Shade stress in various growth phases of peanut genotypes and its effect on agronomic characters and chlorophyll content

AF Hemon*, Sumarjan, and AR Hanafi
Agroecotechnology, Faculty of Agriculture Mataram University, Indonesia

E-mail: faridhemon_1963@yahoo.com

Abstract. Shading stress during plant growth is one of the factors that might decrease the peanut crop productivity. The growth phase of the peanut genotypes has different responses to shade stress. This research aimed to study the effect of shade stress in various growth phases on agronomic character and chlorophyll content of peanut genotypes. Shade stress was regulated in six conditions: no shade, shade from planting to harvest, shade from planting until 25 days after planting (vegetative phase), shade from 26 to 50 dap (flowering phase to pods formation), shade from 51 to 75 dap (seed filling phase), and shade from 75 to 100 dap (the ripening phase of the seeds to harvest). The shade was carried out using parawall which holds 35% of the sun’s rays. The genotypes used were F3 generation plants from crosses (G200-I and G300-II x cv.Domba, Takar 1, Bison). The results showed that there were no significant differences in the interactions between shades in various growth phases and genotypes for agronomic traits, but there were significantly different interactions on the content of chlorophyll-a and total chlorophyll. All 12 peanut genotypes tended to produce more chlorophyll-a and total chlorophyll in shade treatment from planting to harvest. Shade at various plant growth phases had a significant effect on agronomic characters. The shade given from planting beginning to harvest produced the lowest dry pod weight (12.9 g/plant) or yield decreased to 23.7% compared to peanut grown without shading. The genotypes significantly affected agronomic traits and gs genotype (G200-I x cv.Domba); produced the heaviest dry pod weight 17.2 g/plant.

Keywords: growth phases, low light intensity, hybridization, photosynthesis

1. Introduction

At present, the development of peanut plant is mostly directed at marginal land such as dry land and land under plantation crop. Planting peanut under a tree stand is usually intercropped with perennial crops or annual crops such as corn, cassava, or okra [1]. Intercropping planting generates land use that is more efficient and minimizes micro-climate change.

Dry land condition and intercropping system also often causes problems in peanut farming, especially shade and drought stress. The development of intercropping will face with the lack of light (shade stress). Shade stress has a very negative impact on peanut productivity as solar radiation greatly affects peanut growth and productivity through photosynthesis [2].

Plant that are able to grow in low light intensity can adapt through changes in plant morphology and physiology, so that the available light energy can be used efficiently to peanut growth [3]. Lack of light caused an increase in specific leaf area, but in other parameters it could reduce stem diameter and total dry matter. The experiment of that shade intensity up to 75% increased plant height and specific leaf area but reduced leave number, the rate of light absorption, photosynthesis rate, leaf chlorophyll
index, number of filled pods, and weight of seeds per soybean plant. Increased crop leaf area aimed to make light absorption more efficient and photosynthesis could run normally [4].

The use of tolerant cultivar to shade stress is one alternative for increasing peanut production. The experiment of [5] had obtained several F3 generation peanut lines. These peanut lines were generated from crossing between tolerant shade and drought cultivar. The earlier result of the research has produced tolerant mutant lines to drought (G200-I and G300-II) and other researchers have also produced tolerant shade cultivars (Domba, Takar-1, and Bison). Single hybridization has been carried out between G200-I and G300-II (female parents) with cv. Domba, Takar-1, and Bison and the hybridization had produced F3 genotypes. Results of research [5] that each genotype had a different tolerance to shading. Plants that are adaptive to low radiation, usually increasing in leaf area ratio, stem leaf ratio, stem length, and decreased leaf thickness.

The growth phase of the plant has different responses to shade stress. Shade stress causes flowering age and harvest age faster than no shade in the reproductive phase of soybean varieties [6]. Shade application of 50% during plant growth resulted in a decrease of yield between 37 to 74% in soybean [7], and more than 55% in rice [8]. Low light intensity at flowering caused a decrease in carbohydrate, protein, auxin, proline, and cytokinin, but increased gibberellin and dissolved N content in upland rice panicle [9]. Shade also affected the chlorophyll content of soybean leaves. The experiment of [10] reported that at 50% shade chlorophyll-a, chlorophyll-b, and chlorophyll a/b ratio in tolerant varieties showed a higher increase than sensitive soybean varieties. Therefore, the objective of the present study was to study the effect of shade stress given at various stages of plant growth on the agronomic character and chlorophyll content of peanut genotypes.

