Morpho-chemical evaluation of soybean genotypes across tropical agroecosystem

A Krisnawati and M M Adie

Indonesian Legume and Tuber Crops Research Institute, Malang, Indonesia

E-mail: my_ayda@yahoo.com

Abstract. Soybean is the most important legumes crop and source of protein for Indonesia society. The aim of the research was to evaluate the morpho-chemical characters of soybean genotypes across environments. A total of 16 soybean genotypes were evaluated in four different agro-ecosystems from February to May 2015. The experiment at each agro-ecosystem was arranged in a randomized block design with 16 treatments and four replicates. The result showed that the morpho-chemical characters of 16 genotypes were significantly affected by genotype by environment interaction. The average of protein contents in four locations were 39.30%, 38.76%, 39.06%, and 38.14%, respectively; and the oil contents were 19.52%, 19.66%, 19.15%, and 20.54%, respectively. There was an indication that a higher protein content resulted in a lower oil content. The highest protein content was GRRR-12-15 (39.96%), and followed by Anjasmoro (39.84%) and GA-1-4 (39.46%). Genotypes with high oil content were GAAA-6-3 (21.36%), GAA-5-1 (20.72%), and GRRRR-19-7 (20.38%). A drier agro-ecosystem affected to the decrease in protein content. Stability analysis of protein content showed that GA-1-6 as the most stable genotype, followed by Anjasmoro and GA-1-4. Meanwhile, the most stable genotype for its oil content was Grobogan, and followed by GAAA-6-3 and GA-1-6. The high yield productivities were showed by GAAA-6-7 (3.06 t/ha), GA-1-4-2 (2.91 t/ha), and GA-1-4 (2.82 t/ha). The two top yielding genotypes, GAAA-6-7 and GA-1-4, were showed specific adaption, hence they can be recommended to be developed at relatively dry environment to obtain high yield, protein and oil contents.

1. Introduction
Soybean is the most important legume commodity in Indonesia, as well as a source of vegetable protein. Based on 2014 SUSENAS data, soybean consumption in Indonesia reaches 7.13 kg/capita/year. The consumption of tempeh was 6.95 kg/capita/year, whereas the tofu consumption was 7.07 kg/capita/year [1]. The large demand for soybeans in Indonesia for food raw materials caused the provision of soybean varieties with high nutritional contents and morphological characteristics that are in accordance with Indonesia's tropical agro-ecosystem.

Soybean cultivation in Indonesia is carried out in various agro-ecosystems. The differences in soybean agro-ecosystems are in the form of soil types, types of land, planting seasons, cropping patterns, etc. Protein and oil content in soybeans are quantitatively inherited and influenced by the genotype and environment interaction [2]. A study by Yaklicha & Vinyard [3] reported the influence of rainfall and temperature on protein and oil content in soybeans. Research on environmental influences and soybean genotypes at the Central European showed that the highest protein content was...
obtained in conditions of high air temperature and moderate rates of rainfall during the seed filling phase, whereas the protein content was significantly reduced in insufficient nitrogen fixation and followed by high rainfall during the seed filling [4]. A study reported that the protein content was determined by environmental factors, especially during the seed filling period [5]. Another study reported that drought stress and high temperature during the seed filling period will increase the protein content but reduce the oil content and seed yield [6].

The most important economic value of soybean character is the seed yield, which is followed by other characteristics, such as resistance to biotic and abiotic stresses and high seed nutritional content. A high potential yield of a soybean genotype followed by high protein and oil content is an ideal soybean variety. Simultaneous selection of the agronomical characteristics and nutritional content has been carried out in various soybean production centers in the world [7, 8]. According to Mahmoud et al. [9], who studied the breeding activities carried out over 60 years, to obtain an increase in protein content in soybeans was difficult due to the limited source of germplasm. Eskandari et al. [10] suggested the use of soybean marker breeding programs to increase oil content without reducing the protein content and seed yield.

The evaluation of soybean protein and oil content in the Indonesian soybeans are important to obtain the ideal variety which in accordance with the consumers’ preferences. The aims of the research were to evaluate the morpho-chemical characters of soybean genotypes across environments and their yield and yield components.

