Evaluation on morpho-physiological characters of synthetic maize genotypes on low soil nitrogen level condition

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Abstract. A study aimed to evaluate the growth and production of several synthetic maize lines at low nitrogen level was conducted at the Indonesian Cereals Research Institute Experimental Farm in Bajeng District, Gowa Regency, South Sulawesi. The research was carried out from September to December 2018. The study also aimed to determine the nitrogen dose that could be used for selection of synthetic maize genotypes against low nitrogen doses. The study was conducted in the form of an experiment using a Split Plot Design. Five levels of Nitrogen fertilizer were used namely 0, 50, 100, 150, and 200 kg N per hectare. Various genotypes treatment were consisted of 4 synthetic maize genotypes namely Syn 5, Syn 6, Syn 7, Syn 8 and 2 comparative varieties namely Bisma and Lamuru. The results show a significant interaction between the treatment of nitrogen doses and the synthetic maize genotype on the morphological and physiological characters of the maize genotypes. Genotypes gave the best results at nitrogen doses of 100 kg N ha\(^{-1}\) was Syn8 (8.67 tons ha\(^{-1}\)) and 50 kg N ha\(^{-1}\) (6.47 tons ha\(^{-1}\)). Nitrogen doses of 50 kg N ha\(^{-1}\) and 100 kg N ha\(^{-1}\) can be used for the selection of maize tolerant to low nitrogen condition with productivity of 4.57 tons ha\(^{-1}\) and 8.67 tons ha\(^{-1}\), respectively. There is potential for the Syn8 genotype to be developed for low-nitrogen-tolerant maize varieties due to higher production compared to the comparative variety.

1. Introduction
As one of the main food commodities after rice in Indonesia, maize has a strategic and economic values in the country. Maize has the wide opportunity to be developed due to its position as the main source of carbohydrates and protein after rice. Based on the nutrient content, corn has prospects as food, animal feed and industrial raw materials. The use of corn as an industrial raw material will provide added value to the farming business.

The demand for corn production increases from year to year in a fairly high amount to meet the national demand. Corn production in the last five years increased by an average of 12.49% per year, where in 2018 corn production reached 30 million tons of dry kernels. This is also supported by annual harvested area data which averagely increased by 11.06% and average productivity increased by 1.42% [1]. The increase, to some extent, is attributable to the efforts made by the government in the last five years including in maize plant breeding.

One of the outputs of the Indonesian Cereals Research Institute (ICRI) is the selection of ten pure lines extracted from two single cross hybrids, namely CML505 / NEI 9008DMR and CML 538 / DMRYCML. Parental lines of the two hybrids had a far-reaching genetic background. ICRI elite lines
have resistance to downy mildew disease, while the two introduced lines from CIMMYT have tolerance to drought stress, early maturity and high yield production.

Compared to other nutrients, maize require nitrogen nutrients (N) in large quantities. This element has contributed greatly to the increased food production and become a major barrier to crop production in the world, hence the demand for N-source fertilizers such as urea is greater than other fertilizers. Maize plants are known to be sensitive to and acquisitive for nitrogen nutrients causing the application of nitrogen fertilizer is absolutely necessary to support the success of corn cultivation [2].

At certain times, maize plantations even experience a combination of drought stress and nitrogen nutrient deficiency which results in a greater yield decline. The results of Banziger et al. [3], states that a decrease in yield of 80-100% can be expected due to drought stress and low nitrogen fertilization. Nitrogen deficiency is due to its availability in the soil and its low uptake into the soil. This condition is exacerbated by the scarcity of subsidized nitrogen fertilizer that is often experienced by farmers during the growing season and the lack of capital to buy nitrogen fertilizer and seeds [4].

The strategy of developing maize on land that often experiences drought stress conditions is to assemble synthetic corn that is tolerant to drought stress and low N fertilization so that it can utilize dry land with limited water availability and infertile land in this case due to nutrient deficiency N. Synthetic corn varieties can support wider planting of maize and involve farmers with less capital so that it can support an increase in national maize production.

