lines from different crosses. The objective of this study was to investigate the contribution of the agronomic characters and disease incidence level, and the similarity of the lines as the foundation to distinguish F5 CpMMV-resistant soybean lines.

MATERIALS AND METHODS

Plant materials

The plant materials were F5 lines derived from 26 crossings (Table 1). The parents crossing were Argomulyo, Burangrang, Grobogan, Panderman, UM 2.4, UM 3.2, UM 3.4, UM 4.1, UM 6.1, UM 6.2, UM 6.3, UM 7.2, and UM 7.6. Argomulyo, Burangrang, Grobogan, and Panderman were soybean varieties with large seeds, while UM 2.4, UM 3.2, UM 3.4, UM 4.1, UM 6.1, UM 6.2, UM 6.3, UM 7.2, and UM 7.6 were CpMMV-resistant soybean lines.

Study site and experiment design

The experiment was conducted at 8.05568 S, 112.66675 E, and 478 meters above sea level in Srigading Village, Tajinan Sub-district, Malang District, East Java, Indonesia. On February 9, 2023, the process of planting was carried out. The experiment was conducted on augmented design, with a spacing of 40 cm × 25 cm and two plants per hill, the soybean lines were grown 3 m long.

Soil tillage and fertilization

Soil ploughing was done as efficiently as possible to develop the proper soil structure for the growth of soybean plants. Drainage channels were built, and weeds were eliminated with insecticides before planting. To apply fertilizer, 50 kg of urea, 75 kg of SP36, and 75 kg of KCl were used.

Agronomic observation

Agronomic observations were done on plant height (HGT), number of branches (BRC), number of filled and unfilled pods (POF and POU), number of reproductive nodes (NOD), 100-seed weight (SW), and number of seeds (NS), and seed yield (YLD).

Disease inoculation

Before the experiment began, *B. tabaci* was propagated as a CpMMV virus vector in the field. Experimental planting was conducted on the virus vector population so that inoculation occurred naturally.

Disease observation

The disease was scored according to Zubaidah and Kuswantoro (2016).

Data analysis

The contribution, similarity, PCA biplot, and correlation were calculated and visualized using R program version R 4.3.1.

Table 1. Plant materials used in the experiment

Codes	Cross lines
A	Argomulyo × UM 6.3
B	Burangrang × UM 3.4
C	Burangrang × UM 4.1
D	Burangrang × UM 7.2
E	Burangrang × UM 7.6
F	Grobogan × UM 2.4
G	Grobogan × UM 3.2
H	Grobogan × UM 3.4
I	Grobogan × UM 4.1
J	Grobogan × UM 6.1
K	Panderman × UM 3.4
L	Panderman × UM 4.1
M	Panderman × UM 6.2
N	Panderman × UM 6.3
O	UM 2.4 × Grobogan
P	UM 3.2 × Grobogan
Q	UM 3.4 × Panderman
R	UM 3.4 × Burangrang
S	UM 4.1 × Argomulyo
T	UM 4.1 × Burangrang
U	UM 6.1 × Argomulyo
V	UM 6.2 × Argomulyo
W	UM 6.2 × Panderman
X	UM 6.3 × Burangrang
Y	UM 6.3 × Panderman
Z	UM 7.6 × Burangrang

RESULTS AND DISCUSSION

The tested soybean lines showed differences in plant height with an average of 43.97 cm (Figure 1A). Genetic and environmental factors influence plant height (Lee et al. 2015; Cao et al. 2019). The parameter of plant height is also related to the response of plants to pathogenic virus infection. Plants may become stunted and fail to grow in heavy infections, with characteristic symptoms such as wrinkling and leaf malformation. Viral infections cause disturbances such as reduction in chloroplast formation (Singhal et al. 2020). Disruption of plant height growth can also affect the formation of branches because the branches are on the plant stem. In this study, the average number of branches was 4.61, with the lowest number being 1 and the highest being 17. There were 216 soybean genotypes with 1-3 branches, 691 soybean genotypes with 1-3 branches, 90 lines with 6-8 branches, three soybean genoytpes with 9 branches, and one soybean genotypes with 10 and 17 branches (Figure 1B). The number of branches is a parameter that is strongly influenced by genetic and environmental factors and also affects the number of pods and yield per plant (Kuswantoro 2014; Shim et al. 2017).

The number of reproductive nodes ranged from 6-61 nodes, with an average of 27.8 nodes (Figure 1C). The number of plant branches strongly influences the number of reproductive nodes. Development of varieties by selecting candidates based on the number of nodes mapping continues to support increased soybean yields (Orlowski et al. 2016; Yin et al. 2018) because the number of nodes can affect yield (Egli 2013). This phenomenon happens because the pods appear in the reproductive nodes. The number of filled pods of the tested soybean lines ranged from 10 to 198 pods, with an average of 80.30 pods (Figure 1D). The number of filled pods per plant results from the highest number of seeds per plant. The number of filled pods and seeds significantly affects seed yield per plant (Aondover et al. 2013). These characters are strongly associated with increased crop yield per hectare (Karyawati and Puspitaningrum 2021).

