Physiological Response and Tolerance of Genotypes of Shallots (*Allium ascalonicum* L.) under Shade Stress

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Abstract. This study aims to determine several shallot genotypes' physiological responses and tolerance to shade stress. The experimental design used was a completely randomised design (CRD) consisting of 6 genotypes, namely: G1 (Keta Monca), G2 (Ampenan), G3 (Super Philip), G4 (Thailand Nganjuk), G5 (Bali Karet), G6 (Lokananta). Each treatment was repeated five times. Planting was done using polybags measuring 35x35 cm; each polybag planted two bulbs, 2-3 cm deep, with a spacing of 20x20 cm. Shade treatment used 65.00% parent with a shade height of 2 m, a width of 1.5 m, and a shade length of 3 m. The results of the physiological response research showed that the parameters of N-Total, protein, carbohydrates, and carotenoids showed significant differences in the genotype of shallots and indicated an interaction between treatments. The parameters of chlorophyll a, chlorophyll b, and proline showed no significant difference and no interaction between treatments. The genotypes of shallots sensitive to shade resistance were genotype Super Philip, Thailand Nganjuk, and Bali Karet. The moderate tolerant genotype was Ampenan, and the susceptible genotype was Keta Monca.

Keywords: shade; proline; chlorofill.

INTRODUCTION

Shallot (*Allium ascalonicum* L.) is one of the horticultural commodities that have been developed nationally. The prospect of onion cultivation is excellent because it can improve farmers' living standards. It has a high economic and industrial value and export potential [1].

Indonesia's centre of shallot production is Java Island, with a total output of 956,652 tons or about 77.53% of the total national shallot production. Central Java province is the largest shallot producer, producing 519,356 tons or 42.09% of the total national shallot production, followed by East Java and West Java. West Nusa Tenggara is the largest shallot-producing province outside Java, with a show of 117,513 tons or about 9.52% of the total national shallot production, followed by West Sumatra [2].

The productivity influences the productivity of NTB shallots from Bima Regency, designated by the government as a shallot development area. The planted area from 2014 is growing. From 2014 to 2016, they were 8,207, 10,491, and 13,674 ha, respectively, and the production was 91,709, 125,057 and 153,297 tons [2].

Shallots are mostly planted on marginal lands such as dry land, rainfed land, and under tree stands. The ground under tree stands usually has dry land conditions, so the plants get drought stress. Planting plants under tree stands causes plants to get shade stress, where plants experience a lack of sunlight. An understanding of shade stress is essential in onion planting. Shade stress affects all aspects of plant growth, including anatomical, morphological, physiological and biochemical plants. The yield of bulbs in shallots has been reported to be directly related to the amount of water supply and shade.

The primary constraint on the land under the stand is the low light intensity due to the shading factor. Therefore, efforts are needed to obtain adaptive and high-producing plant varieties with low light intensity (shade) conditions. Light has a significant role in physiological processes, such as photosynthesis, respiration, growth and development, closing and opening of stomata, various plant movements and germination [3].

The growth and morphology of shallots cannot be separated from environmental factors that influence them, one of which is shade. The presence of dye causes the intensity of sunlight plants to receive to decrease. Plants will adapt, namely through changes in plant morphological characters. Changes in the morphology of these plants, such as the increase in plant height [4], decreased number of leaves and tillers [5], decreased leaf thickness [6] and increased leaf area [7, 8]. Environmental shade or low light intensity conditions also affect plant physiological conditions [9] and production [10, 11]. Authors [12] stated that physiological conditions include chlorophyll, carbohydrates, nitrogen levels, proteins, etc.

Plant tolerance determines the plants' response to the shaded environment to reduced light intensity. One of the effects of shade on plant morphology is that the plant stems become taller because the plant stems are etiolated [13]. This morphological condition causes the plant to fall, so it can quickly reduce tuber yields. According to [14, 15], shade changed the shallot bulb weight yield and influenced the growth process. Shade by 50 % during growth resulted in a decrease in soybean yield between 37 and 74 % [16, 17], and rice decreased yield productivity by more than 55 % [18].

Based on the description above, this study aimed to determine the physiological response and tolerance of several shallot genotypes to shade stress.

METHOD

The experimental design used was a completely randomised design (CRD) consisting of 6 genotypes, namely: G1 (Keta Monca), G2 (Ampenan), G3 (Super Philip), G4 (Thailand Nganjuk), G5 (Bali Karet), G6 (Lokananta). Each treatment was repeated five times. Planting was done using polybags measuring 35×35 cm; each polybag planted two bulbs, 2-3 cm deep, with a spacing of 20×20 cm. Shade treatment using 65.00% parent with a shade height of 2 m, a width of 1.5 m and a shade length of 3 m.