2. Materials and Methods
2.1. Materials and research location
The research materials were 16 soybean genotypes, which consisted of 14 recombinants inbreed lines and two check varieties (Anjasmoro and Grobogan). The experiment was conducted in four soybean production centers, namely Nganjuk, Mojokerto, Blitar, and Pasuruan. The agro-ecosystems of those four locations were presented in Table 1.

| Code | Location | Soil type | Land type | Altitude (m asl) |
|------|----------|-----------|-----------|-----------------|
| E1   | Jati Kampir Village, Bagor District, Nganjuk | Regosol | Lowland | 58 |
| E2   | Kedunguneng Village, Bangsal District, Mojokerto | Gray | Lowland | 72 |
| E3   | Binangun Village, Kesamben District, Blitar | Alluvial | Upland | 355 |
| E4   | Sumber Banteng Village, Kejayan District, Pasuruan | Brown | Upland | 124 |

2.2. Field trials and data collection
The field trials in Nganjuk and Mojokerto were conducted in lowland (after rice planting) with zero tillage, whereas in Blitar and Pasuruan were conducted in upland with optimal tillage. On each location, the experiment was arranged in a randomized block design with 16 treatments and four replications. The plot was 2.4 × 4.5 m in size, with 40 cm × 15 cm planting distance. Fertilizers were applied at rates of 250 kg Phonska/ha, 100 kg SP 36, and 1 t/ha organic fertilizer which given entirely at the time of planting. The weeds were controlled at two and four weeks after planting. The pests and diseases were optimally controlled. Plants were harvested when reach R8 (full maturity). The observed morphological characters were days to maturity, 100 seed weight, and seed yield. The observed chemical characteristics were oil and protein content of each location.
2.3. Data analysis
The morpho-chemical data were subjected to combined analysis of variance (ANOVA). The stability analysis of oil and protein content were using AMMI method [11].

3. Results and Discussion
3.1. The combined analysis of variance
The analysis of variance for morpho-chemical of 16 genotypes in four locations was presented in Table 2. The performance of morpho-chemical characteristics was significantly influenced by location, genotype, and interaction of location and genotype. The coefficient of variation values vary from 0.79 – 13.62%. The study on the role of genotype and environment of the soybean oil and protein content vary between each soybean production center in the world. The influence of location and genotype on the oil and protein content was determined by the character of the environmental agro-ecosystem used for the study and the differences in the genetic background of the tested soybean genotype.

| Character                  | RepL | Location (L) | Genotype (G) | L × G | CV\(^a\) (%) |
|----------------------------|------|--------------|--------------|-------|--------------|
| Protein content (% dry basis) | 0.3243\(^*\) | 3.8026\(^*\) | 1.6468\(^*\) | 1.1628\(^*\) | 0.79          |
| Oil content (% dry basis)    | 0.3771\(^*\) | 5.8231\(^*\) | 2.3697\(^*\) | 0.8167\(^*\) | 1.19          |
| Days to maturity (days)      | 0.4309\(^{ns}\) | 44.4309\(^*\) | 53.4809\(^*\) | 3.5675\(^*\) | 1.34          |
| 100 seed weight (g)          | 4.0853\(^*\) | 152.9995\(^*\) | 27.6431\(^*\) | 4.9645\(^*\) | 7.79          |
| Seed yield (t/ha)            | 0.0192\(^*\) | 5.4896\(^*\) | 0.07466\(^*\) | 0.4240\(^*\) | 13.62         |

\(^{a}\)CV = coefficient of variation
*= significant at 5 % probability level (p < 0.05) ** = significant at 1 % probability level (p < 0.01), ns = not significant

3.2. Protein content
The average protein content of 16 genotypes in four locations ranged from 37.54 – 39.96% (average of 38.81%). Genotype GRRR-12-15 showed the highest protein content (39.96%), and then followed by Anjasmoro (39.84%) and GA-1-4 (39.46%). Those genotypes have higher protein content than Grobogan (38.87%), but comparable with Anjasmoro (39.84%). Genotype with the lowest protein content was GA-1-6 (37.54%) (Figure 1).

