Growth and production of soybean (Glycine max L. Merril) varieties in response to waterlogging at vegetative (V₅) growth phase by application of gibberellic acid and salicylic acid

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Abstract. Waterlogging can cause damage to soybean plants that can decrease the growth and yield. The objective of this research was to measure the growth and production of three soybean varieties such as Burangrang, Anjasmoro and Argomulyo, imposed on waterlogging at vegetative stage (V₅) along with the application of GA₃ and Salicylic acid. Waterlogging was conducted for 72 h by sinking the planting media inside a pool filled with water. Number of leaves, pod, seed and chlorophyll content were measured. The result showed that foliar application of GA₃ (200 ppm) together with Salicylic acid (150ppm) for each enhanced the number of pod and seed for Burangrang soybean variety on waterlogged condition compared with normal condition. Burangrang and Anjasmoro soybean varieties gave the highest chlorophyll content on waterlogged condition after being applied by the same dose of plant growth regulators. In term of yield (number of pod and seed per plant), Burangrang soybean variety is the most promising variety to plant in waterlogged condition.

1. Introduction
In crops, soybeans are the third most important commodity after rice and corn. In addition, soybean is a commodity that is rich in protein [1]. The need for soybeans in Indonesia every year is always increasing along with population growth and improving per capita income. Therefore, additional supply of soybeans is needed and to fulfill the demands, soybeans must be imported because national production is insufficient [2].

Waterlogging is a major problem in many agricultural areas in the world and soybeans are plants that are sensitive to waterlogging. In Indonesia, soybean can be cultivated in paddy fields after rice. Waterlogged soil conditions (saturated water) due to residual water from rice planting or rainwater often become one of the causes of low productivity of soybeans in paddy fields [3].

Water stress (waterlogging) can cause premature aging like the occurrence of chlorosis, necrosis, and falling leaves as well as stunting the plant growth which decreases yield (productivity). The level of decrease in yield also depends on the soybean variety, plant growth phase, severity of stress, soil texture, and presence of disease [4].

Soybean plants will experience a yield loss of 93% if they experience flooding for 7 days in the flowering stage R₁ (plants begin to flower), as well as the generative phase of R₃ (plants begin to form
pods), and R₅ (plants begin to form seeds) reach 63% yield loss. Whereas at the vegetative phase V₂ (plants forming the second node), waterlogging can reduce yield by 30% [5].

Exogenous application of 50 ppm gibberellin on third and sixth week after planting was able to increase plant height compared to controls and increase the number of pods and seeds in Wilis soybean variety[6]. Exogenous application of GA₃ to soybean can protect the fall of flowers and increase crop production [7]. SA has the effect of protecting the development of anti-stress programs and accelerating the process of normalization of growth after eliminating stress factors [8]. Salicylic acid and its regulatory role in plant physiology include inhibiting ethylene biosynthesis, interfering with membrane depolarisation, blocking wound responses, and an increase in photosynthetic rate and chlorophyll content in soybeans [9]. Elicitors such as chitosan and salicylic acid could be used not only to increase isoflavone concentration of soybean seeds, but also to increase the growth and seed yield [10].

The purpose of this study was to determine the growth and production of several soybean varieties in response to waterlogging in the vegetative (V₅) growth phase with application of GA₃ and salicylic acid.

2. Materials and Methods.

2.1 Experimental design
The present study was conducted in the experimental area of Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia. Three soybean varieties were used Burangrang, Anjasmoro, Argomulyo, planted in polybags that were filled with 5 kg of mixed top soil and compost. Urea (0,44 g/polybag), TSP (0,68 g/polybag) dan KCl/ha (0,44 g/polybag) were used as basic fertilizer. Giberelic and salicylic acid were used in this research. This research used a Randomized Block Design with 3 factors such as varieties, plant growth regulator, and waterlogging with 18 treatment combinations and 3 replicates. Sample of 3 plants were taken at random from each plots to measure number of leaves, pods, and seeds, also chlorophyll content were measured in this research. Number of leaves was measured by only counting the perfectly opened leaf. Number of pods was measured at the harvest time and only pods that contain seeds inside were calculated. Number of seeds was measured by opening the collected pods. Chlorophyll content was measured by using Arnon’s method [11] by crushing 1 g of leaf sample using 85% Acetone. Spectrophotometer with a wavelength of 645 nm and 663 nm was used to determine the chlorophyl content of each leaf sample.

Factor I : Soybean varieties
V₁ : Burangrang
V₂ : Anjasmoro
V₃ : Argomulyo

Factor II : Plant Growth Regulator
K₀ : Control
K₁ : GA₃ (150 ppm) + SA (100ppm)
K₂ : GA₃ (200 ppm) + SA(150ppm)

Factor III: Waterlogging
G₀ : Without waterlogging
G₁ : With waterlogging at V₅ growth phase

2.2 Giberelic acid and Salicylic acid application.
GA₃ and SA were applied with spraying system based on concentration according to the treatment by mixing it with destilled water, with 7-day intervals started at the second week after planting until the beginning of flowering stage.
2.3 Waterlogging method
Waterlogging were done by sinking the planting media into a plastic pool sized 10 m x 2 m that was filled with water, started when plant entered growing phase V₅ (perfectly opened leaf at the fifth node) for 72 hours.

