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Application of silica solubilizing bacteria to improve the water use efficiency of maize

Aplikasi bakteri pelarut silika untuk memperbaiki efisiensi penggunaan air pada tanaman jagung

Indah Puspita SARI[1*)], Yulin LESTARI[2)], HAMIM[2)] & Laksmita Prima SANTI[1)]

[1)]Pusat Penelitian Bioteknologi dan Bioindustri Indonesia (PPBBI), Jl. Taman Kencana No 1, Bogor, 16128, Indonesia
[2)]Institut Pertanian Bogor (IPB), Jl. Raya Darmaga, Bogor, 16680, Indonesia

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Abstrak

Perubahan iklim global mengakibatkan penurunan curah hujan dan peningkatan evaporasi, sehingga diperkirakan frekuensi dan tingkat keparahan cekaman kekeringan akan semakin tinggi. Silika (Si) diketahui dapat meningkatkan ketahanan tanaman terhadap kekeringan dengan cara memperbaiki efisiensi penggunaan air pada tanaman. Meskipun ketersediaanya berlimpah di tanah, sebagian besar Si dalam bentuk yang tidak tersedia bagi tanaman, karena sifat kelarutannya yang rendah. Untuk meningkatkan silika tersedia bagi tanaman, bakteri pelarut silika (BPS) memiliki peranan yang penting. Penelitian ini bertujuan menguji aktivitas pelarutan silika dari tiga koleksi isolat BPS Pusat Penelitian Bioteknologi dan Bioindustri Indonesia (PPBBI) pada sumber silika tidak larut berupa magnesium trisilikat, kuarsa, dan feldspar, serta melihat pengaruh aplikasi BPS terhadap efisiensi penggunaan air pada tanaman jagung yang diberi perlakuan cekaman kekeringan. Aktivitas pelarutan silika diukur menggunakan modifikasi metode standar 4500-SiO2 D Heteropoly blue. Pengendalian kekeringan di rumah kaca mengadaptasi sistem Snow dan Tingey. Rancangan percobaan menggunakan rancangan acak lengkap faktorial dengan kondisi kekeringan dan jenis bakteri BPS sebagai peubah bebas. Efisiensi penggunaan air diukur secara real time dengan sap flow meter. Hasil penelitian menunjukkan bahwa BPS dengan kode Pseudomonas fluorescens-B.41 memiliki aktivitas pelarutan silika tertinggi pada susbtrat Mg-trisilika yaitu 81,93 ppm. Aplikasi BPS menurunkan laju transpirasi jagung dan meningkatkan efisiensi penggunaan air hingga 84% pada cekaman kekeringan sedang dan 46% pada irigasi normal, namun pada cekaman kekeringan parah, dimana larutan hara dipertahankan pada jarak 25 cm dari sistem perakaran efisiensi penggunaan air tidak signifikan. Diduga hal ini disebabkan kondisi kekeringan pada media tanam terlalu ekstrim sehingga BPS yang diaplikasi tidak dapat mempertahankan aktivitasnya.

[Kata kunci: aquaporin, cekaman kekeringan, sistem Snow dan Tingey, BPS, kuarsa]

Abstract

Global climate change will result in decreased rainfall and increased evaporation. Thus, it is estimated that the frequency and severity of drought stress will get worse. Silica increases plant drought resistance by improving water use efficiency in plants. Despite its abundant availability in soil, most silica sources are not available to plants due to their low solubility. Silica solubilizing bacteria (SSB) have an important role in increasing the available silica. This study aims to observe the silica solubilizing activity of three SSB isolates collections of PPBBI on insoluble silica sources, including magnesium trisilicate, quartz, and feldspar, and see their effects on increasing water use efficiency in corn plants via drought experiments. SSB activity was measured using the modified standard method of 4500-SiO2 D Heteropoly blue. Drought control in the greenhouse follows the Snow and Tingey system. The experimental design used a completely randomized design factorial with irrigation conditions and SSB species as variables. Water use efficiency is measured in real-time with a sap flow meter. The results showed that SSB Pseudomonas fluorescens-B41 had the highest silica dissolving activity 81.93 ppm on Mg-trisilicate. The application of SSB can reduce maize transpiration rate and increase water use efficiency up to 84% under moderate drought stress and 46% under normal irrigation, but in severe drought stress, where the nutrient solution was maintained at 25 cm from plant root, water use efficiency was not significant. This is suspected due to the extreme drought conditions in the potting soil so that the applied SSB cannot maintain its activities.