![Figure 1. Protein content of 16 soybean genotypes in four tropical agro-ecosystems.](image-url)
Four locations have different characteristics of agro-ecosystem. Nganjuk and Blitar are locations with not relatively dry climate, on the contrary Mojokerto and Pasuruan are locations that tend to be dry. The average protein contents in Nganjuk and Blitar were 39.30% and 39.06% respectively, meanwhile the average protein content in Mojokerto and Pasuruan were 38.76% and 38.14% respectively. There is an indication that the protein content will be lower if soybeans are developed in areas that tend to be dry climate. This can be seen within the higher average protein content in Nganjuk and Blitar when compared to Mojokerto and Pasuruan.

Soybean oil and protein content were determined by environmental factors [12]. This study obtained a significant interaction between protein content with environment (location), indicating that the protein content between locations will vary in different locations. Therefore, AMMI (Additive Main Effect and Multiplicative Interaction) analysis was used in this study to identify the stability of protein content.

Based on AMMI biplot (Figure 2) which generated using genotypic and environmental scores of the first two AMMI components (IPCA1 and IPCA2), three soybean genotypes were identified have the most stable protein content, namely GA-1-6 (genotype 2), GRR-2-1 (genotype 5), and Anjasmoro (genotype 15). However, the best three genotypes with high protein content were GRRR-12-15, Anjasmoro, and GA-1-4. Genotype GA-1-6 as the most stable protein content showed the lowest protein content, as well as GRR-2-1.

Similar finding on the significance effect of environment on the oil and protein content also obtained in other studies [13, 14].

**Figure 2.** Biplot AMMI (PC1 vs PC2) for protein content of 16 soybean genotypes in four environments (E1-E4).

### 3.3. Oil content

Oil content between genotypes and locations is varied. The average oil content of 16 genotypes in four locations ranged from 18.39 – 20.72% (average of 19.71%) (Figure 3). Three genotypes with highest oil content were GAAA-6-3 (21.36%), GAA-5-1 (20.72%), and GRRR-19-7 (20.38%). The average oil content in Nganjuk, Blitar, Mojokerto, and Pasuruan were 19.52%, 19.15%, 19.66%, and 54%, respectively.
When connecting the data on oil and protein content, it indicated that a higher protein content will caused the lower of oil content. In this study, the correlation between protein and oil content was $r = -0.32^{ns}$. Similarly, Mertz-Henning et al. [15] proving the negative correlation between protein and oil contents in soybean yield.

The study on the stability of oil content was also using AMMI method (Figure 4). The stable genotypes for its oil content were Grobogan (genotype 16), GAAA-6-3 (genotype 10), and GA-1-6 (genotype 2). Genotype GAAA-6-3 was not only produced the highest oil content in four locations, but also showed a stable oil content. Selection in the soybean oil and protein content can be done when genotype and environment interaction is available. Moreover, the important environmental factors were temperature, water availability, and nitrogen supply, especially during the seed filling period [16].

![Figure 4. Biplot AMMI (PC1 vs PC2) for oil content of 16 soybean genotypes in four environments (E1-E4).](image)
3.4. Morphological characteristics

The important economic characters for the soybean development in the tropical agro-ecosystem are the seed yield, early maturity (< 80 days), and large seed size (>14 g/100 seeds). The range of days to maturity of 16 genotypes was 75 – 82 days (average 78 days). A total of twelve genotypes was categorized as early maturity. Grobogan is early maturing and large seeded variety. GRRR-12-15 have similar maturity with Grobogan (75 days). The seed size, which is reflected by 100 seeds weight, varies from 15.26 – 20.65 g (average of 16.72 g/100 seeds). Based on the seed size grouping in Indonesia, all genotypes were classified as large seed size. Grobogan variety (20.65 g/100 seeds) has the largest seed size. The difference between agro-ecosystems appears to have more influence on seed size than the days to maturity.

The variety of agro-ecosystems of four locations has an impact on the differences in the seed yield of each genotype. Location of Nganjuk was able to produce the highest average seed yield (3.03 t/ha), followed by Blitar (2.70 t/ha), Pasuruan (2.51 t/ha,) and Mojokerto (2.35 t/ha). The range of seed yields across agro-ecosystems was 2.31 - 3.06 t/ha (average 2.65 t/ha). The seed yield performance of Anjasmoro (2.31 t/ha) was lower than that of Grobogan (2.43 t/ha). However, overall seed yields of soybean genotypes were able to produce higher yields when compared to the check varieties. Genotype of GAAA-6-7 was able to produce the highest yield (3.06 t/ha) across agro-ecosystems, and it was followed by GA-1-4-2 (2.91 t/ha) and GA-1-4 (2.82 t/ha). On the optimal agro-ecosystem, such as Nganjuk, several genotypes were able to produce over 3.0 t/ha. Meanwhile on the driest agro-ecosystem, such as Mojokerto, the highest seed yield was obtained by GAA-2-8 (2.67 t/ha).