The implementation of the research includes providing seeds, preparing planting media, planting, maintaining, irrigating, fertilising, sampling, harvesting, post-harvesting and observing. There are seven parameters for keeping physiological responses: N-Total, protein, carbohydrates, carotenoids, chlorophyll a, chlorophyll b, and proline. N-Total analysis using the formula (1).

$$Nitrogen\ contetnt, \% = \frac{(V_c - V_b) \times N \times 14}{Sample\ weight} \times 100, \quad (1)$$

Protein analysis using the formula (2).

$$protein\ contetnt, \% = N \times F_k$$

$$N = \frac{(HCl - blank) \times NHCL \times Fk \times 14}{Sample\ weight} \times 100, \quad (2)$$

where V_c, V_b – sample and blank titar, ml; N – normality of standard H_2SO_4 solution; 14 – equivalent weight of nitrogen; *Fk* = water content correction factor = 100/(100 – % moisture content).

Carotenoid analysis using the formula (3).

$$Carotenoids = \frac{(A480 + 0.14A663 - 0.63A645) \times V \times Fp \times 10^3}{112.5W} \quad (3)$$

where *A480* – Absorbance at a wavelength of 480 nm; *A645* – Absorbance at a wavelength of 645 nm; *A663* – Absorbance at a wavelength of 663 nm; *V* – Extract volume, ml; *W* – Sample weight (g); *Fp* – Dilution factor.

Carbohydrate analysis using the formula (4).

$$Carbohydrate, \% = \frac{GT \times Fp}{Sample\ weight} \times 100, \quad (4)$$

where GT – Table Sugar.

Chlorophyll analysis used the calculation formula (5, 6):

$$Chlorophyll\ \mathrm{A} = 13.7A665 - 5.76A649 \quad (5)$$

$$Chlorophyll\ \mathrm{B} = 25.8A649 - 7.60A665 \quad (6)$$

Proline analysis using the formula (7).

$$Y = 0.0336X + 0.0002 \quad (7)$$

where Y – absorbance ; X – concentration.

Calculate the value of sensitivity (S) of shade stress based on the formula (8).

$$S = \frac{1 - \frac{Y}{Y_p}}{1 - \frac{X}{X_p}}, \quad (8)$$

where Y – score the mean of certain variables (e.g. several branches, plant height, and others) on one genotype under shade stress;
Y_p – score the average of these variables in an optimum environmental genotype;
X – score the average of these variables in all genotypes that experienced shade stress;
X_p – score the mean of these variables in all optimum environmental genotypes.

Score or Value: A – Shade stress tolerant genotype, S<0.5; B – Somewhat tolerant, if 0.5<S<1; C – Sensitive, if S > 1.

RESULTS AND DISCUSSION

Physiological Response of Several Genotypes of Shallots to Shade Stress. In Table 1, the analysis results show that the parameters of N-Total, protein, carbohydrates and carotenoids show significant differences in the genotype of shallots and indicate an interaction between treatments.

Table 1 – Physiological responses of several shallot genotypes on seven parameters of treatment

Parameter	Treatment		
	G	JN	G*JN
N-Total	S	S	S
Protein	S	S	S
Carbohydrate	S	S	S
Carotenoids	S	S	S
Chlorophyll a	NS	NS	NS
Chlorophyll b	NS	NS	NS
Proline	NS	NS	NS

Notes: G – Genotype; JN – Shade Type; G*JN – Combination of Genotype with Shade and No Shade Treatment; S – Significant; NS – Non-significant.

The parameters of chlorophyll a, chlorophyll b and proline showed no significant difference, and there was no interaction between these treatments.

Each plant genotype has a different tolerance to shade stress. Plants adaptive to low radiation experience an increase in leaf area ratio, leaf stem ratio, and stem length and a decrease in leaf thickness [19]. Authors [20] reported that the interaction of the soybean genotype with the environment influenced the growth and yield of soybean plants. Research on the response of plant genotypes to shade is mostly carried out using modified shade environments such as a parent or black cloth. Artificial colouring is also widely used to analyse the growth and morphophysiology of soybean plants [21, 22, 23].

In plants experiencing shade stress, plant height, according to [24], is more influenced by environmental factors, including light and water. Plant height showed etiolation symptoms characterised by excessive plant height growth. Authors [25] stated that plants with 50 % shade had higher growth but were etiolated. The hormone auxin influences this etiolation. In low light, auxin will spur higher stem growth, but the plant becomes weak, the stem is not sturdy, the leaves are small, and the plant looks pale. Symptoms of etiolation occur due to the absence or lack of sunlight. This is to the study's results, which showed an interaction between the shading treatment and the genotype of shallot plants on the parameters of N-Total, protein.