Corresponding author: indah4neko@gmail.com

[Keywords: aquaporin, drought stress, Snow and Tingey system, SSB, quartz]

Introduction

Agricultural land conversion into settlements or industries due to increasing population has a significant impact on fulfilling food needs. Therefore, optimizing food production in suboptimal land is crucial to support the achievement of national food security. The largest land resource in Indonesia is suboptimal dry land, as much as 123.1 million hectares (Haryono, 2104). Many strategic agricultural commodities start to utilize suboptimal dry land, such as maize. In dry land, the only water source comes from the rain, so water becomes a limiting factor for maize growth because nutrient availability and the physiological process of maize growth are highly dependent on groundwater availability. In addition, global warming has resulted in decreased rainfall and increased evaporation, causing dry land to be very vulnerable to drought stress (Diedrich *et al.*, 2012). Lack of groundwater will decrease metabolic activity, reduce plant biomass, and reduce photosynthesis rate, thus decreasing productivity or crop failure in maize (Bu *et al*., 2010). According to Vogel *et al*. (2019), climate extremes such as drought explain 18-43% of global maize, soybean, and rice crop yield variations. A two-degree Celsius warming could reduce major crop yields by 3-13% (Wang *et al*., 2020).

The main challenge in understanding drought stress conditions is choosing the suitable simulation method in greenhouses to describe drought conditions that resemble conditions in the field. Methods for applying water reduction control on growing media in greenhouses have been studied for a long time. The most basic method is passive pot-drying, namely by completely stopping irrigation. Still, this method is considered not to fully describe the process of groundwater deficit that occurs naturally because it causes an increase in the level of drought that is too fast (Poorter *et al*., 2012). Another commonly used method is adding an active osmotic agent such as polyethylene glycol (PEG), but in both methods, it is challenging to apply drought conditions with various intensities (Marchin *et al.*, 2020). In this study, the method developed by Snow and Tingey (1985) was chosen to control the water reduction system in a greenhouse. With this system, it is possible to implement a water deficit in a controlled manner with various drought intensities and durations, and it is suitable for long-term drought stress applications (Marchin *et al*., 2020).

Silica is the second most abundant element in the crust, being surpassed only by oxygen, it is also one of the most important trace elements for human health (Martin, 2013) and plays a crucial role in plant health, particularly in alleviating environmental stresses (Aki *et al.,* 2020). In the last few years, the researcher has examined the effect of Si on mitigation stress (Etesami & Jeong, 2018; Luyckx *et al.*, 2017, Meena *et al.,* 2014). According to Antonangelo *et al.* (2017), the role of Si in the soil is to increase the availability of other nutrients that are essential for plants and lower the level of soil acidity. Application Ca-Mg silicate can improve soil acidity and increase pH value so Si, Ca, and Mg are available to plants. Fertoz (2020) states Si is an essential nutrient for soil health, crop production, and mitigation stress. Silica has been widely studied to have a positive effect on increasing plant tolerance to drought stress conditions. It can optimize water use efficiency by reducing leaf transpiration and xylem flow rates in plants (Gharineh & Karmolollachaab, 2013). Silica accumulation results in cell wall strengthening through various mechanisms, supporting plant resistance to abiotic and biotic stress (Malhotra *et al*., 2016). Figure 1 shows the different Si fractions in the soil. Adsorbed Si component is bound to soil particles, Fe and Al oxide or hydroxide, but Si in soil solution is available in the form of monomer or mono silicic acid (H_4SiO_4), which is the only form that can be uptake by plants. Although Si is abundant in the soil, due to its insoluble nature and biogeochemically immobile, the availability of Si in the form of mono silicic acid, which plants easily absorb, is very low.

