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Stomata and Trichome of the Anjasmoro Soybean Cultivar During the Application of Nanosilica and Plant Growth Promoting Rhizobacteria

Stomata dan Trikoma Kedelai Cultivar Anjasmoro Selama Pemupukan Nanosilika dan Plant Growth Promoting Rhizobacteria

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ABSTRACT

Soybean is a very important crop commodity in Indonesia. Increasing soybean production on dryland as cultivation land need to be carried out. Dryland have issues with either of nutrient and water availability which is not supportable for plant growth. Therefore, the application of Nano-silica and Plant Growth Promoting Rhizobacteria (PGPR) are expected to increase nutrient absorption, synthesis of plant growth hormone and increase in plant resistance to drought stress. The growth of drought-resistant plants is able to be showed through the status of stomata and trichomes on leaves. So that, this study aimed to determine the stomata and trichome performance of soybean plants of Anjasmoro variety using Nano-silica and PGPR as fertilizers. The study design used a completely randomized design with 2 factors including Nano silica concentration (0, 100 and 200 ppm) and PGPR concentration (0, 5, 10, and 15%). Results showed that the nanosilica and PGPR applications had no significant effect on stomata and trichome status of anjasmoro varieties except for stomatal aperture and the ratio of the size of the stomata openings. A positive correlation has been found in the study between stomata openings and soybean yields.

Keyword: Stomata;trichome;anjasmoro soybean;PGPR;nanosilica

INTISARI

Kedelai merupakan komoditas pangan yang sangat penting di Indonesia. Peningkatan produksi kedelai melalui pemanfaatan lahan kering sebagai lahan budidaya berpotensi untuk diterapkan. Lahan kering memiliki kendala baik dari unsur hara maupun ketersediaan air sehingga tidak mendukung pertumbuhan tanaman. Oleh karena itu aplikasi pupuk Nano-silica dan Plant Growth Promoting Rhizobacter (PGPR) diharapkan dapat meningkatkan penyerapan unsur hara, sintesis hormon pemacu tumbuh tanaman dan peningkatan ketahanan tanaman terhadap cekaman kekeringan. Pertumbuhan tanaman yang tahan kekeringan dapat dilihat melalui performa stomata dan trikoma pada daun. Oleh karena itu, Penelitian ini bertujuan untuk mengetahui performa stomata dan trikoma tanaman kedelai varietas Anjasmoro menggunakan pemupukan nano-silika dan pupuk hayati PGPR. Rancangan Penelitian menggunakan rancangan acak lengkap dengan

2 Faktor meliputi konsentrasi Nano-silica (0, 100, dan 200 ppm) dan konsentrasi PGPR (0, 5, 10, dan 15 %). Hasil penelitian menunjukkan bahwa perlakuan pemupukan nanosilika dan PGPR memberikan pengaruh yang tidak nyata terhadap status stomata dan trikoma kedelai varietas anjasmoro kecuali pada variable bukaan stomata dan rasio ukuran bukaan stomata. Korelasi positif telah ditemukan di penelitian ini antara bukaan stomata dengan hasil tanaman kedelai.

Kata Kunci: Stomata; trikoma; Kedelai; Anjasmoro; PGPR; Nanosilika

INTRODUCTION

Soybean is one of the most important food crops commodities in Indonesia. Increasing soybean production capacity in Indonesia to be a chore unresolved problem until today. In addition to the intensification of agriculture, the dry land also began to require attention in view of productive agricultural land in Indonesia has declined. Biofertilizer implementation is one of alternatives to improve a soil fertility. Plant Growth Promoting Rhizobacteria (PGPR) Fertilizer is a biological fertilizer that contains bacteria that live in the root zone of plants. Zahedi and Abbasi (2015) showed that the application of PGPR improved plant growth and was able to increase plant resistance to drought stress.