3. Results and Discussion

3.1 Number of leaves
The number of leaves on the fifth week after planting or week before flowering was observed as shown in Table 1

| Treatment | Number of leaves |
|-----------|-----------------|
| V₁K₀G₀    | 8.67            |
| V₁K₀G₁    | 8.83            |
| V₁K₁G₀    | 9.00            |
| V₁K₁G₁    | 9.33            |
| V₁K₂G₀    | 8.83            |
| V₁K₂G₁    | 9.33            |
| V₂K₀G₀    | 8.67            |
| V₂K₀G₁    | 8.83            |
| V₂K₁G₀    | 9.67            |
| V₂K₁G₁    | 9.00            |
| V₂K₂G₀    | 9.33            |
| V₂K₂G₁    | 9.00            |
| V₃K₀G₀    | 8.67            |
| V₃K₀G₁    | 9.00            |
| V₃K₁G₀    | 8.67            |
| V₃K₁G₁    | 10.00           |
| V₃K₂G₀    | 9.33            |
| V₃K₂G₁    | 9.00            |

For variety V₁ (Burangrang), the highest number of leaves was on waterlogged condition as much as 9.33 leaves both on V₁K₁G₁ and V₁K₂G₁ treatment where control of this variety showed the lowest number of leaves. For variety V₂ (Anjasmoro), the highest number of leaves was as much as 9.67 leaves on V₂K₀G₀ treatment, where no waterlogging stress was given. The control of variety V₂ (Anjasmoro) showed the lowest number of leaves. For variety V₃ (Argomulyo), the highest number of leaves was as much as 10 leaves on V₃K₁G₁ treatment where waterlogging stress was given. The lowest number of leaves on variety V₃ (Argomulyo) were achieved by control and V₃K₁G₀ Treatment.

From the data collected from 3 soybean varieties, the control of those 3 varieties showed the lowest number of leaves. Application of Gibberellic acid and Salicylic acid could increase number of leaves. The results goes along with Hasanah et al [10] literature that states elicitors such as salicylic acid can be used to increase the growth of plant, including height and number of leaves.

3.2 Number of pod
The number of pod was measured by calculating all the formed pods that contained seeds on each plant as shown in Table 2.
| Treatment | Number of Pod |
|-----------|--------------|
| V1K0G0   | 17.17        |
| V1K0G1   | 20.67        |
| V1K1G0   | 17.83        |
| V1K1G1   | 22.00        |
| V1K2G0   | 25.83        |
| V1K2G1   | 22.33        |
| V2K0G0   | 21.33        |
| V2K0G1   | 16.50        |
| V2K1G0   | 22.50        |
| V2K1G1   | 16.00        |
| V2K2G0   | 20.00        |
| V2K2G1   | 17.50        |
| V3K0G0   | 21.67        |
| V3K0G1   | 21.17        |
| V3K1G0   | 21.50        |
| V3K1G1   | 17.33        |
| V3K2G0   | 15.33        |
| V3K2G1   | 15.83        |

For Variety V1 (Burangrang) the highest number of pod per plant was V1K2G1 as much as 22.33 pods for waterlogged condition and V1K2G0 as much as 25.83 pods without waterlogging. Burangrang variety could give higher yield on waterlogged condition after being applied with K1.

For variety V2 (Anjasmoro) the highest number of pod per plant was V2K2G1 as much as 17.50 pods for waterlogged condition and V2K1G0 as much as 22.50 pods without waterlogging. For Variety V3 (Argomulyo) the highest number of pod per plant was V3K0G1 as much as 21.17 pods for waterlogged condition and V3K0G0 as much as 21.67 pods without waterlogging, which both are control without any plant growth regulator application.

Variety V1 (Burangrang) and V2 (Anjasmoro) showed great results after being applied with GA3 (200 ppm) and SA (150 ppm) and it goes along with Sumarno et al [10] statement that states exogenous application of Gibberellic acid could increase number of pod (research using Willis soybean variety). Based on the results above, Variety V1 (Burangrang) and V2 (Anjasmoro) can promise better yield after being treated with plant growth regulators, but not with variety V3 (Argomulyo).