A previous study was conducted using 16 synthetic corn lines from a number of parental lines [5]. From the study, 8 synthetic corn lines tolerant of drought stress and N nutrients were obtained namely Syn1-1, Syn1-2, Syn1-4, Syn1-7, Syn1-8, Syn-10, Syn1-15, and Syn1-16. The recent study aimed to conduct a further testing to determine its adaptability to a variety of different environmental conditions by selecting four synthetic corn genotypes and two corn varieties as a comparison

2. Methodology
The study was conducted at the ICRI Experimental Farm (5°18’21.5” S latitude; 119° 28’38.6” E longitude) in Bajeng District, Gowa Regency at an altitude of 27.2 m above sea level (asl). Study site is characterized with rainfall of 210.67 mm/year on average of 11 days of rain, relative humidity of 86% - 97%, and average daily temperature of 24.85 °C. The study was conducted from September to December 2018.

2.1. Experimental design
The study used a split plot design with the dosages of nitrogen as the main plot and synthetic maize genotypes as the subplot. Nitrogen dosage treatment consisted of five levels, namely 0, 50, 100, 150, and 200 kg N per hectare, while subplots were four genotypes of synthetic maize (Syn 5, Syn 6, Syn 7, Syn 8) and two maize varieties as comparisons (Bisma and Lamuru). Each treatment was repeated 3 times resulted in 90 experimental units. The size of each bed was 3 m x 4 m with the height and the distance between beds were 10 cm and 50 cm, respectively.

2.2. Seed preparation
The seed used were previously selected for healthy seeds that free from pests and diseases with viability of at least 80%, sharp, shiny, and pure both physically and genetically. Prior to planting, the seeds were treated with metalaxyl fungicide. Seeds were planted with a planting distance of 80 cm x 20 cm using a planting stick. Each hole was planted with one corn seed and given furadan to avoid pest attacks then covered with soil.

2.3. Fertilization
Two sources of nitrogen were used, namely NPK and Urea fertilizers. In addition to nitrogen elements, the maize plants also fertilized with phosphor and potassium nutrients. Hence, three types of fertilizers were used in this study, namely NPK and Urea for the source of Nitrogen element, SP36 for phosphate element, and KCl for potassium element. Fertilization were carried out three times during the growing
period. Fertilization was conducted by placing the fertilizer based on the treatment levels in an approximately 5 cm deep holes between the maize plants. Dosages of the nitrogen given are shown in table 1.

**Table 1. Dosages of nitrogen fertilization and application method of the fertilizer used**

| Nitrogen dose (kg N ha\(^{-1}\)) | Type of Fertilizer | Fertilization time |
|----------------------------------|--------------------|--------------------|
|                                  |                    | I (7-10 DAP)       | II (30-35 DAP) | III (50-55 DAP) |
| 0                                | KCL                | 75 kg ha\(^{-1}\) (90 g/plot) | 75 kg ha\(^{-1}\) (90 g/plot) | 50 kg ha\(^{-1}\) (60 g/plot) |
|                                  | SP36               | 200 kg ha\(^{-1}\) (240 g/plot) | - | - |
| 50                               | KCL                | - | 50 kg ha\(^{-1}\) (60 g/plot) | 50 kg ha\(^{-1}\) (60 g/plot) |
|                                  | NPK                | 133.33 kg ha\(^{-1}\) (160 g/plot) | 100 kg ha\(^{-1}\) (120 g/plot) | 100 kg ha\(^{-1}\) (120 g/plot) |
|                                  | SP36               | 100 kg ha\(^{-1}\) (120 g/plot) | - | - |
| 100                              | NPK                | 266.67 kg ha\(^{-1}\) (320 g/plot) | 200 kg ha\(^{-1}\) (240 g/plot) | 200 kg ha\(^{-1}\) (240 g/plot) |
| 150                              | NPK                | 266.67 kg ha\(^{-1}\) (320 g/plot) | 200 kg ha\(^{-1}\) (240 g/plot) | 200 kg ha\(^{-1}\) (240 g/plot) |
|                                  | Urea               | - | 58 kg ha\(^{-1}\) (70 g/plot) | 50 kg ha\(^{-1}\) (60 g/plot) |
| 200                              | NPK                | 266.67 kg ha\(^{-1}\) (320 g/plot) | 200 kg ha\(^{-1}\) (240 g/plot) | 200 kg ha\(^{-1}\) (240 g/plot) |
|                                  | Urea               | - | 117 kg ha\(^{-1}\) (140 g/plot) | 100 kg ha\(^{-1}\) (120 g/plot) |
| DAP = days after planting.       |                    |                    |                    |                    |