In addition, the use of Nano-silica fertilizers is another alternative that is also able to increase plant resistance to drought (Ahmed et al., 2011). Nano-silica fertilizer reduced the rate of transpiration in plants that water deficit (Silva, 2012). In addition, Nano silica also accelerated the germination and growth enhancement of *Vicia faba* (Roohizadeh et al., 2015). It becomes very interesting as a combined fertilizer because Nano silica is not toxic to PGPR, it could actually increase the total amount of soil microbes and maintain soil pH (Karunakaran et al., 2013). Therefore, this approach was conducted to see the drought resistance through stomata and trichomes status of Anjasmoro soybean.

MATERIALS AND METHODS

The research was conducted at the Research and Education Garden of the Stiper Agricultural Institute, Yogyakarta, Indonesia, in March - August 2018. Anjasmoro soybeans cultivar was obtained from Food Crop and Horticulture Seed Development Center, Gunung Kidul, Yogyakarta, Indonesia. Nano-silica was obtained from Indonesian Institute of Sciences, Tangerang, Indonesia. PGPR was made using bamboo and *Mimosa pudica* rhizosphere as a source of Rhizobacteria inoculum. Soybean seeds were sown in polybags that have been filled with sand soil media (regosol).

Nano silica and PGPR Fertilization

Nano silica and PGPR applications were carried out on 14 DAP (Day After Planting) plants every 2 weeks. Their concentration of solution was made in accordance with the treatment. Nano-silica fertilizer was applied to plants with a dose of 50ml/plant. Meanwhile, PGPR fertilizer was performed with a dose 100ml/plant. Watering was carried out once a week until field capacity.

Research Variables

Variables measured in this study were stomata density (Nstomata / mm2), trichome density (Ntrichome/ mm^2), epidermal number (Nepid / mm^2), stomata opening width, length/width ratio, stomatal aperture width, Stomatal Index and seed weight (yield). Stomata samples were taken during the vegetative phase using a replica method. *OptiLab Professional* and *image raster software Ver. 1.3.1b* were used for measurement of stomata. Seed weight was measured when it was dried physiologically.

Research Design and Data Analysis

Factorial complete randomized design consisting of 2 factors with 3 replications was used in this study. The first factor was the Nano-silica concentration consisting of N0 = 0ppm, N1 = 100ppm, and N2 = 200ppm. The concentration of PGPR included P0 = 0%, P1 = 5%, P2 = 10%, and P3 = 15% as the second factor. The data obtained were analyzed using ANOVA test (α0.05). If there was an influence, it followed by the Duncan test. A regression test was conducted on the yield (seed weight) to find out the relationship between variables.

RESULTS AND DISCUSSION

Results showed that Nano silica and PGPR had separate effects on epidermal density and stomatal index (Table 01). Soybean treated with PGPR had epidermis density greater than without PGPR. However, stomatal index without PGPR has a higher value compared with the PGPR treatment. Meanwhile, Nano silica and PGPR did not affect the density of stomata and trichomes. Although the response of stomatal aperture to water deficit has been well studied, little is known about the effect of water conditions on stomatal development in terms of density and size.

Tabel 1. Effect of PGPR and Nano-silica Fertilizer separately on the stomatal density (N_{stoma}), trichomes density ($N_{trichomes}$), and epidermal density (N_{epid}) and Stomatal index.

Treatments	$N_{stomata}$ /mm^2		$N_{trichome}$ /mm^2		N_{epid} /mm^2		Stomatal index	
PGPR 0%	204.98 ±	15.36a	2.48 ±	0.50a	858.33 ±	82.33b	19.51 ±	0.80a
PGPR 5%	207.36 ±	19.43a	1.79 ±	0.14a	1091.67 ±	96.32a	15.94 ±	0.49b
PGPR 10%	217.94 ±	10.97a	1.72 ±	0.20a	1029.17 ±	51.00ab	17.48 ±	0.30b
PGPR 15%	194.86 ±	11.73a	2.15 ±	0.23a	995.83 ±	26.94ab	16.31 ±	0.65b
Nano-silica 0ppm	191.07 ±	12.71p	1.79 ±	0.21p	931.25 ±	93.51p	17.43 ±	0.87p
Nano-silica 100ppm	222.18 ±	11.11p	2.33 ±	0.38p	1046.88 ±	51.63p	17.55 ±	0.67p
Nano-silica 200ppm	205.61 ±	11.90p	1.99 ±	0.15p	1003.13 ±	33.55p	16.94 ±	0.58p

Note: The numbers in rows and columns with ± Standard error (SE) followed by the same letters indicate no significant differences based on the DMRT test (α=0.05).