In contrast to filled pods, unfilled pods can reduce soybean yields. The number of unfilled pods in this study was scarce, with an average of 2.93 pods and a range of 0-13 pods per plant (Figure 1E). Environmental and/or genetic factors can influence unfilled pods on plants. A low number of unfilled pods provides opportunities for high yields and shows a new genotypic quality that efficiently suppresses the formation of unfilled pods (Suhre et al. 2014). Unfilled pods occurred due to obstacles in pod filling, therefore, increasing yields were a consequence of increasing the number of filled pods and decreasing the number of unfilled pods. The more filled pods, the greater the number of seeds. In addition, the minimum number of seeds per plant was 16, and the maximum was 440, with an average of 173.31 (Figure 1F). The number of branches and filled pods strongly influences the number of seeds. Several studies have consistently reported a positive relationship between the number of branches, pods, and seeds per plant (Orlowski et al. 2016; Xu et al. 2021).

The size of soybean seeds plays a vital role in the soybean food industry in Indonesia. Harsono et al. (2021) stated that varieties weighing >14 g/100 seeds were the most in demand. The minimum weight of 100 seeds in the present study was 2.53 g, and the maximum was 22.59 g, with an average of 15.46 g (Figure 1G). Large seed size affects the increase in crop yield. Gaweda et al. (2020) stated that an increase in seed weight of 1 g/m^2 per plant resulted in an average increase in seed yield of 0.3 t/ha. Seed yield was closely related to agronomic characteristics such as the number of filled pods and seed size. Seed yield ranged from 3.05 to 70.84 grams, with an average of 27.55 (Figure 1H). Berhanu et al. (2021) reported that an increase in plant agronomic characteristics affects the number of seeds per plant. Several yield components, such as the number of nodes and pods, also support increasing soybean yield (Ferrari et al. 2018).

The incidence of CpMMV infection scoring in soybeans showed that the average infection score was 2, with the lowest score being 1 and the highest being 3 (Figure 1I). Scoring 2 was based on the symptoms observed in the field. The selection of resistance of soybean lines to CpMMV starting from the F2 generation can reduce the symptoms of CpMMV attack in this F5 generation. The selected plants were plants with mild symptoms so that the symptoms became milder in later generations.

Based on the boxplot obtained, the crosses with the highest plant heights were obtained in the N and U crosses (Figure 2A). Other crosses with the highest plant heights were H, C, and S. The K cross, which consisted of only one selected individual, had a height of 55 cm. Crosses with the shortest plant height were obtained on D and E. This study had extremely high individuals, namely in Q and N crosses. These individuals were G668 (98 cm) and G331 (94 cm) in the Q and G316 crosses (88 cm) and G314 (83 cm) in N cross. In a previous study using the F3 generation, a similar plant height was also obtained, namely an average of 50 cm (Badiaraja et al. 2021a) and 53 cm in the Grobogan/UM 3-2 cross, and 56 cm in UM 3-2/Grobogan crosses (Badiaraja et al. 2021b). The height of plant supports many branches because, with the height, the branches may develop properly. Crosses with the highest number of branches per plant were obtained in cross V. There was not much difference between crosses, but from 26 crosses, it was divided into three parts. Result revealed that 12 crosses were classified as having many branches, 9 crosses with medium number of branches, and 7 crosses with a small number of branches (Figure 2B). The R cross had individuals with many extreme branches, reaching up to 17 branches as indicated by G252. In this study, the average number of branches per plant was 5.6, which was higher than the study by Badiaraja et al. (2021a,b) and Mustofa et al. (2021), who reported an average number of branches of 2.1-3.85. Crosses with the most reproductive nodes per plant were obtained in the M cross (Figure 2C). The Panderman × UM 6.2 cross in the F1 generation only reached three nodes (Isnaini et al. 2020). Other crosses with the most reproductive nodes were L, N, and V. The cross with many extreme individuals was U cross, with 73 nodes achieved by G232. The M cross had the highest number of filled pods (Figure 2D). The

Panderman × UM 6.2 cross in the F1 generation only reached six pods, but the reciprocal reached 70 pods (Isnaini et al. 2020). Other crosses with the highest number of filled pods were N and Y. The extremely high number of filled pods was achieved by crossing N, Q, and S. The cross with the lowest number of filled pods was R. The highest number of unfilled pods was obtained in L cross, followed by A cross. The lowest number of unfilled pods was observed by R cross, followed by Z and E. The extremely high number of unfilled pods was recorded in V, O, and A (Figure 2E). M cross had the highest number of seeds per plant, followed by N cross (Figure 2F). In N cross, there was one line with the highest number of seeds, namely G481 (440 seeds) and G356 (399 seeds) in N cross. This number of seeds was higher than that reported by Staniak et al. (2021), which reached 50 seeds, and Badiaraja et al. (2021b), which got 70 seeds. Badiaraja et al. (2021a) achieved 153 seeds by crossing UM 6.1 x Grobogan. The large number of seeds occurred because they were planted wide so that soybeans can grow optimally. The X, Z, and R achieved crosses with the lowest number of seeds.