2.4. Parameter observation
Parameter observed consisted of growth and production parameters including plant height, number of leaves, ear height, weight of 1000 kernels, and productivity. In addition, physiological parameter observed was leaves chlorophyll index.

2.5. Data analysis
The observed parameter data were analyzed using two ways analysis of variance (ANOVA) for a split plot design. If there is a significant effect of the treatment in ANOVA, further tests are performed to analyze the differences of means between treatments using the Least Significant Difference Test (LSD) at a confidence level of 5%.

2.6. Stress Tolerance Index (STI)
Stress tolerance index was calculated based on kernel production using the equation proposed by Fischer and Maurer (1978) in Fernandez [6]:

\[
STI = \frac{(Y_{pi} \times Y_{si})}{Y_{p}^2}
\]

where:
- \(Y_{pi}\) = The average of a genotype that was not exposed to low nitrogen stress;
- \(Y_{si}\) = The average of a genotype exposed to low nitrogen stress;
- \(Y_{p}\) = The average of all genotypes that was not exposed to low nitrogen stress.

The criteria for determining the level of tolerance to low nitrogen stress is if the value of STI ≤ 0.5 as sensitive, 0.5 < STI < 1.0 as medium tolerant, and STI ≥ 1.0 as tolerant.
2.7. Heritability analysis

Estimated heritability and criteria were based on Elrod & William [7] using the equation:

\[ h^2 = \frac{\sigma^2_G}{\sigma^2_P} \times 100\% \]

where,

- \( \sigma^2_G \) = Genotypic variance;
- \( \sigma^2_P \) = phenotypic variance.

Heritability is categorized as low if \( 0 < h^2 < 20 \); medium if \( 21 < h^2 \leq 50 \); and high if \( 50 < h^2 < 100 \).

3. Results

3.1. Response of synthetic maize genotypes to different nitrogen levels on growth parameters

Response of the maize synthetic genotypes to different low levels of nitrogen are shown in table 1. Variance analysis show that there was a highly significant interaction effect between the maize genotypes and the level of nitrogen fertilizer applied. Plant height, leaves number and the height of the ear were varied between the synthetic maize genotypes and the soil nitrogen levels condition.

**Table 2.** Average of plant height, number of leaves and ear height of synthetic maize genotypes on different level of nitrogen.

