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Growth and yield performance of drought-stressed soybean (*Glycine max* L.) treated with neem-mediated silver nanoparticles

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Abstract. The utilization of nanotechnology in agriculture has demonstrated significant potential, particularly in enhancing crop resilience to environmental stress. This study investigated the effect of silver nanoparticles (AgNPs) in seed treatment on soybean performance under drought conditions. The study used a factorial CRD with four replicates. The experimental factors included seed treatments (control, AgNP priming, and AgNP coating) and drought stress levels represented by the field water capacity (FWC) of 80%, 60%, and 40%. The results demonstrated that seed treatment with AgNPs significantly increased plant growth in AgNP coatings based on plant height but did not significantly increase all soybean yield variables. FWC of 40-60% showed severe and moderate stress effects on soybean, which significantly decreased the leaf number but increased the leaf green index. In conclusion, AgNP seed treatment had a limited effect on soybean production but could be a potential approach to enhance drought tolerance.

1 Introduction

Climate change is a serious problem in the agricultural sector of many countries worldwide. Climate change has led to various consequences, including drought stress, which diminishes soil moisture and substantially impacts the development and yield of agricultural crops, such as soybean seed production. This impact can occur during the vegetative and generative phases, decreasing soybean yield [1]. However, the generative phase is the most sensitive to drought stress because flowering and pod filling occur during this phase.

Plant adaptation to drought stress is required to achieve the expected productivity. Various techniques can solve drought problems, including creating drought-resistant varieties through breeding programs and plant biotechnology. This can also be achieved by

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precision watering or by providing water reservoirs as reserves. In addition, seed technology offers a solution for plant adaptation, namely, seed priming [2].

Seed priming improves plant performance from germination and growth to seed production [3]. In principle, seed priming stimulates early metabolism, so the seeds are better prepared to grow faster and more uniformly when planted. Seed priming also gives seeds a memory of certain stresses, making them more adaptable to suboptimal growth environments [4]. Various methods are used for seed priming, including physical, biological, and chemical methods. The latest method is seed priming with nanomaterials.

Nanotechnology is the latest approach used by various sectors, such as medicine, manufacturing, food and beverage, and agriculture, to increase the value of products [5]. Silver nanoparticles (AgNPs) are among the most commonly utilized nanomaterials in various applications. In the agricultural sector, AgNPs are typically used as plant growth stimulants, fungicides to prevent diseases, and agents to improve fruit ripening. AgNPs have good conductivity, chemical stability, and unique optical and electrical properties; therefore, they are used as catalysts, sensors, and antibacterial, antiviral, and antifungal agents [6].

Biological synthesis methods can use microorganisms and plant extracts as reducers, which are believed to be more environmentally friendly. The basic principle is that plant extracts act as reducers, converting Ag^+ ions to uncharged and stable Ag^0. The neem plant (*Azadirachta indica*) is known to contain abundant secondary metabolites, such as azadirachtin and nimbin [7]. In synthesizing AgNPs, neem extract acts as a reducing and capping agent.

Research examining the impact of AgNPs on soybean growth and yield, especially under drought-stress conditions, remains limited [8]. Therefore, this investigation sought to explore the potential benefits of AgNPs, produced using neem leaf extract, in enhancing soybean plant development and productivity during drought stress. The study focused on applying these nanoparticles through seed priming and coating methods.

2 Materials and methods

2.1 Experimental procedure

AgNPs were synthesized by mixing 1 mM $AgNO_3$ solution with neem leaf extract of 9:1 (v/v). The solution mixture was stirred using a hotplate magnetic stirrer at 60 °C for 20 min until the solution turned reddish-brown. The characteristics of the AgNPs used were a z-average size of 55 nm, polydispersity index (PDI) of 0.449, and zeta potential of -28.8 mV.

The experiment utilized a two-factor factorial, completely randomized design with four replicates. The first factor was the seed treatment (control, AgNP priming, and AgNP coating). The second factor was the field water capacity (80%, 60%, and 40%). Field water capacity describes the level of drought stress.

The seeds used were Grobogan soybean varieties obtained from BPSI Tanaman Aneka Kacang, Malang, East Java. The initial viability and vigor of the seeds were 84.5% and 62.5%, respectively. Priming of soybean seeds was carried out by soaking the seeds in a 10% AgNPs solution for 6 h at 25 ºC, followed by air-drying to the initial moisture content. The ratio of seeds to the AgNPs solution was 3:10 (w/v). The selection of a 10% AgNP concentration was based on a previous study, which resulted the highest increase in soybean seed vigor (radicle emergence) compared to 2.5-7.5% AgNPs.

The seeds were manually coated using a jar containing a 1% NaAlg–10% AgNP mixture. The jar was shaken for 10 min until the mixture was evenly distributed over the entire seed surface and aerated before planting. The ratio of seeds, AgNPs, and NaAlg was 10:1:1 (w/v). The seeds in the control treatment were not treated with AgNPs solution.