The Y cross had the largest 100-seed weight. Other crosses with the largest 100-seed weight were Q and O (Figure 2G). The highest 100-seed weight was obtained by G828 with 22.59 g, followed by G736 from the Q cross with 22.24 g in S cross. There were seven lines with a weight of 100 seeds greater than 20 g in the Q cross, namely seven lines. In this study the lowest 100-seed weight was obtained in X, U, and F crosses. In F crosses, there were lines with tiny 100-seed weights. The number of lines with seed size in the Y and Q crosses was influenced by their parents, namely Panderman, while Grobogan influenced the O crosses. Panderman seed size can reach 22.7 g (Soverda et al. 2012), and Grobogan can get 23.3 g (Badiaraja et al. 2021a).

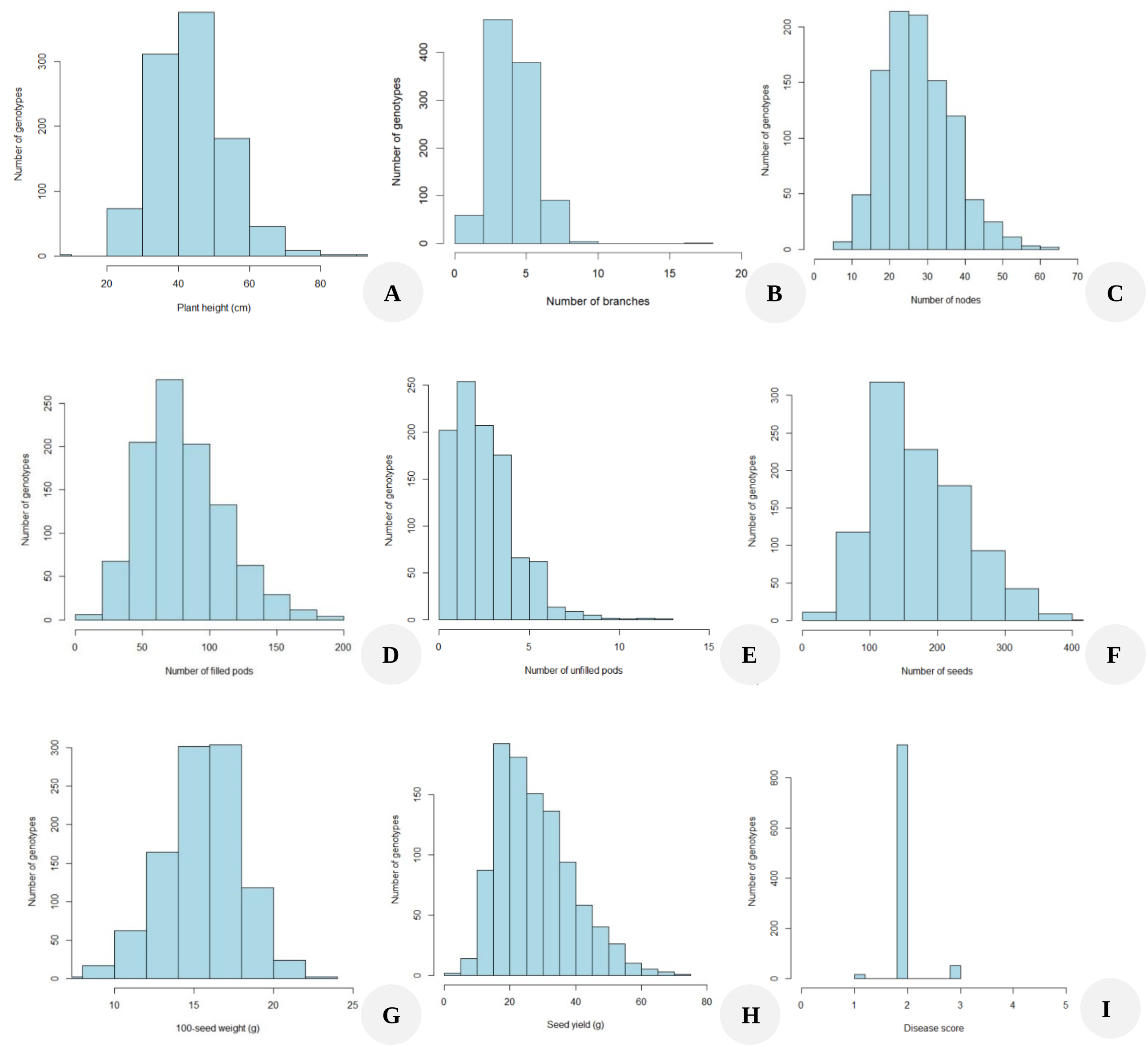

Figure 1. Agronomic characters and disease scores of 1000 soybean lines derived from different crossing