The results showed no interaction between the provision of shade and the genotype of shallot on the proline observation parameter. Proline compounds function to regulate cell osmotic pressure (osmotic adjustment). Accumulating proline can reduce the osmotic potential, reducing the water potential in cells without limiting enzyme function and maintaining cell turgor [26]. Authors [27] stated that there would be an increase in the proline content in the plant crown when the plant is under stress.

One of the critical external factors that can affect the growth and production of a plant is light intensity. Sunlight is one of the essential factors that can affect the photosynthesis rate in plants with chlorophyll. According to [28], sunlight has various effects on plant growth and provides a source of energy for photosynthesis. The absence of light will affect the physiological status of plant tissue so that the carbohydrate content will be reduced at low light intensity or dark. Changes in the level of endogenous hormones or other physiological components can be affected by light intensity, duration, or quality of light. Light intensity is one of the critical factors for plant growth. Shade treatment can affect the chlorophyll content because the amount of light absorbed by plants is lower. The results showed that the chlorophyll observation parameter did not experience any interaction between the shading treatment and the onion genotype.

Table 2 – Average Value of Advanced Test Results (BNJ 5%) combination of shade and no shade treatments on the parameters of N-total, Protein, Carbohydrates and Carotenoids

Shade	Parameter			
	N-Total	Protein	Carbohydrate	Carotenoids
N_0	0.22[a]	1.35[a]	4.63[b]	104.59[b]
N_1	0.16[b]	1.02[b]	5.09[a]	112.42[a]
BNJ 5%	0.01	0.07	0.10	2.45
G1	0.17[bc]	1.02[c]	5.33[a]	103.91[c]
G2	0.23[a]	1.43[a]	4.32[d]	114.36[a]
G3	0.15[c]	0.93[c]	4.41[d]	104.68[c]
G4	0.22[a]	1.37[a]	4.78[c]	101.65[c]
G5	0.19[b]	1.18[b]	5.06[b]	109.67[b]
G6	0.19[b]	1.17[b]	5.29[a]	116.76[a]
BNJ 5%	0.02	0.12	0.18	4.25

Notes: Number-numbers followed by the same letter in the same column show no significant difference based on the BNJ test at a 5% significance level. G1= keta monca; G2= ampenan ; G3= super philip ; G4= tailland sulking; G5= rubber back; G6= lokananta.

The study's results (Table 1) showed that the effect of N-Total, protein, carbohydrates and carotenoids was significantly different on the genotype of shallot plants and indicated an interaction between the genotype treatment of shallot and the shade treatment. Shallots treated with no shade showed a better effect on N-Total and protein parameters than carbohydrates and carotenoids. The results showed that shading increased stem height, accelerated flowering time and reduced dry stover weight per plant. The increase in stem height is an attempt by plants to increase light absorption because plants cannot raise their leaves above the canopy. The research of [29] reported that shading caused an increase in stem height and leaf number of soybean plants.

The earlier flowering process occurs due to protein and carbohydrates (Table 1), which are readily soluble (phytochromes). Shaded environmental conditions can change the pigments (phytochromes) in shaded soybean plants into forms that initiate flowering induction [30]. The decrease in the dry weight of plants due to shading is due to shady conditions where plants experience a limited amount of solar energy that can be absorbed for the optimal photosynthesis process, resulting in a decrease in the dry weight of the plants produced. As stated by [31], the dry weight is the net accumulation of carbon dioxide during plant growth.

The sunlight captured by chlorophyll raises the energy level of the electrons resulting from the oxidation of water in the process of photosynthesis. Plant energy can be used for biological purposes, macromolecular synthesis, and cell division. For example, division in the ontogenetic stomata system is based on how the guard stem cell divides, which is related to the direction of the cell wall that cuts that part of the meristemoid [32].

Plants that grow in low light-intensity environments have smaller roots, are few and are composed of thin-walled cells. This occurs due to inhibiting the translocation of photosynthetic products from the roots. Longer plant stems are composed of thin-walled cells, larger intercellular spaces, and less transport and reinforcement tissue. The leaves are more significant and thinner, and the size of the stomata is more critical; the epidermal cells are light, but the number of leaves is less, and the space between cells is more. Experiments with iris leave grown at different intensities showed that the number of stomata decreased with decreasing light intensity. Responses to increased light intensity differ between plants suitable for shaded conditions (shade plants; indoor plants); with plants that can grow in unshaded conditions. Shade-suitable plants show meagre photosynthetic rates at high light intensities [33]. The photosynthetic rate of shaded plants reaches saturation point at lower light intensities, higher photosynthetic rates at very low light intensities, and lower light compensation points than open-fit plants. From the above description, plants suitable for shade can survive in shaded conditions (low light intensity), while plants suitable for open cannot survive [33].

