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Effect of benzyl amino purin (BAP) and gibberellin acid (GA₃) to chlorophyll and antioxidant enzymes of soybean (*Glycine max* (L) Merrill.) genotypes in response to inundation conditions

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Abstract. Indonesia's climate change issues cause the significant potential for inundation to occur when rainfall is too high in the area of soybean cultivation, which can give an impact on soybean productivity. The objective of this research was to measure the effect of growth regulators which can respond to soybean plant adaptation to inundation stresses. The influence of Benzyl Amino Purin (BAP) and Gibberellin Acid (GA₃), and their mixture on the chlorophyll and antioxidant defense system of three (Grobogan, Willis, and Detam-1) soybean genotypes has been conducted subjected to inundation condition in March 2018. The chlorophyll total by GA₃ was significantly increased at Detam-1 soybean genotype, while no change in other two soybean genotypes in GA₃, BAP, and the mixture compared to controls. Exogenous GA₃ enhanced the activity of superoxide dismutase (SOD) in Grobogan and Detam-1 soybean genotypes in response to inundation (72 h). On the other hand, peroxidase (POD) activity was enhanced on Grobogan with GA₃, and Detam-1 soybean genotypes with BAP applications when compared to control. Our result reveals that SOD together with POD activities was participate to protect Detam-1 soybean genotype from inundation condition.

1. Introduction

Food security is under severe dread because of the growing world residents. Response for crop production growth up to 100-110% was predicted from 2005 to 2050 [1]. Soybean is an important crop plant as the primary source for protein and oil, and to recover in assembly food-need in Indonesia. Soybeans are mostly cultivated in paddy fields after rice, and some are on dry land during the rainy season. In both planting systems, part of the growing phase of soybean plants will be faced with high soil moisture. The condition of moist growth media will inhibit plant growth which in turn reduce yield [2].

The low production of soybean in Indonesia is caused by several factors such as soil, climate, pests and diseases, as well as poor management practices. Soybean is regarded as a waterlogging-sensitive crop [3]. Soybeans are C₃ plants that cannot withstand drought and waterlog. The ability to survive under low oxygen conditions is related to the activation of complex molecular and metabolic responses [4]. The good groundwater condition for soybean plants is groundwater in a field capacity since the plants grow to full pods, then dry condition before harvest [5]. The stagnant soil conditions (saturated water)



ranged between 15-25% at the age of 15-30 days (vegetative phase) could be more prominent reduced seed yield 50 %. The condition of flooded soil for two weeks during the flowering phase (R_2) of some soybean genotypes can significantly reduce floral bud numbers, and seed yields by at least 37%, also even caused plant death [6]. Therefore, the effective way for inundation tolerance in soybean is needed to make certain food production, although variation in sensitivity within and among their genotypes exists.

Plant growth regulators found in many types of plants. BAP is a synthetic compound cytokine type which also plays a role in root formation, cell division and sprout organ formation [7]. Several studies have shown that the use of a combination of NAA and BAP can increase orchid seed germination. Luan V.Q et al. [8] reported that the use of a combination of NAA 1 mg.l^{-1} and BAP 0.5 mg.l^{-1} is good for germination of *Dendrobium sp. in vitro*. The application of GA_3 very effectively used in increasing seed yield, stem elongation, increase the number of panicles, accelerate flowering period in rice seeds. According to Susilawati et al. [9], exogenous GA_3 200 ppm can increase plant height, panicle number, flowering age and panicle length and production of rice. Bishnoi and Krishnamoorthy [10], in peanut plants which were submerged for 7 and 14 days could reduce leaf area, the amount of chlorophyll and photosynthesis rate but slightly reduce the potential of leaf water with the GA_3 application.

Planting of soybeans on wet soil will inhibit germination and initial growth, due to lack of oxygen for seed growth and plant roots. Regarding those unfavourable conditions, affected growth and reduced photosynthetic activities of the plant. Beside of that, the antioxidant enzyme system has been known in plants producing numerous varieties of activated oxygen species, causing membrane lipids, protein and DNA damage, and photosynthetic pigments destruction [11]. In summarizing, superoxide dismutase (SOD) is primarily activated to change superoxide to oxygen and hydrogen peroxide, and together with another three responsible enzymes, namely catalase (CAT), glutathione peroxidase (GPx), and ascorbate peroxidase (APX) developed antioxidant defense system, in order to control hydrogen peroxide (H_2O_2), a dangerous oxygen-containing free radical in to water molecules [12]. H_2O_2 has a role as a molecular signal in the plant. It is connected in the adjusting of various abiotic and biotic stresses and at high amount has a significant role in cell death and throughout the final stages of aging [13]. Peroxidases (EC.1.11.1.7.) are enzymes which catalyse redox reactions together with H_2O_2 as an electron acceptor and a variety of substrates in which oxygen, water or both are released. They are steady heat enzymes and are found in plants and animals [14].

In our previous study, Grobogan soybean genotype is more responsive to growth when treated in waterlogging conditions during germination and growth of embryo genic callus in *in vitro* hypoxic conditions [15]. Conversely, other reports showed no significant correlation between germination and seedling stages and yield in flood-tolerant soybeans [16]. This study was conducted to examine the influence of exogenous applications of GA_3 , BAP, and their mixture on chlorophyll content, and antioxidant enzyme activities in soybean genotypes (Grobogan, Willis, and Detam-1) under inundation conditions.