The M cross achieved the highest seed yield. Another cross with the highest seed yield was N (Figure 2H). Although the W cross did not have high seed yield, but it had the line with the highest seed yield, namely G151, with 70.84 g. G356 and G481 also obtained the lines with the highest seed yield from the N cross with 68.75 and 67.74 g. The crosses that produced the lines with the lowest seed yields were the X, Z, and F crosses. Badiaraja et al. (2021b) reported a seed yield of 11.33 g in the Grobogan × UM 3-2 cross. Whereas with the UM 6.1 × Grobogan cross, it reached 7-19 g (Badiaraja et al. 2021a).

In general, the disease scores of the 26 crosses were a score of 2. G crosses showed crosses with a score of 1-3, while those with scores of 2-3 were shown by W and Z crosses (Figure 2I). This low score occurred because the soybean lines tested had been selected many times. CpMMV attack can reduce plant height, number of pods, seed size, and seed yield (Barreto da Silva et al. 2020). In the initial test, the soybean lines can experience a heavy score (Zubaidah and Kuswantoro 2016). Systemic mottling, mosaic, numerous types of leaf deformation, including crinkling, blistering, and leaf defoliation, as well as plant stunting, are signs of the mosaic disease brought on by CpMMV (Yadav et al. 2023).

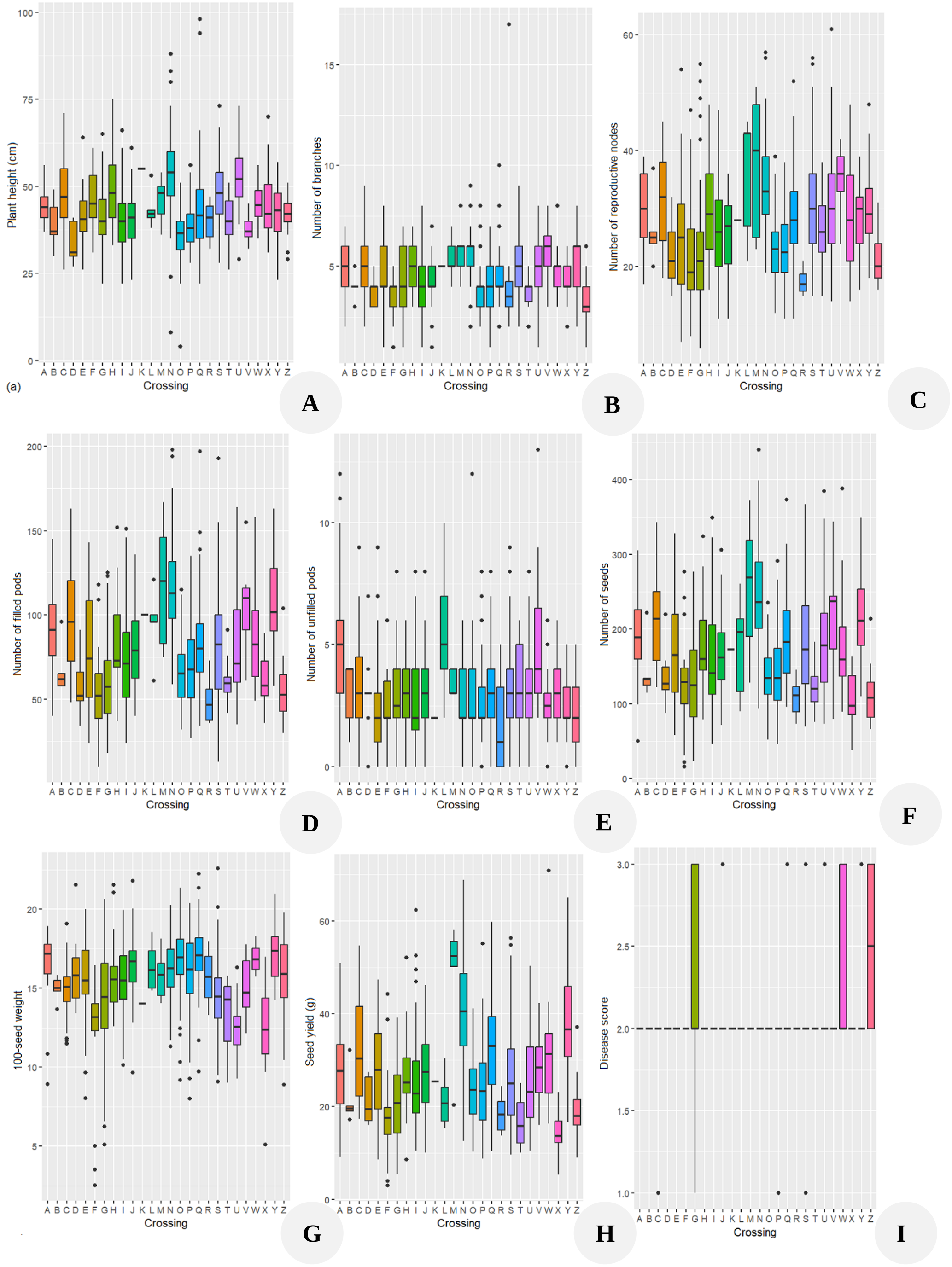

Figure 2. Agronomic characters and disease scores of 26 soybean crossings

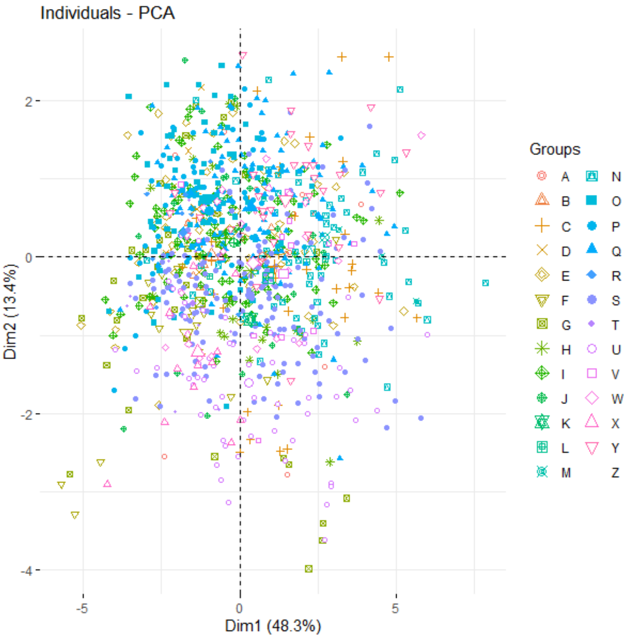

Figure 3. Distribution of 1000 soybean lines derived from different crossing

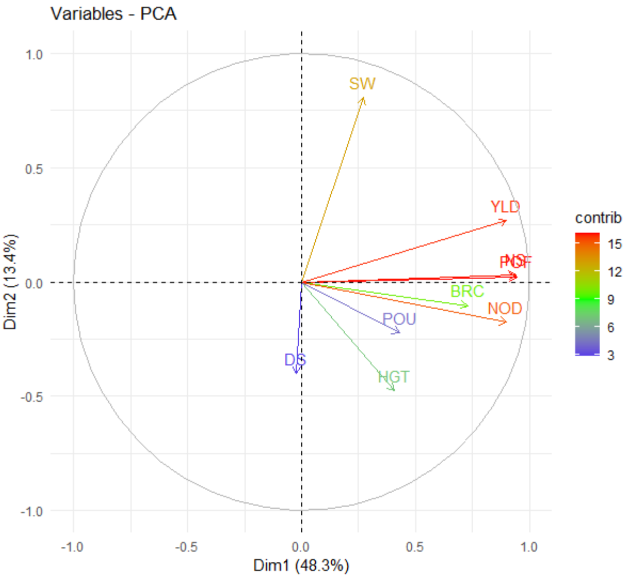

Figure 4. Contribution of characters of 1000 soybean lines derived from different crossing

