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Involvement of humic acid in production and physiology of soybean (*Glycine max* L.) under drought stress conditions

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ABSTRACT

Introduction: This study aims to determine the best effect of soybean plant production and physiology on humic acid application under drought-stress conditions. **Methods:** This study used a factorial complete randomized design with three levels of humic acid (0 ppm, 500 ppm, and 1000 ppm) and three levels of drought stress (80% KL, 60% KL, and 40%). The parameters analyzed were the number of flowers, pod fresh weight, pod dry weight, number of seeds, leaf chlorophyll, relative water content (RWC), and stomatal density. **Results:** The highest number of flowers was in the 80% KL drought stress treatment; the highest pod fresh weight and pod dry weight were in the 80% KL drought stress treatment; the highest number of seeds was in the 80% KL drought stress treatment; the highest leaf chlorophyll was in the 1000 ppm humic acid treatment and 80% KL drought stress; the highest relative water content (RWC) was in the interaction between 1000 ppm humic acid and 80% KL drought stress; and the highest stomatal density was in the 80% KL drought stress treatment. **Conclusion:** Humic acid application affects leaf chlorophyll and relative water content (RWC). Drought stress affects the number of flowers, pod fresh weight, pod dry weight, number of seeds, leaf chlorophyll, relative water content (RWC), and stomatal density. There was an interaction effect between humic acid application and drought stress on the relative water content (RWC) parameter.

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INTRODUCTION

Soybean is one of the leading food crop commodities in Indonesia. The protein content in soybean seeds of superior varieties reaches 37-43% (Liana *et al.*, 2023). Soybeans are widely used in the food industry such as soy milk, tofu, tempeh, flour, and soy sauce, and in the non-food sector such as making animal feed. Demand for soybeans has increased yearly but is not matched by soybean production. One of the causes of the decline in soybean production is the low productivity of soybeans due to the reduction in productive land area. Based on data from the Central Bureau of Statistics (2021), the highest productivity of soybeans is grown on irrigated rice fields, reaching 18.45 ku/ha while the national soybean requirement is 2.7 million tons annually to meet domestic needs (Triastono *et al.*, 2020).

Factors that influence the low production of soybean crops can come from the plant and the environment. Climate change is one of the factors causing the decline in soybean production in Indonesia. Temperature and rainfall intensity have an impact on dry land which causes a decrease in soybean production (Ruminta *et al.*, 2020). The dry season causes the land to become dry or drought stress which results in low soybean production (Saputra *et al.*, 2015). Limited water availability in the dry season disrupts the irrigation system. Unfulfilled irrigation systems in the dry season cause a decrease in soybean production considering that soybean plants are more sensitive to drought (Suryaningrum *et al.*, 2016). Drought conditions can cause anatomical and physiological changes in plants. Roots are one part of the plant affected by physiological disorders (Fenta *et al.*, 2014). The root response in the face of drought stress is to change the number and size of cells. Mechanical tolerance of plants in the face of drought stress is done by reducing the diameter of the stele and xylem (Makbul *et al.*, 2011).

The low productivity of soybeans can be overcome by tillage and the addition of nutrients to plants. The addition of biostimulants can be used as one way to overcome the problem of drought. Biostimulants play a role in increasing the efficiency of nutrient absorption in the soil, maintaining water content in the soil, increasing plant tolerance to stress, and increasing production yields and plant biomass (Pajrita *et al.*, 2023). Humic acid is a biostimulant composed of organic compounds that undergo a humification process and affect plant growth. Humic acid indirectly affects soil fertility or directly affects plant metabolism. Humic acid has biological, chemical, and nutritional benefits. Humic acid biologically affects the activity of microorganisms and increases root growth, chemically absorbs and binds plant nutrients, and nutritionally provides nitrogen, phosphorus, and sulfur for plants and microorganisms (Nuarini and Zahro, 2020).