| Genotypes   | Nitrogen doses (kg ha\(^{-1}\)) | LSD\(_{0.05}\) (n) |
|-------------|---------------------------------|--------------------|
|             | 0 (n0) 50 (n1) 100 (n2) 150 (n3) 200 (n4) |
| Plant height (cm) |
| g1 (syn 5)  | 136.47\(^{c}\) 163.53\(^{b}\) 173.47\(^{a}\)       | 16.33\(^{b}\)       |
| g2 (syn 6)  | 154.00\(^{b}\) 158.07\(^{b}\) 169.90\(^{a}\)       | 173.07\(^{a}\)       |
| g3 (syn 7)  | 142.80\(^{d}\) 155.40\(^{c}\) 167.60\(^{b}\)       | 179.53\(^{a}\)       |
| g4 (syn 8)  | 165.37\(^{c}\) 178.00\(^{b}\) 186.33\(^{a}\)       | 165.87\(^{c}\)       |
| g5 (Bisma)  | 157.20\(^{b}\) 159.07\(^{a}\) 180.33\(^{a}\)       | 177.60\(^{p}\)       |
| g6 (Lamuru)| 146.67\(^{b}\) 172.67\(^{a}\) 177.40\(^{a}\)       | 176.47\(^{a}\)       |
| LSD\(_{0.05}\) (g) | 7.99 |
| Number of leaves (leaves) |
| g1 (syn 5)  | 11.80\(^{b}\) 12.60\(^{a}\) 11.87\(^{b}\)       | 11.73\(^{b}\)       |
| g2 (syn 6)  | 12.07\(^{a}\) 12.20\(^{a}\) 12.13\(^{a}\)       | 11.27\(^{a}\)       |
| g3 (syn 7)  | 12.07\(^{a}\) 12.20\(^{a}\) 12.20\(^{a}\)       | 12.07\(^{a}\)       |
| g4 (syn 8)  | 12.27\(^{a}\) 12.33\(^{a}\) 11.47\(^{b}\)       | 12.47\(^{a}\)       |
| g5 (Bisma)  | 11.93\(^{b}\) 11.87\(^{a}\) 12.07\(^{a}\)       | 12.13\(^{a}\)       |
| g6 (Lamuru)| 11.40\(^{c}\) 12.20\(^{p}\) 12.73\(^{a}\)       | 11.93\(^{b}\)       |
| LSD\(_{0.05}\) (g) | 0.71 |
3.2. Response of synthetic maize genotypes to different nitrogen levels on physiological parameter

Physiological parameter of the synthetic maize genotypes, i.e. leaf chlorophyll index, decreased with the nitrogen fertilization doses (table 2). There was a very high significant interaction effect between the two treatments on this parameter. Low soil nitrogen level decreased the ability of the plant to synthesize chlorophyll and the responses were varied with the decrease of the nitrogen level.

3.3. Responses of synthetic maize genotypes to different nitrogen levels on production parameters

A highly significant interaction effect were found between the maize genotypes and the level of nitrogen fertilizer applied on the production parameter components. Weight of 1000 kernels (table 3) and productivity (table 4) of the synthetic maize were found to be varied with the soil nitrogen levels condition.

The synthetic maize tested in this study could be categorized tolerant to sensitive to the low soil nitrogen condition. Based on table 4, all synthetic maize genotypes tended to have varied ability in responding to the growing condition with declining nitrogen levels in the soil. Genotypes Syn7 and Bisma seems to shift its tolerance level from tolerant to medium tolerant when the nitrogen fertilizer dose decreased from 200 kg ha$^{-1}$ to 100 kg ha$^{-1}$. On the other hand, Lamuru variety was more responsive to the decrease of nitrogen level in the soil when nitrogen dose was reduced to 150 kg ha$^{-1}$. 

Table 3. Average of leaf chlorophyll index of synthetic maize genotypes on different level of nitrogen.

| Genotypes | Nitrogen doses (kg ha$^{-1}$) | LSD$_{0.05}$ (g) |
|-----------|-------------------------------|-----------------|
|           | 0 (n0) | 50 (n1) | 100 (n2) | 150 (n3) | 200 (n4) |                 |
| g1 (syn 5) | 123153.4$^b$ | 123679.2$^b$ | 124204.7$^a$ | 122980.9$^b$ | 125317.2$^a$ | 1533 |
| g2 (syn 6) | 124421.4$^a$ | 119848.2$^c$ | 121927.8$^b$ | 122363.7$^a$ | 123453.8$^a$ | 1569 |
| g3 (syn 7) | 120837.0$^b$ | 117954.8$^b$ | 118465.6$^b$ | 120420.4$^a$ | 121271.1$^a$ | 1569 |
| g4 (syn 8) | 122896.1$^a$ | 121801.8$^q$ | 124565.4$^a$ | 124848.3$^p$ | 121849.9$^b$ | 1569 |
| g5 (Bisma) | 122437.6$^c$ | 120344.9$^b$ | 119579.9$^b$ | 120183.2$^b$ | 120205.3$^b$ | 1569 |
| g6 (Lamuru) | 125841.1$^a$ | 121996.4$^q$ | 124604.4$^b$ | 123943.3$^p$ | 121422.6$^c$ | 1569 |