The soil moisture content at field capacity was determined using a gravimetric method based on Haridjaja *et al.* [9]. This information was used as the basic moisture content value for the drought stress treatment and the benchmark volume of water to be supplied. The field water capacity (80%, 60%, and 40%) was kept stable by weighing the growing media every two days. The field water capacity treatment was applied for one month at the beginning of the generative period.

Soybean planting was conducted in the greenhouse of BPSI Tanah & Pupuk in Bogor, West Java. During the experiment, the maximum temperature per day ranged from 31.3-45 ºC and the minimum temperature per day was 20.4-25.6 ºC, with an average temperature of 29.5 ºC. Seeds primed and coated were planted with as many as three seeds per polybag (40 × 40 cm). The planting medium consisted of soil and compost in a ratio of 2:1. Fertilizers were applied 1 week and 4 weeks after planting, with doses of urea 25 kg/ha, SP-36 50 kg/ha, and KCl 75 kg/ha. The pest control used insecticide (deltamethrin 25 g/L) at a concentration of 0.5 mL/L.

2.2 Plant growth parameters

The number of branches, leaves, and leaf greenness index were counted after the field water capacity treatment ended. Shoot height, shoot dry weight, root dry weight, and root volume were measured at harvest. The measurement of leaf greenness index was conducted using a SPAD meter on fully open young leaves.

2.3 Plant yield parameters

Harvesting is performed after soybeans enter the physiological maturity phase, characterized by the fact that most leaves begin to dry and fall off, the pods are fully filled, and the pod skin changes colour to brownish yellow. The yield variables observed were the pod number, pod weight, seed number, and seed weight per plant.

2.4 Statistical analysis

Microsoft Excel 2021 and R-studio were utilized to process and analysed the experimental data. The statistical evaluation employed analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) at the 5% level.

3 Results and discussion

Some growth variables responded significantly to the seed treatment and field water capacity. Seed treatment affected shoot height, whereas field water capacity affected leaf number and leaf greenness index. The interaction between the treatments did not significantly affect any of the growth variables. Seed treatment, field water capacity, and their interactions had no significant effect on yield variables (Table 1).

Applying AgNPs to plants through seed treatment is expected to improve the germination, growth, and production performance of soybean plants. This experiment investigated the effects of two seed treatment methods: seed priming and coating. Seed treatment did not significantly affect the field emergence, but was still high in all treatments (>70%). Furthermore, seed treatment with AgNPs did not significantly increase shoot dry weight, root dry weight, and number of branches. Seed treatment with AgNPs also did not significantly decrease root volume and number of leaves (Table 2).

The shoot height of soybean showed significantly different responses to the seed treatments. AgNP coating significantly increased shoot height by 16.3%, whereas AgNP priming increased shoot height by 7.2%, although not significantly compared to the control (Table 2). Nanoparticles enhance plant growth through the ROS pathway, which initiates various enzyme activities, including an increase in dehydrogenase, rubisco enzyme, antioxidant enzymes, proline, and aquaporin [10]. The green synthesis of AgNPs had a beneficial impact on the growth and development of tomato plants, resulting in increased plant height through the promotion of cell division and elongation [11].

Table 1. Analysis of variance analysis (ANOVA) for soybean growth and yield parameters

Variables	ST	FWC	ST x FWC	CV (%)
Field emergence (%)	0.438 ^{ns}	-	-	17.48
Shoot height (cm)	0.026 *	0.064 ^{ns}	0.256 ^{ns}	12.81
Shoot dry weight (g)	0.938 ^{ns}	0.322 ^{ns}	0.933 ^{ns}	37.87
Root volume (cm^3)	0.670 ^{ns}	0.369 ^{ns}	0.965 ^{ns}	40.63
Root dry weight (g)	0.640 ^{ns}	0.094 ^{ns}	0.476 ^{ns}	32.07
Number of branches	0.236 ^{ns}	0.607 ^{ns}	0.582 ^{ns}	22.17
Number of leaves	0.559 ^{ns}	0.016 *	0.054 ^{ns}	19.97
Leaf greenness index	0.566 ^{ns}	0.019 *	0.169 ^{ns}	5.17
Pod weight per plant (g)	0.938 ^{ns}	0.297 ^{ns}	0.550 ^{ns}	11.82
Seed weight per plant (g)	0.829 ^{ns}	0.524 ^{ns}	0.393 ^{ns}	13.78
Pod number per plant	0.288 ^{ns}	0.641 ^{ns}	0.938 ^{ns}	13.42
Seed number per plant	0.545 ^{ns}	0.408 ^{ns}	0.746 ^{ns}	18.21

Symbol (*) represents statistical significance, (ns) indicates non-significant. CV is the coefficient of variance. ST is the seed treatment, and FWC is the field water capacity.