Previous research conducted by Simanjuntak *et al.* (2015) on the growth and production of soybeans under drought stress showed that drought stress decreased total leaf area (25.40%), root volume (44.87%), crown wet weight (38.01%), and dry weight of 100 seeds (23.32%). The ability of soybean plants to grow and develop under drought-stress conditions is also influenced by soybean plant varieties. This is per the research of Saputra *et al.* (2015) on the effect of drought stress on the growth and seed production of five soybean varieties showed the results that grobogan soybean varieties are tolerant to drought stress of 65% KL. Increasing soybean growth can be done by giving biostimulants in the form of humic acid. Wahyuningsih *et al.* (2016) applied humic acid with a concentration of 1200 ppm which resulted in increased P uptake and soybean plant growth. Wijayanto and Sucahyo (2021) conducted research and showed that the provision of humic acid increased the productivity of soybean plants by 127.78%. Based on the research that has been done on the effect of drought stress and the provision of humic acid on the growth and production of soybean plants, the update of this research is to examine how the interaction between drought stress and humic acid concentration affects the production and physiology of soybean plants (*Glycine max* L.). The purpose of the study was to determine the best production and physiology with the addition of humic acid in drought-stressed soybean plants.

METHODS

Experimental design and planting materials

The research was conducted from August to October 2022 at the Greenhouse of the Faculty of Animal Husbandry and Agriculture, Diponegoro University, Tembalang, Semarang City. The research used a 3 x 3 factorial completely randomized design (CRD) with 3 replications. The first factor was the level of drought stress consisting of 3 levels, namely K0 = 80% KL, K1 = 60% KL, and K2 = 40% KL. The second factor was the application of humic acid concentration consisting of 3 levels, namely H0 = 0 ppm, H1 = 500 ppm, and H2 = 1000 ppm.

The experiment used planting material of soybean variety Grobogan (BALITKABI). Seeds were planted as many as 3 seeds per pot. The planting medium was made from a mixture of 6 kg soil, 12 g/pot husk, and 43.2 g/pot manure. Basic fertilization consisted of urea 0.261 g/pot, SP36 1 g/pot, and KCl 0.522 g/pot. Thinning was done at the age of 7 hst by leaving 1 plant per pot. Drought stress application was carried out starting at the age of 4 weeks after planting by watering until the limit of drought stress treatment level. Humic acid application was done twice at the age of 4 weeks and 7 weeks by spraying on the leaves. Maintenance consisted of watering, and controlling pests, diseases, and weeds. Harvesting was done at the age of 70 hst when most of the leaves had begun to turn yellow and fall and the stems began to dry.

Observation parameters

The parameters observed included growth, yield, and physiological components related to drought stress, namely: number of flowers (flower plant-1), pod fresh weight (gram plant-1), pod dry weight (gram plant-1), number of seeds (seed plant-1), leaf chlorophyll (mg gram-1), RWC (%), and stomatal density. Parameters of number of flowers and leaf stomata were observed at 6 weeks after planting (MST). The parameters of pod fresh weight, pod dry weight, number of seeds, leaf chlorophyll, and RWC were observed at harvest time or 70 Days After Planting (HST).

Data analysis

The data obtained will be analyzed using analysis of variance (ANOVA) to determine the effect of treatment. If the research data shows the effect of treatment, it is continued with the Duncan Multiple Range Test (DMRT) at the 5% level to determine the difference between treatments.

RESULTS AND DISCUSSION

Numbers of flowers

Based on Duncan's multiple range test, the results showed that drought stress conditions significantly affected the number of flowers but humic acid application and the interaction between drought stress and humic acid application did not significantly affect the number of flowers. The 80% KL drought stress condition was not significantly different from the 60% KL drought stress condition but significantly different from the 40% KL drought stress condition. Humic acid treatments of 0 ppm, 500 ppm, and 1000 ppm did not show significant differences but there was an increase in the number of flowers as the concentration of humic acid increased (Table 1).

Table 1. Number of flowers of soybean (*Glycine max* L.) under drought stress conditions and humic acid application.