2. Materials and Methods
2.1. Experimental design
The experiment was carried out using a Completely Randomized-Split Plot design in the greenhouse with shade stress at various phases as the main plot and peanut genotypes as the subplot. Shade stress was regulated in six conditions: n0= no shade, n1= shade from planting to harvest, n2= shade from planting until 25 days after planting (dap) (vegetative phase), n3= shade from 26 to 50 dap (flowering phase to pods formation), n4= shade from 51 to 75 dap (seed filling phase) and n5= shade from 75 to 100 dap (the ripening phase of the seeds to harvesting). Shading was carried out using black paranet with a sunlight barrier of 35%. The genotypes used were genotype (G200-IxTakar1)n0, (G200-IxTakar1)n1, (G200-IxTakar1)n2, (G200-IxDomba)n3, (G200-IxDomba)n4, (G200-IxBison)n5, (G300-IxBison)n6, and (G300-IxBison)n7, cv. Takar-1, Domba, and Bison. Each treatment was made in 5 replications.

2.2. Procedures
Two seeds of each genotype mixed with Furadan 3G were planted in the center of a polybag filled with 10 kg sieved soil fertilized with 3.2 g Ponska (75 kg ha⁻¹). The polybags of each genotype were arranged according to treatment with spacing distance of 40x20 cm. The black paranet was set at height of 1.5 m above the plant, the position expected perfectly to reduce the sunlight 35%. Plants were watered every three days and maintained according to standard cultivation. Plant maintenance included soil blow up, weed control, pest and disease control, and water. At the age of the plant 25 dap, the soil medium of peanut plant was blown up and cleaned of weeds.

Chlorophyll content was measured using Spectrophotometric methods. Calculation of chlorophyll content (mg.L⁻¹) was determined according to the calculations of Wintermans and De Mots (1965) in [11]: Chlorophyll-a (mg.L⁻¹) =13.7(OD 665)-5.76 (OD 649); Chlorophyll-b (mg.L⁻¹)=25.8(OD 649) - 7.7(OD 665); Total chlorophyll (mg.L⁻¹)=20.0(OD 649)+6.1 (OD 665), (OD = Optical density = absorbance value).

3. Results and Discussion
The result of experiment showed that shading at various growth phases with genotypes had no significant interaction on agronomic traits, but had significant interactions on chlorophyll-a and total chlorophyll content. This showed that the shading stress given at various growth phases had the same effect on some agronomic characters of peanut genotypes. The result of the previous experiment
showed that some peanut mutant genotypes expressed different responses to shading stress and drought [5]. Chlorophyll-a and total chlorophyll content of peanut leaves were significantly influenced by shading stress and genotype. Peanut genotypes gave different responses to shade stress in producing chlorophyll-a and total chlorophyll content. Peanut genotypes that were tolerant to shade stress produced higher content of chlorophyll-a and total chlorophyll than sensitive genotypes. Research of [12] showed that tolerant soybean plants have higher chlorophyll-a than sensitive shade soybean.

Single-factor shading stress on various phases of peanut plant growth significantly affected all agronomic characters, chlorophyll-a, b, and total content. Peanut genotype significantly affected agronomic traits such as plant height, number of leaves, pod dry weight, number of pods, number of filled pods, chlorophyll-a, and total chlorophyll.

Figure 1 showed that treatment shade stress for the whole life of plants resulted in the highest plant while the shortest one was observed in shade stress during 75-100 dap. Plants grown in shade stress for the whole of their life, in addition, produced less number of leaves and longer stem segments (Figure 2). These results are in accordance with those of [13] who reported that shade intensity up to 75% increased plant height and reduced the number of leaves in soybean plant. Research of [14] explained that plants have many ways to control the influence of shade, like through changes in plant height and number of leaves.

Figure 1. Effect of shade stress at various stages of plant growth on plant height of peanut

Explanation: The same letters at same plant age point showed plant height were not significantly different at 5% level by Duncan test.

The effect of plant genotypes on plant height and number of leaves was only observed at harvest time (Table 1). The highest and the lowest plants were observed on cv. Takar-1 and genotypes (G200-IxDomba)5, respectively. While Genotype (G300-IxBison)2 produced the largest number of leaves. Different peanut genotypes express genes to produce agronomic characters [5].