Numbers followed by different letter in the same row (a,b,c,d) and column (p,q,r,s,t) means significantly different based on LSD at a level of 5%.
Although some genotypes did not show changes in their tolerance levels consistently, all genotypes could be categorized as medium tolerant when the level of nitrogen was reduced to 100 kg ha\(^{-1}\). Genotypes showed medium tolerance even in the lowest level of nitrogen were Syn7, Syn8, and Bisma.

| Genotypes | Nitrogen doses (kg.ha\(^{-1}\)) | LSD\(_{0.05}\) |
|-----------|---------------------------------|----------------|
|           | 0 (n0)  | 50 (n1) | 100 (n2) | 150 (n3) | 200 (n4) | (n) |
| g1 (Syn 5) | 37.87\(c\) pq | 39.27\(bc\) pq | 38.03\(bc\) q | 40.43\(b\) q | 44.03\(a\) p | 44.03\(a\) p |
| g2 (Syn 6) | 35.17\(c\) q | 34.67\(c\) r | 43.33 a | 42.33 a | 38.10 \(b\) q | 38.10 \(b\) q |
| g3 (Syn 7) | 38.23\(ab\) p | 39.23\(a\) pq | 36.70 \(b\) q | 40.90 \(a\) pq | 36.37 \(b\) r | 36.37 \(b\) r |
| g4 (Syn 8) | 35.57\(c\) q | 37.37\(bc\) q | 44.83\(a\) p | 38.93 \(b\) q | 39.83 \(b\) q | 39.83 \(b\) q |
| g5 (Bisma) | 36.00\(c\) pq | 41.03\(a\) p | 35.87 \(b\) q | 43.20 \(a\) p | 43.17 \(a\) p | 43.17 \(a\) p |
| g6 (Lamuru) | 33.77\(c\) q | 38.93\(a\) pq | 37.20 \(a\) q | 37.37 \(a\) q | 37.17 \(a\) r | 37.17 \(a\) r |

Numbers followed by different letter in the same row (a,b,c) and a column (p,q) means significantly different based on LSD at a level of 5%.

3.4. Heritability

Heritability values of several corn genotypes at various nitrogen doses given are shown in table 5. Parameters with high heritability values are plant height and leaf chlorophyll index, with value of 53% and 73%, respectively. While the heritability values that are classified as being moderate are shown by
the parameters of ear height (40.57). On the other hand the heritability value of other parameters i.e. number of leaves, weight of 1000 kernels and productivity categorized in low category with values less than 20.

| No | Characters                          | Heritability (h²) | Category |
|----|------------------------------------|-------------------|----------|
| 1  | Plant height                       | 52.17             | High     |
| 2  | Number of leaves                   | -5.51             | Low      |
| 3  | Ear height                         | 40.57             | Medium   |
| 4  | Leaf chlorophyll index             | 73.17             | High     |
| 5  | Weight of 1000 kernels             | -9.58             | Low      |
| 6  | Productivity                       | 8.78              | Low      |

0 ≤ h² ≤ 20 (low); 21 ≤ h² ≤ 50 (medium); 50 < h² ≤ 100 (high).

4. Discussion

The results of statistical analysis show that the treatment of nitrogen fertilizer and corn genotypes significantly affected the parameters of plant height, number of leaves, the height of the ear location, leaf chlorophyll index, weights 1000 kernels, and productivity.

Application of 150 kg Nitrogen per hectare is a dose that can be used as a reference in selecting corn tolerant genotypes for low nitrogen condition. In this condition, it is able to select maize genotypes that really have the potential to maintain the growth and production when the growing condition in the field failed to sub optimum due to low level of nitrogen in the soil. This indicates that the availability of low nitrogen is an agronomic environment condition being one of the selection criteria in producing nitrogen-efficient corn varieties [8]. In the recent study, genotype that showed best response with the highest production was Syn8, both in control or higher nitrogen dose. Based on this, the genotype is a potential candidate for low nitrogen fertilization genotypes. This is supported by several observational parameters such as plant height, leaf chlorophyll index, weight of 1000 kernels, and productivity.