Drought stress (KL)	Humic Acid (ppm)			Average
	0	500	1000	
	----------- (flower/plant) -----------			
80%	17	37	42	32[a]
60%	22	41	23	29[a]
40%	15	9	21	15[b]
Average	18	29	29	

Notes: Numbers followed by different superscripts in the row mean, column mean, and interaction matrix indicate significant differences in Duncan's α = 5% test.

Drought stress affects plant development in the generative phase. Parameters that appear due to drought conditions in the generative phase can be seen in the flowering phase which can cause flower fall so that no pods are formed (Maimunah *et al.*, 2018). Humic acid application is expected to be more responsive to increased phosphorus so that it can spur soybean growth (Wahyuningsih *et al.*, 2016). Phosphorus plays an important role in the generative growth of soybeans. Phosphorus is needed by plants during flower and fruit formation, stimulating root development, preparing nucleic acids, and accelerating maturation (Fauziah *et al.*, 2018). The role of humic acid in this study had no significant effect, presumably due to the suboptimal absorption of humic acid.

Pod fresh weight

Based on Duncan's multiple range test, the results showed that drought stress conditions significantly differed on pod fresh weight but humic acid application and the interaction between drought stress and humic acid application did not significantly differ on pod fresh weight. Drought stress condition of 80% KL was not significantly different from 60% KL but significantly different from 40% KL. Humic acid treatments of 0 ppm, 500 ppm, and 1000 ppm showed no significant differences (Table 2).

Table 2. Fresh weight of soybean pods (*Glycine max* L.) under drought stress conditions and humic acid application

Drought stress (KL)	Humic acid (ppm)			Average
	0	500	1000	
	------------ g / plant ------------			
80%	25,531	26,182	20,247	23,987[a]
60%	20,982	22,464	18,059	20,502[a]
40%	10,022	18,982	10,532	13,179[b]
Average	18,845	22,543	16,279	

Notes: Numbers followed by different superscripts in the mean row, mean column, and interaction matrix show significant differences in Duncan's α = 5% test.

Drought stress is an essential factor that affects the fresh weight of soybean pods. Lack of water and high temperatures at the beginning of flowering to ripening cause acceleration of the pod filling period and reduce yield weight (Kobraei *et al.*, 2011). Foliar application of humic acid is expected to increase plant N and P uptake. Nutrients are more quickly absorbed by plant tissues, especially the crown, and more quickly enter the cellular metabolic process (Alshaal & ElRamady, 2017).

Pod dry weight

Based on Duncan's multiple range test, the results showed that drought stress conditions were significantly different on pod dry weight but humic acid application and the interaction between drought stress and humic acid application were not significantly different on pod dry weight. The drought stress condition of 80% KL was significantly different from 40% KL but the drought stress condition of 60% KL was not significantly different from 80% KL and 40% KL. Humic acid treatment of 0 ppm, 500 ppm, and 1000 ppm had no significant effect but there was an increase in pod dry weight as the concentration of humic acid increased (Table 3).

Plant water requirements will increase as the plant ages. Higher levels of drought stress received by plants will reduce crop yields because the water requirements of the plant decrease (Saputra et al., 2015). Humic acid is a biostimulant that contains elements that can increase plant growth. Research conducted by El-Bassion et al. (2010) showed that spraying humic acid significantly increased plant growth and yield in the form of the number of pods per plant, pod weight, and protein content. However, under drought stress conditions, pod weight did not increase significantly with the application of humic acid.

In addition, humic acid is also known to have a positive effect on soil structure and nutrient availability, which in turn can support better plant growth. Under drought conditions, humic acid can help plants retain soil moisture and increase water use efficiency. Therefore, although pod weight may not increase significantly under drought stress conditions, the use of humic acid still provides important benefits for overall plant health and productivity.

Table 3. Dry weight of soybean pods (*Glycine max* L.) under drought stress conditions and humic acid application.

Drought stress (KL)	Humic acid (ppm)			Average
	0	500	1000	
	------------ g / plant ------------			
80%	15,561	15,008	14,351	14,973[a]
60%	11,128	13,798	11,976	12,301[ab]
40%	8,741	7,010	10,668	8,806[b]
Average	11,810	11,939	12,332	

Notes: Numbers followed by different superscripts in the row mean, column mean, and interaction matrix indicate significant differences in Duncan's α = 5% test.