Table 1. Plant height (cm), branch number, and leaf number of peanut genotypes.

| Genotypes           | Plant height | Leaf number |
|---------------------|--------------|-------------|
| g1= Takar-1         | 51.1 a       | 55.8 abc    |
| g1= (G200-IxTakar1)5| 47.4 ab      | 50.2 bcd    |
| g1= (G200-IxTakar1)10| 48.9 ab    | 48.2 cd     |
| g1= (G200-IxTakar1)20| 43.3 ab     | 49.8 bcd    |
| g1= (G200-IxDomba)1| 45.1 ab      | 41.6 d      |
| g1= (G200-IxDomba)2| 44.9 ab      | 44.0 cd     |
| g1= (G200-IxDomba)3| 42.3 b       | 43.9 cd     |
| g1= (G300-IxDomba)5| 43.0 ab      | 47.9 cd     |
| g1= Bison           | 45.6 ab      | 63.5 ab     |
| g1= (G200-IxBison)25| 46.7 ab     | 57.5 abc    |
| g1= (G300-IxBison)25| 42.8 b      | 68.6 a      |
| g1= (G300-IxBison)35| 45.0 ab     | 58.1 abc    |

*The number followed by the same letters at the same column were not significantly different at 5% level by Duncan test

Shade stress at various phases of plant growth had significant effects on the age of flowering plants, length of the main stem segment, leaf area, plant biomass dry weight, root dry weight, and root
nodules dry weight. Table 2 shows that plants with shading stress for the whole of their life were flowering later than those with shorter or no shade stress. Sunlight directly affects flowering and photosynthetic activity. Lack of sunlight will inhibit the flowering of peanut plant. Moreover, the formation of new structures in higher plants is controlled by light such as the regulation of flower bud formation and the accumulation of food reserves by the cell in leaf bases [15]. In spite of the fact that light influences many phases of plant growth and development, relatively few photoresponses are probably involved [16]. Plants treated with shade stress from planting time to harvest also generated narrower peanut leaves, lighter plant biomass dry weight, root dry weight, and root nodule dry weight. Specifically, for plant biomass dry weight, all plants growing in shading stress produced lighter biomass dry weight than those growing in no shading stress. Shade stress inhibited plant biomass dry weight, root dry weight, and root nodule dry weight.

Separately, both shade stress and genotypes had significant effects on the number of filled pod and pod dry weight (Table 3). Plants growing under shade stress for the whole of their life resulted in the lowest number of pod and pod dry weight. The percentage of reduction in pod dry weight of these plants was 23.7% (data not presented) compared to those without shade stress. Genotype g6 = (G200-IxDomba) tended to produce the heaviest pod dry weight of 17.2 g per plant.

| Shade stress | Age begin flowering (day) | Length of main stem segment (cm) | Leaf area (cm²) | Plant biomass dry weight (g) | Root dry weight (g) | Root nodule dry weight (g) |
|--------------|---------------------------|---------------------------------|----------------|-----------------------------|--------------------|---------------------------|
| n0           | 24.9 b c                | 3.2 c                         | 46.8 a         | 12.9 a                      | 0.8 a              | 0.07 b                    |
| n1           | 34.6 a                   | 4.7 a                         | 37.2 b         | 4.7 c                       | 0.2 c              | 0.03 c                    |
| n2           | 31.9 a                   | 3.7 b                         | 43.2 ab        | 9.9 b                       | 0.6 b              | 0.08 b                    |
| n3           | 27.5 b                   | 3.7 b                         | 47.2 a         | 8.7 b                       | 0.5 b              | 0.07 b                    |
| n4           | 24.9 bc                  | 3.0 c                         | 45.9 a         | 9.4 b                       | 0.5 b              | 0.08 b                    |
| n5           | 24.1 c                   | 2.5 d                         | 44.3 a         | 10.2 b                      | 0.6 b              | 0.12 a                    |

*) The number followed by the same letters at the same column were not significantly different at 5% level by Duncan test.