The information about this subject is somewhat contradictory among studies or crop species, suggesting that there is a complex regulation of stomatal development in response to the environment (Casson and Gray 2008). For soybean, their stomatal density increased under drought conditions compared with well-watered conditions (Tanaka and Shiraiwa 2009). It was also found in this study, that the number of stomata was more than the epidermis which is shown in the stomatal index value that was high in control (0% PGPR). Lower stomatal index has more resistance to drought because it is able to reduce the rate of transpiration. In limited water conditions, the plant will carry out stomata closure to regulate water loss and CO_2 capture which is important for the availability of CO_2 fixation during photosynthesis (Taiz and Zeiger, 2002).

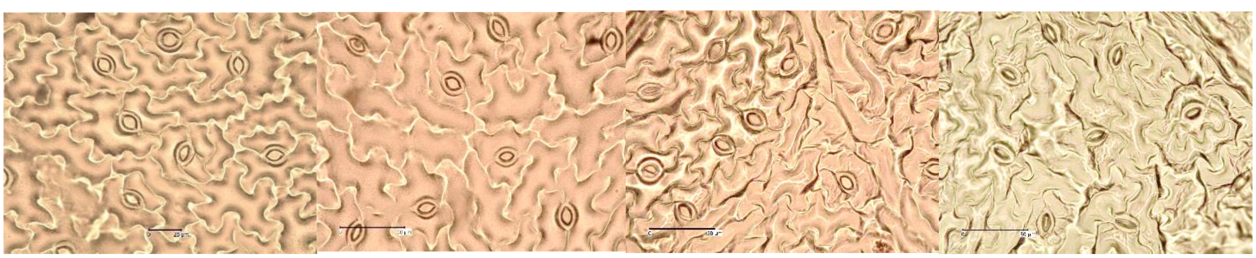

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Figure 1. Photomicrograph of stomata with 400x magnification a) PGPR10%+Nano-silica 0ppm b) PGPR 15%+ Nano-silica 0ppm c) PGPR15%+Nano-silica 200ppm d) PGPR 0%+Nano-silica 0 ppm

In Table 02, results generally indicated that the application of PGPR affected stomatal aperture width. Soybean plants that were resistant to drought conditions had a high stomatal aperture width. It could be showed in figure 1, soybeans with PGPR application had high stomatal aperture more than without PGPR. The influence of PGPR application seemed to give an effect over than nano silica in this study. Stomatal L/W ratio indicate the proportion of pore aperture. Their significant aperture is occurred if it gives a low value close to 1. In contrary, the greater values show their closure of stomata. Based on this study, plant which were untreated with PGPR gave high L/W ratio (table 02) from 1.90 – 2.63.

Table 2. Effect of PGPR and Nano-silica on stomata width and Stomatal L/W (Length/ width) ratio

Treatments	Stomata width (µm)			L/W ratio		
PGPR 0% +Nano-silica 0 ppm	6.87 ±	0.17	d	1.91 ±	0.14	bcd
PGPR 0% + Nano-silica 100ppm	6.92 ±	0.71	d	1.90 ±	0.21	bcd
PGPR 0% + Nano-silica 200ppm	4.31 ±	0.24	f	2.63 ±	0.13	a
PGPR 5% + Nano-silica 0ppm	7.86 ±	0.32	bcd	1.64 ±	0.07	cd
PGPR 5% + Nano-silica 100ppm	7.41 ±	0.36	cd	1.75 ±	0.13	bcd
PGPR 5% + Nano-silica 200ppm	7.36 ±	0.83	cd	1.98 ±	0.35	bcd
PGPR 10% + Nano-silica 0ppm	10.04 ±	0.24	a	1.44 ±	0.04	d
PGPR 10% + Nano-silica 100ppm	6.68 ±	0.86	de	1.96 ±	0.29	bcd
PGPR 10% + Nano-silica 200ppm	8.14 ±	1.14	bcd	2.17 ±	0.31	abc
PGPR 15% + Nano-silica 0ppm	9.23 ±	0.46	ab	1.44 ±	0.08	d
PGPR 15% + Nano-silica 100ppm	5.11 ±	0.16	ef	2.35 ±	0.07	ab
PGPR 15% + Nano-silica 200ppm	9.16 ±	0.37	abc	1.50 ±	0.09	d