2. Materials and Methods

2.1. Plant material and treatments

The plant materials that were used in this study include soybean seeds namely Grobogan, Willis, and Detam-1, which were obtained from Research Centres for tubers and seeds (Balitkabi) Malang, Indonesia. The seeds of each genotype were surface sterilized with 40% (v/v) Sodium hypochlorite solution for 5 min and thoroughly washed with sterilized distilled water and were sown in a shallow container containing farm soil. Germinated seeds were placed into plastic polybags filled with 5 kg of the mixture of organic nutrient and soil, and grown in the field. Treatments were arranged in a factorial randomized block design consisting of three factors, with three replicates as follows:

- Factor I: Genotypes tested.
G₁: Grobogan
G₂: Willis

- G₃: Detam-1
- Factor II: Growing Regulatory Substances
 - K₀: control
 - K₁: 30 ppm BAP
 - K₂: 150 ppm GA₃
 - K₃: 30 ppm BAP + 150 ppm GA₃
- Factor III: Inundation condition
 - I₀: control
 - I₁: Inundation (72 h)

All treated seedlings were transferred to a water pool (5 m x 2 m x 80 cm) filled with water until 5-10 cm above the soil surface for 72 h submerged when the control soybean plants reached the V₄ stage (perfectly opened leaf at the fourth node).

2.2. BAP and GA₃ application

BAP and GA₃ were applied with spraying system based on the concentration according to the treatment with 7-day intervals started at the second week after planting until the beginning of the flowering stage.

2.3. Response data collection

Chlorophyll contents were estimated according to the method described by Arnon, [17]. SOD activity was analysed according to the identifying of Steward and Bewley [18], using nitro blue tetrazolium (NBT), and POD activity was examined with a spectrophotometer with wavelength at 470 nm using guaiacol as a phenolic substrate with H₂O₂ [19].

3. Results and Discussion

3.1. Total chlorophyll

The chlorophyll total can be seen in Table 1.

Table 1. Effect of foliar BAP and GA₃ on Chlorophyll total (mg/g)

Treatment	G ₁ ^a	G ₂ ^b	G ₃ ^c
C ^d	34.93	34.23	35.50
B ^e	37.30	33.11	33.63
G ^f	32.8	34.37	43.42
B+G ^g	37.90	38.23	37.87

^aGrobogan

^bWillis

^cDetam-1

^dcontrol

^eBAP

^fGA₃

^gMixture of BAP and GA₃

The total chlorophyll showed various profiles depending on the genotypes. Shortage of O₂ is often occurring during various plant developmental processes. The ability to live through low oxygen conditions is closely connected to the activation of fast and influence on molecular and metabolic responses [4]. Relative to the control, a marked promote in chlorophyll was observed in waterlogged

plants for Grobogan genotype added with BAP, also for Detam-1 genotype which added with GA₃. It was found that the application of mixtures (BAP and GA₃) showed a powerful physiological effect on chlorophyll content soybean in inundation condition (Table 1). This could be the role of BAP together with gibberellin signalling promote growth, due to the increase in photosynthesis responses to abiotic stress. Significantly, exogenous application of GA₃ and proline in stressed plants improve the endogenous proline content. Therefore, the changes of chlorophyll content positively correlated to the total of proline contents [20, 21].

3.2. Antioxidant enzymes

SOD and POD enzyme activities of soybean seedlings considerably were changed during inundation conditions after applying in exogenous BAP, GA₃, and mixture BAP and GA₃ (Table 2). SOD and POD enzyme activities in Grobogan and Detam-1 soybean genotypes leaves were promoted only by the sole application of either BAP or GA₃, whereas their mixture was similar to the respective control. In contrast, the tremendous reduction of both SOD and POD enzyme activities was recorded in Willis soybean genotype after pre-treatment with either BAP or GA₃, and their mixtures compared to control (Table 2). The antioxidant defense system gathered a non-enzymatic and an enzymatic system including superoxide dismutase (SOD) and Peroxidase (POD). A significant increased in SOD and POD activities in Grobogan and Detam-1 soybean genotypes, showed a key in response to inundation condition, it may have resulted due to the formation of O₂⁻ and toxic levels of H₂O₂. There was an achieved performed action between SOD and POD in inundation condition, indicated existing at the same phase of the plant cells to protect opponent the stress [14, 22].

Table 2. Effect of foliar application of BAP and GA₃ on both SOD and POD enzyme activity

Treatment	SOD			POD		
	G ₁ ^a	G ₂ ^b	G ₃ ^c	G ₁ ^a	G ₂ ^b	G ₃ ^c
C ^d	12.53	19.32	15.12	2.71	9.56	2.97
B ^e	10.63	14.49	16.15	7.81	7.52	2.02
G ^f	20.29	11.27	19.60	5.82	6.09	10.80
B+G ^g	12.53	17.94	12.01	2.40	6.05	2.12

^aGrobogan

^bWillis

^cDetam-1

^dcontrol

^eBAP

^fGA₃

^gMixture of BAP and GA₃

4. Conclusions

The present study showed enhancement of SOD and POD activities led to the proper protection of plants against inundation conditions for Grobogan and Detam-1 soybean genotypes

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