The distribution of the tested lines was generally in quadrant II. The closer to the center of the quadrant, the more lines were found. However, no lines were found that were precisely in the center of the quadrant. Quadrant II was mostly occupied by lines from crosses I, O, and P. Quadrant I was mostly occupied by lines from crosses N, Q, and Y. Crosses C had a wide distribution in quadrant I. In quadrants III and IV, the distribution of lines was more expansive than in quadrants I and II. The lines with a wide distribution in quadrants III and IV were the lines from crosses G, F, and U (Figure 3).

Based on character contribution, the seed sizes of lines in quadrant I were larger. The height of plants was higher in the lines of quadrant IV. The lines around the border of quadrants I and IV contained many reproductive nodes, pods, seeds, and seed yield. The disease score was at the edge of quadrants III and IV. The disease score in contrast to seed size was in quadrant I (Figure 4). In different crosses, Kuswantoro et al. (2020) also reported a unidirectional contribution from the number of productive nodes, number of filled pods, number of unfilled pods, and seed yield per plant. Badiaraja et al. (2021a) reported that the number of seeds and seed yield per plant have a unidirectional contribution, while other characters' contributions vary. The direction of the arrow depicts the position of a character's contribution, while the size of the arrow depicts the size of the character's contribution. This means that if the direction of the arrow is in quadrant 1 then the genotype in quadrant 1 which is adjacent to the direction of the arrow has a large value based on that character. The size of the arrow describes the size relative to other characters.