Note: The numbers in rows and columns with ± Standard error (SE) followed by the same letters indicate no significant differences based on the DMRT test (α=0.05).

The mechanism of plant growth promoting rhizobacteria varied physically and chemically. Rhizobacteria according to (Subramaniam *et.al*, 2016) produce aminocyclopropane-1-carboxylate (ACC) deaminase which is able to degrade ethylene (a hormone that inhibits the growth of roots and stems in drought condition). Physically, rhizobacteria produce extracellular matrix containing oligo and polysaccharides (important in retaining water) so that the soil is more adaptive to drought (Naseem and Bano 2014; Timmusk et al., 2014). Because of water availability in the media was sufficient, the stomata in the leaves will open so that photosynthesis is able to perform properly. This occurrence will impact on crop yields. The weight of soybean seeds can be seen in table 03.

Tabel 3. Seed weight (g / plant) Anjasmara soybean fertilized using PGPR and Nano-silica

Treatments	Seed weight (g/plant)
PGPR 0% +Nano-silica 0 ppm	1.31 ± 0.08d
PGPR 0% + Nano-silica 100ppm	2.34 ± 0.30cd
PGPR 0% + Nano-silica 200ppm	1.52 ± 0.23d
PGPR 5% + Nano-silica 0ppm	5.40 ± 0.08ab
PGPR 5% + Nano-silica 100ppm	1.39 ± 0.82d
PGPR 5% + Nano-silica 200ppm	5.74 ± 2.29ab
PGPR 10% + Nano-silica 0ppm	8.12 ± 0.96a
PGPR 10% + Nano-silica 100ppm	3.84 ± 1.94bc
PGPR 10% + Nano-silica 200ppm	8.57 ± 0.23a
PGPR 15% + Nano-silica 0ppm	2.36 ± 0.59cd
PGPR 15% + Nano-silica 100ppm	2.26 ± 0.81cd
PGPR 15% + Nano-silica 200ppm	5.91 ± 0.08ab

Note: The numbers in rows and columns with ± Standard error (SE) followed by the same letters indicate no significant differences based on the DMRT test (α=0.05).

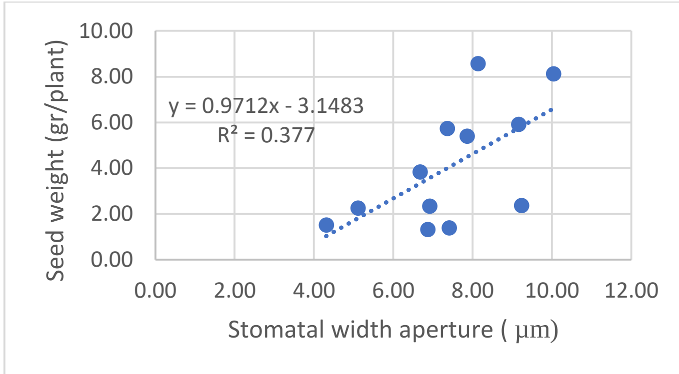

Figure 2. Graphic of Correlation on stomatal width aperture-seed weight

Stomatal width aperture had a positive correlation (R=0.61) with seed weight that drought-tolerant plants (with high stomatal aperture) produced a better seed weight. It also showed a positive relationship between the two variables. (y=0.9712x – 3.1483). These results indicated that the wider the stomata openings resulted the higher its yield.

CONCLUSION

Nanosilica and PGPR fertilizer application were able to increase the stomatal aperture width but it had no effect on the densities of stomata, trichomes and epidermis. Increased stomatal aperture width was able to increase soybean yield.

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