3.3 Number of seed

Table 3 shows about the number of seed per plant based on the treatments. Application of K2 or GA3 (200 ppm) and SA (150 ppm) gave the highest number of seed per plant on Burangrang soybean variety at waterlogged and normal conditions as much as 33.83 and 37.67 pods per plant respectively. Anjasmoro soybean variety with K1 (GA3 (150 ppm) + SA(100ppm)) gave the same number of seed per plant as much as 31.67 with K2, which are the highest on normal condition, but only with K2 treatment that gave the highest number of seed on waterlogged condition as much as 28.33 seeds. Argomulyo variety showed highest result on control treatment as much as 31.83 seeds.

Variety V1 (Burangrang) and V2 (Anjasmoro) showed great results after being applied with plant growth regulators, which goes along with Yennita [7]; Sakhabutdinova et al [8]; Hasanah and Sembiring [10] statements that state application of Gibberellic acid can protect from falling flowers and increase crop production, as Salicylic acid has the effect of protecting the development of anti-stress programs and accelerating the process of normalization of growth after eliminating stress factors and also can increase isoflavone concentration of soybean seeds, growth and seed yield.
Table 3. Number of seed per Plant of three soybean varieties

| Treatment   | Number of Seed |
|-------------|---------------|
| V₁K₀G₀      | 27.83         |
| V₁K₀G₁      | 32.67         |
| V₁K₁G₀      | 28.83         |
| V₁K₁G₁      | 32.83         |
| V₁K₂G₀      | 37.67         |
| V₁K₂G₁      | 33.83         |
| V₂K₀G₀      | 30.33         |
| V₂K₀G₁      | 26.83         |
| V₂K₁G₀      | 31.67         |
| V₂K₁G₁      | 24.33         |
| V₂K₂G₀      | 31.67         |
| V₂K₂G₁      | 28.33         |
| V₃K₀G₀      | 31.83         |
| V₃K₀G₁      | 29.33         |
| V₃K₁G₀      | 30.33         |
| V₃K₁G₁      | 24.83         |
| V₃K₂G₀      | 21.67         |
| V₃K₂G₁      | 22.33         |

3.4 Chlorophyll content

Table 4 shows about chlorophyll content per plant after being treated with plant growth regulators and waterlogging conditions.

Table 4. Chlorophyll content per plant of three soybean varieties

| Treatment   | Chlorophyll Total (mg/g) |
|-------------|--------------------------|
| V₁K₀G₀      | 4.20                     |
| V₁K₀G₁      | 3.30                     |
| V₁K₁G₀      | 4.48                     |
| V₁K₁G₁      | 3.71                     |
| V₁K₂G₀      | 4.13                     |
| V₁K₂G₁      | 4.74                     |
| V₂K₀G₀      | 3.91                     |
| V₂K₀G₁      | 3.44                     |
| V₂K₁G₀      | 4.30                     |
| V₂K₁G₁      | 4.25                     |
| V₂K₂G₀      | 3.88                     |
| V₂K₂G₁      | 4.93                     |
| V₃K₀G₀      | 4.25                     |
| V₃K₀G₁      | 2.77                     |
| V₃K₁G₀      | 3.82                     |
| V₃K₁G₁      | 3.91                     |
| V₃K₂G₀      | 4.11                     |
| V₃K₂G₁      | 4.10                     |

For variety V₁ (Burangrang), the highest chlorophyll content was on waterlogged condition as much as 4.74 mg/g on V₁K₂G₁ treatment where V₁K₀G₁ treatment of this variety showed the lowest
chlorophyll content. For variety V₂ (Anjasmoro), the highest chlorophyll content was on V₂K₂G₁ as much as 4.93 mg/g., and the lowest was on V₂K₀G₁ treatment. For variety V₃ (Argomulyo), highest chlorophyll content was achieved by the control or V₃K₀G₀ as much as 4.25 mg/g.

Variety V₁ (Burangrang) and V₂ (Anjasmoro) showed increase in chlorophyll content after being applied with plant growth regulator, and it goes along with Raskin [9] statement that states salicylic acid and its regulatory role in plant physiology include inhibiting ethylene biosynthesis, interfering with membrane depolarisation, blocking wound responses, and an increase in photosynthetic rate and chlorophyll content in soybeans.

4. Conclusions
Application of GA₃ (200 ppm) and SA (150ppm) gave the highest number of pod and seed per plant on Burangrang and Anjasmoro soybean varieties, both in waterlogged and normal condition. Application of plant growth regulator did not give the best result on Argomulyo variety as the control gave the highest number of pod, seed, and chlorophyll content. In term of yield (number of pod and seed per plant), Burangrang soybean variety is the most promising to plant in waterlogged condition, followed by Anjasmoro soybean variety.

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Acknowledgments
This work was supported by project Higher education for basic research (Penelitian Dasar Unggulan Perguruan Tinggi (PDUPPT), Ministry of Research, Technology and Higher Education Republic of Indonesia (Grant No.38/UN5.2.3.1/PPM/KP-DRPM/2018). Moreover, the authors acknowledge the Universitas Sumatera Utara, Medan, Indonesia for the support of scientific labs.