When viewed in more detail from the five crosses, the A cross spread at quadrants I and IV, and the B cross spread at quadrant III. In quadrants III and IV, there were several lines of C cross. D cross was mostly found in quadrant II, while E cross was spread evenly in all quadrants (Figure 5A). In these five crosses, the character contribution observed (Figure 5B) was similar to the character contribution in all crosses (Figure 4), where seed yield, number of filled pods, number of seeds and number of fertile nodes were at the border of quadrants I and IV.

Most lines of the F-N crosses were in quadrants I and II (Figure 6A). Quadrant I was occupied by many lines of N and I crosses. Quadrant II was occupied by many lines of I, J, and G crosses. Many lines of the G cross were also in quadrant III. In quadrant IV, many lines of N, G, and I crosses exist. Character contributions to the F-N crosses (Figure 6B) were similar to all (Figure 4).

The lines of O-T crosses were divided into two parts. Most O, P, and Q crosses were in quadrants I and II, while S and T crosses were mostly in quadrants III and IV (Figure 7A). In contrast to other crosses, many lines of R crosses were in quadrant II. The character contribution to this cross (Figure 7B) was also similar to that of all crosses (Figure 4), except that the disease score had the same direction as 100-seed weight (Figure 7B).

There was also a division into two parts on the lines of U-Z cross. Most of the lines of U cross were in quadrants I and II, while the lines of Y, W, and Z crosses were mostly in quadrants III and IV (Figure 8A). In contrast to other crosses, the line from cross X was in quadrants I and IV. In this U-Z cross, the pattern of character contributions was similar to that of all crosses. The direction of disease score was different than all agronomic characters (Figure 8B). This difference in order indicates that the lines in quadrants II and III had higher seed yields, number of filled pods, and number of seeds than lines in quadrants I and IV. The difference in direction is also detailed when reported by Kuswantoro et al. (2020) and Badiaraja et al. (2021a). This phenomenon occurred because the population used were different, so the direction of contribution was also different. Correlation between characters shows that, in general, there was a significant correlation between agronomic characters (Figure 9). No significant correlation was obtained in 100-seed weight with all agronomic characters, except seed yield. No significant correlation was also obtained for plant height with the number of unfilled pods, 100-seed weight, and seed yield. This study is different from the study of Lü et al. (2016) and Yin et al. (2017), which stated that plant height is an essential agronomic character closely related to soybean yield. Nevertheless, this study agrees with Xue et al. (2021), who reported that an increase in the number of branches affected the number of pods per plant and contributed to an increase in yield. An increase in the number of branches leads to a high number of nodes, seeds, and seed yields, as shown by robust correlations. This study is also in line with Abugalieva et al. (2016). Bhor et al. (2014) stated that plant height, number of pods, and 100-seed weight can be used as criteria for selecting superior varieties.

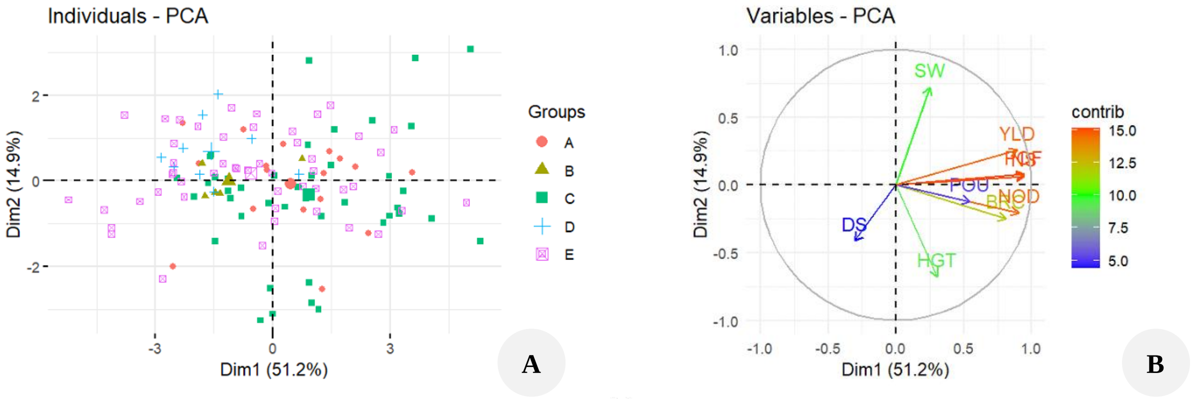

Figure 5. A. Lines distribution. B. Characters contribution of 1000 soybean lines derived from different crossing