Corn plants that experience nitrogen deficiency can experience stunted growth, lower number of leaves and leaves turn yellow due to lack of chlorophyll. This is consistent with the opinion of Rashidi and Seyfi [9] that nitrogen-deficient corn plants will show yellow colour and curl up leaves. This shows that nitrogen deficiency conditions determine the ability of plants to maintain generative growth and yield. Nitrogen dose affected the plant height and lower nitrogen supply suppressed plant growth such as in the genotypes Syn5 and Syn7. Compared to these genotypes, Syn6 and Syn8 seemed able to maintain its growth in plant height. Genotypes that grow in high nitrogen nutrient conditions will produce optimal plant growth. The more nitrogen nutrients, the better for plant height growth so that the reception and absorption of sunlight can take place optimally. The higher the plant, the more leaves are formed so that the reception and absorption of sunlight can be maximized [9]. Maximum absorption of sunlight, followed by optimal absorption of nutrients, resulted in optimized process of photosynthesis [10].

The application of nitrogen fertilizer plays a role in stimulating overall growth, especially stems, branches and leaves. In addition, nitrogen also plays an important role in the formation of leaf green which is very useful in the process of photosynthesis [11]. The element of nitrogen helps plants to form chlorophyll compounds or leaf green substances which function to assist plants in providing the amount of assimilate which will be used as energy for growth and development of plant organs. Plants that experience nitrogen deficiency will experience rapid aging because the amount of leaf green matter formed is very low compared to the normal conditions. According to Banziger et. al. [3, 12] and Omoigui et. al. [13], the ability of corn genotypes to delay senescence not only in drought stress
conditions, but also in the low availability of soil nitrogen is related to the ability of roots to absorb water and nitrogen which is quite high [14].

Based on the results in the recent study, Syn8 was the genotype with the best efficiency of using nitrogen as shown in the use of nitrogen dose of 100 kg N per hectare. The higher the kernels produced the more efficient the genotype in using the nitrogen element in producing the kernels. This proves that some genotypes have capability in utilizing nitrogen to produce high yield. According to Nasaruddin and Musa [15], plants show response to nitrogen treatment, because nitrogen is the main nutritional element for amino acids synthesis. Nitrogen concentration in plants reflects the supply of nitrogen in the root media, and increases yield as a result of increasing internal nitrogen concentrations in the plant tissue. The Syn8 could be suggested as a tolerant genotype to a low level of nitrogen indicated by other observational characters such as plant height, weight of 1000 seeds and productivity.

Criteria for a particular corn genotype that is classified as tolerant based on the STI value is that the greater the STI value of a corn genotype, the greater the productivity of the corn genotype produced under unfavourable growing conditions. The selection of hybrid corn based on STI can screen the potential tolerant hybrid corn genotype with high yield under stress conditions [16].

Heritability is an important parameter in plant breeding [17]. The high value of heritability of a trait indicates that the correlation between phenotype diversity and genetic diversity is high [18]. This is in accordance with the opinion of Basir [19] that characters with high heritability will increase the effectiveness of selection because the observed characters are a reflection of the influence of genetic factors compared to the environmental factors. Quantitative characters that have high heritability will produce a selection progress for desirable traits, whereas if low heritability is less effective to be used as material.

5. Conclusions

- Syn8 genotype is the genotype that has the highest potential productivity (8.67 tons ha\(^{-1}\)) at a nitrogen dose of 100 kg N ha\(^{-1}\).
- Nitrogen doses of 50 kg N ha\(^{-1}\) and 100 kg N ha\(^{-1}\) can be used for selection of corn tolerant to low nitrogen across a range of nitrogen levels 4.57 tons ha\(^{-1}\) and 8.67 tons ha\(^{-1}\), respectively.
- There is potential for the Syn8 and Syn7 genotypes to be developed for low nitrogen-tolerant varieties because they provide higher production than comparative varieties (Lamuru  varieties).

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