Number of seeds

Based on Duncan's multiple range test, the results showed that drought stress conditions were significantly different on the number of seeds but the provision of humic acid and the interaction between drought stress and humic acid were not significantly different on the number of seeds. The 80% KL drought stress condition was significantly different from the 40% KL drought stress condition but the 60% KL drought stress condition was not significantly different from the 80% KL and 40% KL drought stress conditions. Humic acid treatments of 0 ppm, 500 ppm, and 1000 ppm were not significantly different from the drought stress conditions (Table 4).

Table 4. Soybean (*Glycine max* L.) seed yield under drought stress and humic acid application

Drought stress (KL)	Humic acid (ppm)			Average
	0	500	1000	
	------------ biji / plant ------------			
80%	16,00	18,33	17,67	17,33[a]
60%	12,00	15,33	16,33	14,56[ab]
40%	13,67	14,00	11,33	13,00[b]
Average	13,89	15,89	15,11	

Notes: Numbers followed by different superscripts in the row mean, column mean, and interaction matrix indicate significant differences in Duncan's α = 5% test.

Lack of water needed by plants causes inhibition of physiological and metabolic processes such as nutrient absorption, reduced photosynthetic yields, and inhibition of photosynthate transportation which causes an increase in empty pods. The increase in the number of empty pods causes a decrease in seed yield (Rahardian, 2013). Humic acid application is expected to increase plant production under drought-stress conditions. The success of optimal humic acid absorption is influenced by environmental factors including temperature and sunlight intensity. Insufficient light intensity causes the role of humic acid in increasing the rate of photosynthesis to be disrupted so that photosynthate yield is less than optimal.

Chlorophyll

Based on Duncan's multiple range test, the results showed that drought stress conditions and humic acid application significantly affected leaf chlorophyll but the interaction between drought stress and humic acid application did not significantly affect leaf chlorophyll. The 80% KL drought stress condition was not significantly different from the 60% KL drought stress condition but significantly different from the 40% KL drought stress condition. The application of 0 ppm humic acid was significantly different from the application of 500 ppm and 1000 ppm humic acid but the application of 500 ppm and 1000 ppm humic acid was not significantly different (Table 5).

Table 5. Chlorophyll of soybean (*Glycine max* L.) leaves under drought stress conditions and humic acid application

Drought stress (KL)	Humic acid (ppm)			Average
	0	500	1000	
	-------------------- mg/g --------------------			
80%	0,0010449	0,0011087	0,0009809	0,0010449[a]
60%	0,0005250	0,0011767	0,0012947	0,0009988[a]
40%	0,0004732	0,0006616	0,0008793	0,0006714[b]
Average	0,0006811[b]	0,0009823[a]	0,0010516[a]	

Notes: Numbers followed by different superscripts in the row mean, column mean, and interaction matrix indicate significant differences in Duncan's α = 5% test.

Plant responses to drought stress are modulated by many factors including osmotic regulation, environmental signaling, photosynthesis, hormone regulation, CO2 concentration, and respiration (Gong *et al.*, 2020). The process of photosynthesis is also inhibited when plants are under drought stress. During drought stress, plants also experience a decrease in the rate of photosynthesis caused by stomatal closure and a decrease in electron transport and phosphorylation capacity in leaf chloroplasts (Saputra *et al.*, 2015). Humic acid has several effective roles in plant growth such as making leaves thick, shiny, and dark green in color, increasing chlorophyll content, and improving plant performance (Selladurai and Purakayastha, 2016). Humic acid application through the leaves is more easily absorbed because the chance of interaction between ions is much lower. The availability of nutrients such as Zn, Fe, Mn, and Mg as precursors and catalysts in chlorophyll formation is more easily absorbed through the stomata (Selim *et al.*, 2012).

Relative water content (RWC)

Based on Duncan's multiple range test, the results showed that drought stress conditions, humic acid application, and the interaction between drought stress and humic acid application significantly affected the relative water content (RWC).