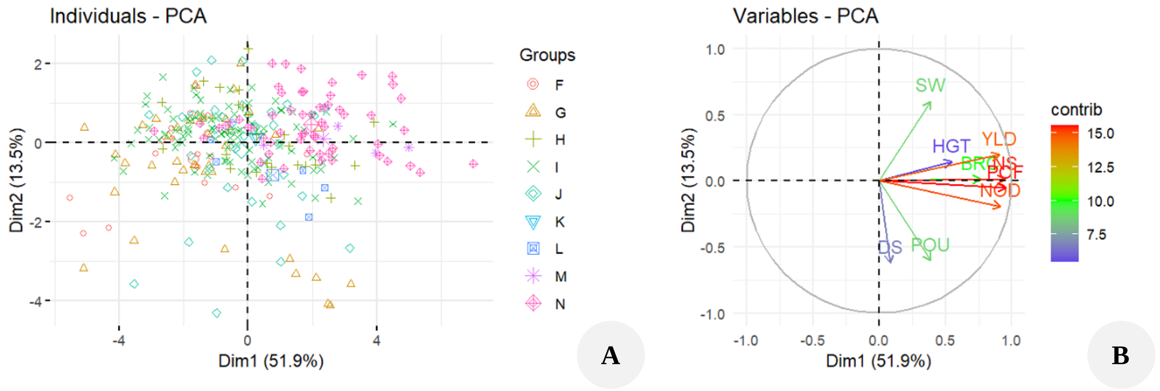

Figure 6. A. Lines distribution. B. Characters contribution of 1000 soybean lines derived from different crossing

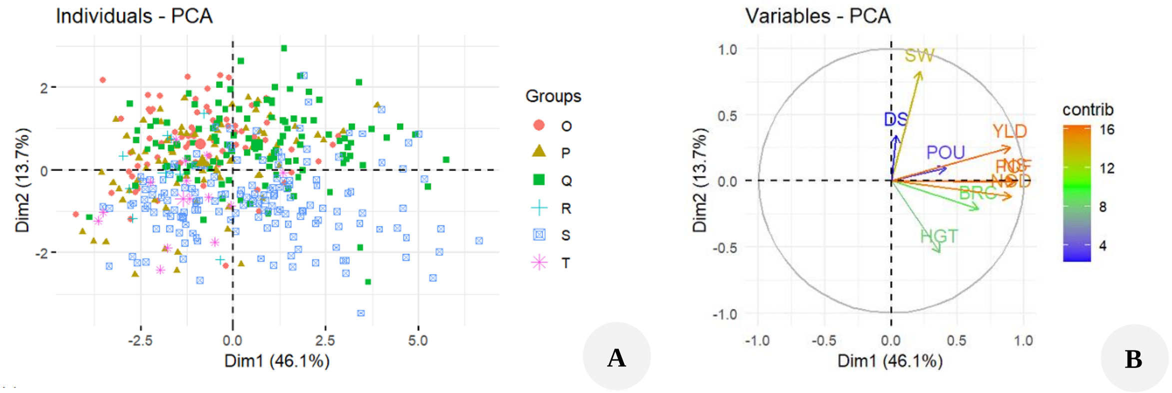

Figure 7. A. Lines distribution. B. Characters contribution of 1000 soybean lines derived from different crossing

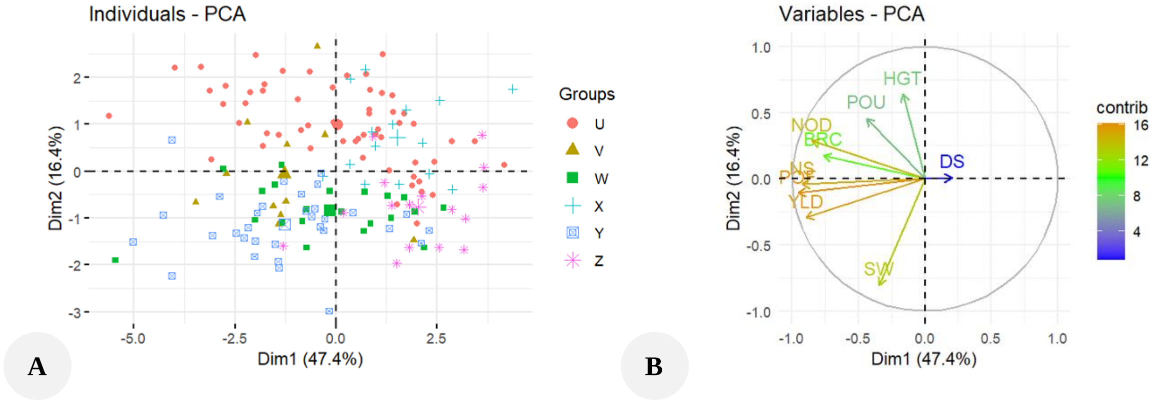

Figure 8. A. Lines distribution. B. Characters contribution of 1000 soybean lines derived from different crossing