3.5. Selection of ideal genotype

The ideal genotype for Indonesian tropical agro-ecosystem is characterized not only by the morphological character with economic value (such as high yield, early maturity and large seed size), but also has a high nutritional content, especially protein and oil. This is because most of the soybeans in Indonesia are used as industrial raw materials and has become a source of food crop with important vegetable protein content.

In soybean breeding, special attention is given to develop cultivars that have high protein and oil content, in addition to high and stable yields. In this study, genotype with the highest yield productivity was GAAA-6-7 (3.06 t/ha), which has morphological characteristics of medium maturity and large seed size. The protein content was relatively high than other tested the genotypes, i.e. 39.51%, and the oil content was 20.26%. Other genotype, GA-1-4, the yield was 2.82 t/ha, and having morpho-chemical characteristics as early days to maturity, large seed size, protein and oil content were 39.46% and 20.30%, respectively. GA-1-4 was suggested to become an alternative for users if they need early maturing soybeans. In addition, it has the potential to be developed in dry agro-ecosystems. Soybean genotypes GAAA-6-7 and GA-1-4 are selected as ideal genotypes due to their morpho-chemical characteristics, which is not only suitable to be developed in the Indonesian tropical environment but also in accordance with the industrial preference of soy-based food (Table 3).
Table 3. Morpho-chemical characters of 16 soybean genotypes in four tropical agro-ecosystems.

| No | Genotype  | Seed yield (t/ha) | Days to maturity (days) | 100 seed weight (g) | Protein (% dry basis) | Oil (% dry basis) |
|----|-----------|------------------|------------------------|---------------------|----------------------|------------------|
| 1  | GAAA-11-2 | 2.76             | 78                     | 16.05               | 38.58                | 19.39            |
| 2  | GA-1-6    | 2.76             | 77                     | 17.60               | 37.54                | 19.92            |
| 3  | GAAA-7-1  | 2.49             | 76                     | 16.29               | 39.14                | 18.39            |
| 4  | GAA-5-1   | 2.62             | 78                     | 17.17               | 38.26                | 20.72            |
| 5  | GRR-2-1   | 2.58             | 79                     | 15.93               | 39.08                | 19.52            |
| 6  | GA-1-4    | 2.82             | 78                     | 16.36               | 39.46                | 20.30            |
| 7  | GA-1-2    | 2.76             | 78                     | 15.35               | 38.65                | 19.13            |
| 8  | GAA-2-8   | 2.75             | 76                     | 18.26               | 37.71                | 19.01            |
| 9  | GRRR-12-15| 2.70             | 75                     | 17.35               | 39.96                | 19.09            |
| 10 | GAAA-6-3  | 2.31             | 80                     | 15.97               | 37.54                | 21.36            |
| 11 | GRRRR-19-7| 2.72             | 77                     | 16.40               | 39.37                | 20.38            |
| 12 | GA-1-7    | 2.40             | 79                     | 16.55               | 38.37                | 20.03            |
| 13 | GAAA-6-7  | 3.06             | 80                     | 16.29               | 39.51                | 20.26            |
| 14 | GA-1-4-2  | 2.91             | 78                     | 15.26               | 39.18                | 19.49            |
| 15 | Anjasmoro | 2.31             | 82                     | 16.04               | 39.84                | 19.18            |
| 16 | Grobogan  | 2.43             | 75                     | 20.65               | 38.87                | 19.28            |
|    | Average   | 2.65             | 78                     | 16.72               | 38.81                | 19.71            |

4. Conclusion
The performance of morphological (seed yield, days to maturity, seed size) and chemical characteristics (protein and oil) were significantly affected by genotype and the differences in agro-ecosystem. Although the drier agro-ecosystem tends to cause a decrease in the protein content, this condition may also result in an increase in the oil content. Soybean genotypes of GAAA-6-7 and GA-1-4 are potential to be developed at Indonesia’s tropical agro-ecosystems.

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