Table 6. The relative water content of soybean (Glycine max L.) under drought stress conditions and humic acid application

Drought stress (KL)	Humic acid (ppm)			Average
	0	500	1000	
	-------------------- (%) --------------------			
80%	262,50^{abc}	263,21^{ab}	264,89^{a}	263,53^{a}
60%	212,50^{d}	247,50^{abc}	260,71^{abc}	240,24^{b}
40%	175,30^{e}	231,67^{cd}	234,17^{bcd}	213,71^{c}
Average	216,77^{b}	247,46^{a}	253,26^{a}	

Notes: Numbers followed by different superscripts in the row mean, column mean, and interaction matrix indicate significant differences in Duncan's α = 5% test

Soybean plants under 80% KL drought stress conditions produced RWC which was not significantly different in the treatment of humic acid 0 ppm, 500 ppm, and 1000 ppm. The relative water content of leaves is a plant response to drought stress. Water content in the leaves describes the water status and turgor pressure of leaf cells, especially when plants experience a decrease in water potential (Dewi *et al.*, 2019). In soybean plants under 60% KL drought stress conditions, 500 ppm humic acid treatment produced RWC which was not significantly different from 1000 ppm humic acid treatment but significantly different from 0 ppm humic acid treatment. The decrease in leaf-relative water content can be used as an indicator to determine the balance between water content and leaf transpiration rate (Aziza *et al.*, 2002). In soybean plants in 40% KL drought stress conditions, 500 ppm humic acid treatment produced RWC which was not significantly different from 1000 ppm humic acid treatment but significantly different from 0 ppm humic acid treatment. Soybean plants under 40% KL drought stress conditions produced the smallest average RWC. Problems in plants with small water content will be more complex. Lack of water in plants reduces the water potential and turgor of plant cells, increasing the concentration of solutes in the cytosol and extracellular matrix. Decreased cell development leads to growth inhibition and reproductive failure (Table 6).

Stomata density

Based on Duncan's multiple range test, the results showed that drought stress conditions significantly affected stomatal density but the provision of humic acid and the interaction between drought stress and humic acid did not significantly affect stomatal density. The 80% KL drought stress condition was not significantly different from the 60% KL drought stress condition but significantly different from the 40% KL drought stress condition. Humic acid treatments of 0 ppm, 500 ppm, and 1000 ppm were not significantly different but there was an increase in stomatal density as the concentration of humic acid increased (Table 7).

Table 7. Stomata density of soybean (*Glycine max* L.) under drought and humic acid stress conditions.

Drought stress (KL)	Humic acid (ppm)			Average
	0	500	1000	
	------------------- stomata/mm^2 -------------------			
80%	1,98	2,19	2,37	2,18^{a}
60%	1,92	2,18	2,35	2,15^{a}
40%	1,43	1,36	1,64	1,48^{b}
Average	1,78	1,91	2,12	

Notes: Numbers followed by different superscripts in the row mean, column mean, and interaction matrix indicate significant differences in Duncan's α = 5% test

Closure of leaf stomata in drought-stressed plants is done to retain the water needed by plants. Reduction of water loss by closing stomata in plants with drought stress conditions is done to withstand evapotranspiration (Shahriari *et al.*, 2022). Humic acid treatment was not significantly different in various treatments presumably because drought stress conditions cause limited open stomata. Closed leaf stomata have an impact on the application of humic acid through the leaves (Maulana *et al.*, 2014). The limitation of open-leaf stomata causes humic acid to not be absorbed optimally.

CONCLUSION

Humic acid application with concentrations of 500 ppm and 1000 ppm affected leaf chlorophyll and relative water content (RWC) compared to plants without humic acid treatment. Drought stress affected the decrease in the number of flowers, pod fresh weight, leaf chlorophyll, and stomatal density at 40% KL. There was an interaction effect between the provision of humic acid and drought stress on the parameters of relative water content (RWC) with the highest results being the provision of 1000 ppm humic acid under conditions of 80% KL.

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