Seed yield did not correlate with CpMMV disease scores (Figure 9). CpMMV infection did not affect seed yield in soybean genotypes. Under conditions of heavy CpMMV infection, plants are hampered in producing filled and unfilled pods (Sutrisno and Kuswantoro 2016). The decreased number of unfilled pods indicated that pathogenic infections did not impede plant growth. The 100-seed weight affects the increase in the soybean harvest index (Hakim and Suyamto 2017). Karyawati and Puspitaningrum (2021) stated that seed yield is only directly correlated with the 100-seed weight and indirectly correlated with the number of pods filled and the number of seeds per plant. Berhanu et al. (2021) reported that seed yield significantly correlated with the number of branches, nodes, seeds, and filled pods. Faot et al. (2019) reported a correlation between disease scores, plant height, number of reproductive nodes, unfilled pods, seed size, and seed yield. These results show that these characteristics can be considered in developing soybean plants for high seed yield. Infection with mild symptoms did not cause significant disruption to plant growth, but if there was a severe infection, it hindered growth. CpMMV infection can cause high attack rates ranging from 27-86% (Sutrawati et al. 2021), affecting crop performance and yield. There was no correlation between CpMMV infection scores on all growth parameters and seed yield, possibly due to genotypic tolerance and resistance. Zubaidah and Kuswantoro (2016) determined the pattern of plant resistance observed in three observations, where susceptible plants became resistant and recovered from infection as the plants grew older. This phenomenon can occur in plants with resistant characteristics. Based on the observed characters, four groups of crosses were obtained (Figure 10). These four groups were more influenced by the number of seeds and filled pods than any other character.

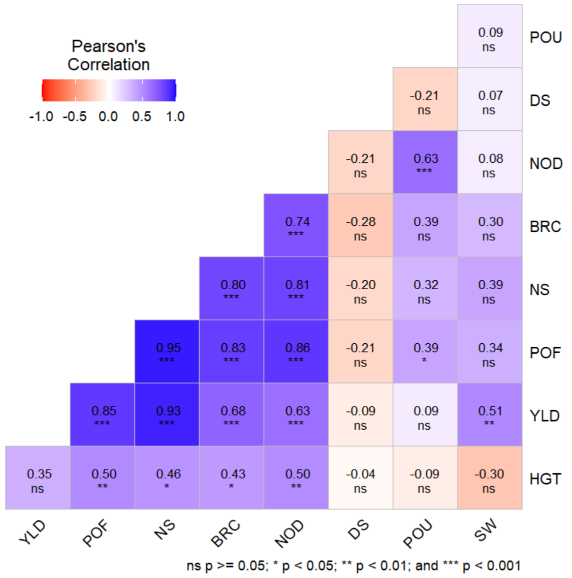

Figure 9. Correlation among characters of 1000 soybean lines derived from different crossing.

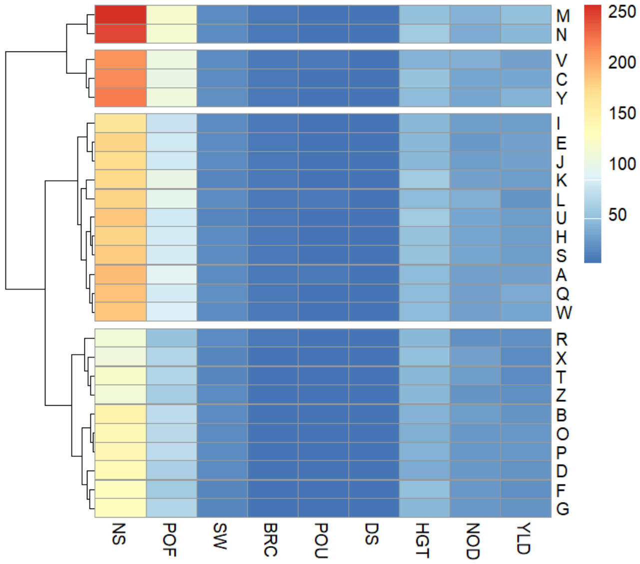

Figure 10. The similarity of 1000 soybean lines derived from different crossing

Plant height also affected the formation of these four groups. Characters with less influence on group formation were disease scores, number of unfilled pods, and number of branches. In the first group, M cross was very similar to the N cross and was a separate group. The second group consisted of V, C, and Y crosses, where C and Y crosses were more similar than V crosses. The third group included 11 crosses. These 11 crosses were divided into two groups consisting of three and eight crosses. The fourth group consisted of 10 crosses. Of these ten crosses, they were divided into two groups consisting of four crosses and six crosses. These groups describe the similarity of the crosses based on their characters.

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