Tolerance of several Shallot Genotypes to Shade Stress. Tolerance is a mechanism for plant resistance in a state of shade stress and affects production, reducing crop yields or productivity. Based on the formula of [34], the genotype of shallots tolerant of shade stress was obtained. Table 3 shows that the sensitive plant genotypes (P) to shade stress are G3, G4, and G5. The moderately tolerant genotype (AT) was G2, and the tolerant genotype (T) was G1.

Table 3 – Value of Sensitivity Index (S) of six Shallot Genotypes tested under Shade Stress and No Shade Stress

Genotype	Sensitivity Value (S)		Average	Phenotype
	BKU	JU		
G1	0.21	0.29	0.25	T
G2	1.35	0.48	0.92	AT
G3	0.15	3.31	1.73	P
G4	1.40	0.60	1.00	P
G5	2.33	0.34	1.34	P
G6	0.61	1.04	0.83	AT

Notes :S=Sensitivity Index Value; T= Tolerant (< 0.5); AT= Somewhat Tolerant (>0.5 S< 1); P=Sensitive (S>1); BKU= Bulb Dry Weight; JU= Number of Bulbs.G1= keta monca; G2= ampenan ; G3= super philip ; G4= tailland sulking; G5= rubber back; G6=lokananta.

From Table 3, the G1 genotype (Keta Monca) shows a genotype that is tolerant to shade stress. Keta Monca is a local variety of shallots that develops mainly in the Bima Regency, West Nusa Tenggara Province. Production is relatively suitable for local types, has a distinctively spicy taste, and is highly flavourful and savoury. This shallot has a pretty good market, especially in big cities in Indonesia. The shallot cultivation business in Indonesia uses two sources of planting material: local and imported. Farmers prefer to use local varieties because the age of harvest is in line with expectations, and by the conditions of the agroecosystem, the ease of farmers in managing, and plant care that is by the habits of farmers.

In onion plants, shade is leaf protection from damage by exposure to rainwater. This Keta Monca genotype is also a plant resistant to Fusarium wilt disease [35]. When watering, it is adjusted to plant conditions (field capacity) and the weather at the planting time because shallot plants are susceptible to disease if the soil is too wet or too dry. The regulation of osmotic pressure in plants is the work of several compounds that act as osmo-regulators, one of which is proline. Proline Accumulation can reduce osmotic potential, thereby reducing water potential in cells without limiting enzyme function and maintaining cell turgor [36].

Shallots require full light intensity with irradiation of more than 12 hours [37] and air temperatures between 20–30 °C with an optimal average temperature of 24 °C [38].

Sunlight is also thought to affect the growth of shallot plants. Shade conditions prevent sunlight from entering the research site. This results in a lack of sunlight received by plants, thereby inhibiting the process of photosynthesis for plant growth. According to [39], sunlight is an essential factor for the ongoing process of photosynthesis, while photosynthesis is very important for plant metabolism. Added to [40], the effect of sunlight on plants is focused on vegetative and generative growth. Plant response to light is determined by green leaf synthesis, stomata activity (respiration, transpiration), anthocyanin formation, temperature from surface organs, absorption of mineral nutrients, permeability, respiratory rate, and protoplasm flow. Theoretically, the greater the amount of available energy will increase photosynthesis. Lack of sunlight will interfere with photosynthesis and growth, although the need for light depends on the type of plant itself.

Authors [41] stated that the level of shading treatment had no significant effect on the production of fresh matter and dry matter production of forage. Authors [42] noted that the performance of mung bean yields in the 75 % shade reduced the output of mung bean to 65.21% when compared to no shade, but when compared to 50 % shade, the yield of mung bean only decreased by 34.01%.

The results of [43] also showed that shading stress applied from the beginning of the plant to harvest resulted in long stem segments of peanut plants. Authors [44] reported that the intensity of shading up to 75 % increased plant height and reduced the number of leaves on soybean plants.

In addition, lack of light during plant development will cause etiolation symptoms, where plant stems will grow faster but weaker, and the leaves are small, thin and pale (not green). A lack of light causes symptoms of etiolation, or the plant is in a dark and shaded place. Light can also act as an inhibitor in the growth process. This is because it can stimulate the diffusion of auxin to plants not exposed to sunlight. The light that is an inhibitor is caused by the absence of light so that it can maximise the function of auxin to support plant cells.

On the contrary, plants that grow in bright places cause plants to grow more slowly with relatively short, more comprehensive, greener conditions, look fresher, and the plant stems are stronger because sunlight is essential and influences plant growth and development [45].

CONCLUSIONS

The results of the physiological response research showed that the parameters of N-Total, protein, carbohydrates and carotenoids showed significant differences in the genotype of shallots and indicated an interaction between treatments. The parameters of chlorophyll a, chlorophyll b and proline showed no significant difference and no interaction between treatments. The tolerant shallot genotypes (T) were G1 (Keta Monca), and the slightly tolerant genotypes (AT) were G2 (Ampenan) and G6 (Lokananta).

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