Table 2 shows that the AgNP coating insignificantly increased all yield variables, whereas AgNP priming increased three yield variables. These results suggest that AgNP coating has a better potential to increase soybean crop yield than AgNP priming. Compared to their bulk form, nanoparticles exhibit superior characteristics in terms of reactivity, durability, and efficacy when influencing the physiological functions of plants [10]. Seed coating with polymers provides and releases the appropriate number of nanoparticles required by the plant to facilitate metabolism and other biological activities [12].

Table 2. Effects of seed treatment on plant growth and yield parameters

Variables	Control	Seed Treatment			
		Priming	Deviation (%)	Coating	Deviation (%)
Field Emergence (%)	84.03	79.17	-4.9	86.80	2.8
Shoot height (cm) *	133.3 ^{a}	142.9 ^{ab}	7.2	155.0 ^{b}	16.3
Shoot dry weight (g)	7.33	7.82	6.7	8.3	13.2
Root volume (cm^3)	1.96	1.73	-11.7	1.73	-11.7
Root dry weight (g)	0.95	1.01	6.3	0.97	2.1
Number of branches	4.4	4.9	11.4	5.2	18.2
Number of leaves	26.4	24.2	-8.3	25.3	-4.2
Leaf greenness index	46.2	46.6	0.9	45.5	-1.5
Pod weight per plant (g)	13.36	13.34	-0.1	13.55	1.4
Seed weight per plant (g)	9.01	9.17	1.8	9.33	3.5
Pod number per plant	37.0	37.3	0.8	40.1	8.4
Seed number per plant	53.0	57.5	8.5	56.1	5.8

Mean followed by the same letters within each column are non-significantly different based on DMRT ($p < 0.05$). Symbol (*) represents statistical significance.

The number of leaves showed significantly different responses to the field water capacity treatments. FWC 60% and 40% reduced the number of leaves by 17.9-20.0% compared to the control (Table 3). Water scarcity causes a reduction in the water potential of leaves, and reducing new leaf development is a strategy for adapting to suboptimal environmental conditions. [13] stated that reducing the number of leaves is a lateral adaptive mechanism for reducing water loss; thus, further cell damage can be avoided. Water deficits allow plants to adapt by lowering the number of leaves and stomatal openings, thus disrupting the exchange of CO_2 and H_2O, leading to impaired photosynthetic processes and the distribution of assimilates to reproductive organs [1].

The leaf greenness index showed a significant response to field water capacity treatments. The leaf greenness index increased at FWC 60% and 40%, which amounted to 4.5-6.3% compared to the control (Table 3). [14] stated that the increase in the leaf greenness index after water restriction expresses the plant's conservative strategy under drought conditions to adapt and does not affect plant yield.

Table 3. Effects of field water capacity on plant growth and yield parameters

Variables	FWC 80% (Normal)	Drought Stress Level			
		FWC 60% (Moderate)	Deviation (%)	FWC 40% (Severe)	Deviation (%)
Shoot height (cm)	153.8	142.1	-7.6	135.4	-12.0
Shoot dry weight (g)	9.1	7.5	-17.9	6.9	-24.7
Root volume (cm^3)	2.0	1.6	-20.1	1.7	-14.7
Root dry weight (g)	1.1	0.9	-18.0	0.9	-18.0
Number of branches	4.7	4.8	2.1	5.1	8.5
Number of leaves *	29 [b]	23.2 [a]	-20.0	23.8 [a]	-17.9
Leaf greenness index *	44.5 [a]	47.3 [b]	6.3	46.5 [b]	4.5
Pod weight per plant (g)	13.5	13.9	2.7	12.9	-4.9
Seed weight per plant (g)	9.1	9.5	3.7	8.9	-2.8
Pod number per plant	38.8	38.6	-0.5	37.0	-4.6
Seed number per plant	58.8	54.3	-7.6	53.6	-8.8

Mean followed by the same letters within each column are non-significantly different based on DMRT ($p < 0.05$). Symbol (*) represents statistical significance.

Table 3 shows that the 60% FWC treatment had a moderate stress effect on soybean plants, as some plant yield variables increased insignificantly. In contrast, 40% FWC resulted in severe stress effects on soybean plants, which insignificantly reduced all plant yield variables. [15] stated that the severe stress category impaired the effects observed in moderate stress, and FWC below 50% had a deplorable impact on plant yield.

4 Conclusions

AgNP priming and coating did not significantly increase field emergence. AgNP priming did not significantly increase plant growth, but an increase was observed in the AgNP coating based on shoot height. FWC of 40-60% showed severe and moderate stress effects on soybean, which significantly decreased the leaf number but increased the leaf green index. AgNP priming and coating did not significantly increase any of the yield parameters under drought stress conditions. Although AgNP seed treatment had a limited effect on soybean production, it could be a potential approach to enhance drought tolerance by optimizing the AgNP concentration and treatment method.

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