| Treatment | Number of filled pod | Filled pod dry weight |
|-----------|----------------------|-----------------------|
| Shade stress |                      |                       |
| n0        | 13.6 a               | 16.9 a                |
| n1        | 10.0 c               | 12.9 c                |
| n2        | 12.6 ab              | 17.1 a                |
| n3        | 12.3 ab              | 16.3 b                |
| n4        | 12.1 b               | 15.3 b                |
| n5        | 12.1 b               | 16 ab                 |

Genotypes
g1 = Takar-1 11.1 cd 15.7 ab
g2 = (G200-IxTakar1)6 11.6 bcd 14.9 ab
g3 = (G200-IxTakar1)8 11.0 d 14.5 b
g4 = (G200-IxTakar1)10 11.9 bcd 15.8 ab
g5 = Domba 11.4 cd 16.1 ab
g6 = (G200-IxDomba)1 12.2 abcd 17.2 a
g7 = (G200-IxDomba)3 10.9 d 15.6 ab
g8 = (G300-IxDomba)1 11.3 cd 16.2 ab
g9 = Bison 13.6 ab 15.2 ab
g10 = (G200-IxBison)1 12.7 abcd 16.5 ab
g11 = (G300-IxBison)2 13.1 abc 15.2 ab
g12 = (G300-IxBison)9 14.1 a 16.3 ab

*) The number followed by the same letters at the same column were not significantly different at 5% level by Duncan test.

There were significant differences in interaction between shading stress and genotypes on chlorophyll-a and total chlorophyll. Plants of all peanut genotypes growing in shade stress for the whole of their life produced the highest chlorophyll-a and total. These plants tended to produce higher chlorophyll-a and total chlorophyll than genotypes growing in less shade stress (Table 4 and 6).
Decreasing light intensity led to an increase in chlorophyll content. Shade levels above 30% limited carbon assimilation and led to decreased essential oil content and plant growth in Sage plant (*Salvia officinalis* L.) [17]. Peanut genotypes that were tolerant to shade stress tended to adapt by forming higher chlorophyll-a content than sensitive genotypes. However, the chlorophyll a, chlorophyll b, and chlorophyll a + b, photosynthetic and chlorophyll fluorescence characteristics were improved as shade decreased in soybean, and maximum values were observed in 25% shade and no shade [18]. Table 5 shows that shading stress significantly affected to the chlorophyll-b content and peanut plant without shade produced the lowest chlorophyll-b content and the highest was on shading stress given from the age of 26 dap to 50 dap (flowering phase until pods formation). Shade stress at the age of 26 dap to 50 dap required more chlorophyll-b for the flowering process until the pods formation.

**Table 4.** The effect of shade stress given at various stages of plant growth and genotype on Chlorophyll-a content (mg/L).

| Genotypes | Chlorophyll-a content |
|-----------|------------------------|
|           | m0 | m1 | m2 | n0 | n1 | n2 |
| g1= Takar-1 | 14.50 aB5 | 22.47 aA4 | 15.42 aAB5 | 17.14 abAB7 | 22.45 aA4 | 21.58 aA5 |
| g2= (G200-IxTakar1)h | 16.08 aAB | 21.49 aA | 17.15 aAB | 21.43 aA | 13.43 bB | 21.65 aA |
| g3= (G200-IxTakar1)h | 13.12 aB | 20.87 aA | 16.87 aAB | 16.86 abAB | 14.86 abAB | 18.27 abAB |
| g4= (G200-IxTakar1)h | 15.94 aB | 25.02 aA | 17.50 aB | 19.73 abAB | 20.29 aAB | 16.77 abB |
| g5= Domba | 17.87 aAB | 23.43 aAB | 19.61 aAB | 16.72 abAB | 25.07 aA | 24.28 aA |
| g6= (G200-IxDomba)4 | 14.11 aB | 22.10 aA | 17.45 aAB | 17.55 abAB | 19.41 aAB | 18.14 abAB |
| g7= (G200-IxDomba)4 | 16.38 aB | 25.74 aA | 14.14 aAB | 15.03 abB | 16.39 aB | 21.24 aA |
| g8= (G300-IxDomba)4 | 16.15 aB | 26.00 aA | 17.71 aB | 16.06 abB | 18.11 aB | 11.35 bB |
| g9= Bison | 16.50 aAB | 24.39 aA | 17.63 aAB | 12.77 bB | 21.32 A | 15.06 bB |
| g10= (G200-IxBison)7 | 16.93 aBC | 24.59 aA | 16.43 aBC | 21.18 aAB | 21.55 aAB | 13.15 bC |
| g11= (G300-IxBison)7 | 17.88 aA | 21.70 aA | 19.17 aA | 18.33 abA | 23.86 aA | 17.71 abA |
| g12= (G300-IxBison)7 | 15.33 aAB | 21.93 aAB | 14.75 aAB | 18.84 abA | 18.79 aAB | 12.95 bB |

*The number followed by the same small letters at the same column were not significantly different or the number followed by the same capital letters at same row were not significantly different at 5% level by Duncan test.

**Table 5.** The effect of shade stress given at various stages of plant growth and genotype on Chlorophyll-b content (mg/L).

| Shade stress | Chlorophyll-b content |
|--------------|------------------------|
| n0 | 6.55 e<sup>-7</sup> |
| n1 | 13.25 b |
| n2 | 7.92 de |
| n3 | 15.08 a |
| n4 | 10.03 c |
| n5 | 8.11 bd |

<sup>*7*</sup>The number followed by the same letters at the same column were not significantly different at 5% level by Duncan test.

**Table 6.** The effect of shade stress given at various stages of plant growth and genotype on Chlorophyll-total content (mg/L).

| Genotypes | Chlorophyll-total content |
|-----------|---------------------------|
|           | n0 | n1 | n2 | n3 | n4 | n5 |
| g1= Takar-1 | 20.57 aB | 35.25 aA | 22.54 aAB | 25.37 bAB | 33.97 abA | 31.88 aAB<sup>-7</sup> |
| g2= (G200-IxTakar1)h | 22.88 aA | 32.40 aA | 25.12 aA | 31.74 abA | 20.92 aA | 32.89 aA |
| g3= (G200-IxTakar1)h | 18.52 aB | 33.09 aA | 24.57 aAB | 24.64 bAB | 21.89 bcAB | 26.45 abAB |
| g4= (G200-IxTakar1)h | 22.86 aB | 41.53 aA | 2586 aB | 29.08 bAB | 30.37 abcAB | 24.90 abcB |
| g5= Domba | 25.33 aB | 36.07 aAB | 28.67 aAB | 25.11 bB | 39.34 aA | 37.33 aAB |
| g6= (G200-IxDomba)7 | 19.83 aB | 33.18 aA | 25.46 aAB | 25.90 bAB | 29.08 abcAB | 13.48 aAB |
| g7= (G200-IxDomba)7 | 23.45 aB | 41.79 aA | 26.53 abB | 22.34 bB | 24.74 bcB | 32.54 abAB |
| g8= (G300-IxDomba)7 | 22.87 aB | 43.44 aA | 25.81 abB | 23.79 bB | 26.35 bcB | 16.68 bB |
| g9= Bison | 23.39 aB | 38.08 aA | 25.78 aAB | 19.10 bB | 33.03 abcAB | 21.91 bcB |
| g10= (G200-IxBison)7 | 24.00 aBC | 38.31 aA | 23.99 aBC | 38.37 aA | 32.53 abcAB | 19.29 bcB |
| g11= (G300-IxBison)7 | 25.43 aA | 33.81 aA | 28.33 aA | 26.71 aA | 37.07 abA | 25.75 bcA |
| g12= (G300-IxBison)7 | 21.65 aB | 33.81 aA | 21.76 aB | 27.74 bAB | 28.33 abcAB | 18.77 bcB |

<sup>*7*</sup>The number followed by the same small letters at the same column were not significantly different or the number followed by the same capital letters at same row were not significantly different at 5% level by Duncan test.
4. Conclusion
While there were no significantly different results in agronomic traits, the interaction between shade stress and genotypes resulted in significant differences in chlorophyll-a content and total chlorophyll. All 12 peanut genotypes growing in shade stress for the whole of their life tended to produce higher chlorophyll-a content and total chlorophyll. Shade stress treatments significantly affected agronomic characters of peanut. Plants growing under shade stress for the whole of their life produced the lowest dry pod weight (12.9 g/plant) or yield decreased to 23.7% compared to those growing without shade stress. The genotypes had significant effects on agronomic traits and genotype (G200-I x cv.Domba) produced the heaviest dry pod weight 17.2 g/plant.

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