Weathering is a term that describes the general process by which rocks are broken down into sediment, clays, soils, and substances that are dissolved in water (Zaharescu *et al*. 2020). Microorganisms have an important role in the process of mineral weathering (Bio weathering). Many studies report the ability of silica solubilizing bacteria to dissolve silicate minerals. Santi and Goenadi (2017) reported on the ability of *Burkholderia cenocepacia* KTG, *B. vietnamiensis* ZEO3, and *Aeromonas punctata* RJM3020 accelerated the dissolution process of Si from quartz minerals. Various types of *Bacillus, Rhizobium, Pseudomonas, and Kosakania* sp. also report having the ability to dissolve silicate minerals in the form of quartz, muscovite, illite, feldspar, and biotite through acidification, redox, and other biochemical processes (Vasanthi *et al*., 2016; Wang *et al*. 2014; Chen *et al*. 2016; Hu *et al*. 2019). Although various studies have been reported the weathering of silicates by bacteria increases Si plants uptake (Chandrakala *et al*., 2019) and have a positive impact on plants under various stress conditions through better absorption of this mineral (Lee *et al.*, 2019), the correlation of increasing water use efficiency in maize under drought conditions needs to be further studied. The purposes of this study were to determine (i) the activity of dissolving Si from 3 bacterial collections of PPBBI *i.e., Pseudomonas fluorescence*-B41, KI-19, and *Pantoea dispersa*-IGD on sources of insoluble silica magnesium

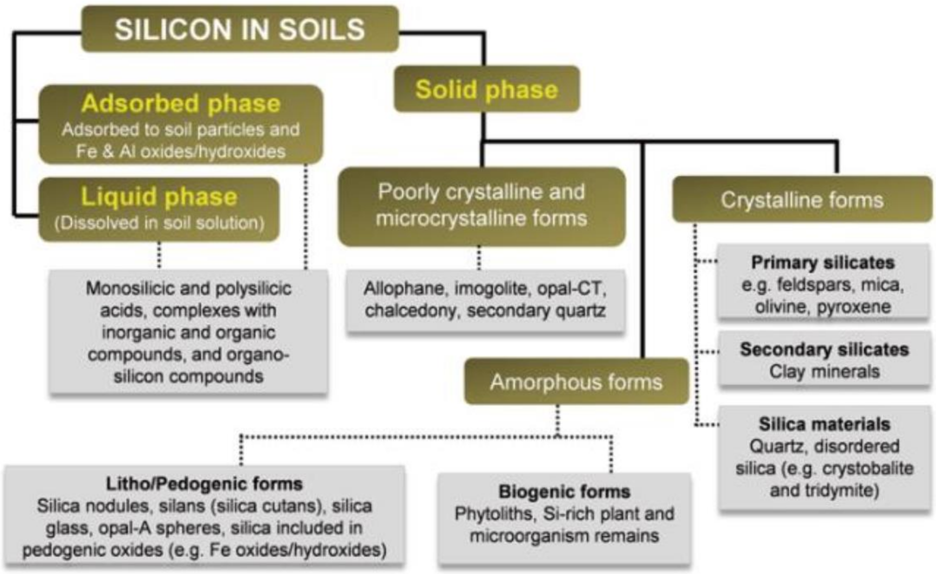


Figure 1. Various silica fraction in the soil (Tubana & Heckman, 2015)
Gambar 1. Berbagai jenis fraksi silika di tanah (Tubana & Heckman, 2015)

trisilicate, feldspar, and quartz, and (ii) to see effect application silica solubilizing bacteria (SSB) improve water use efficiency in maize with difference irrigation condition.

Materials and methods

Silica solubilizing bacteria (SSB) and silicate source

Microorganisms used in this study were SSB *i.e.*, *Pseudomonas fluorescence*-B41, KI-19, and *Pantoea dispersa*-IGD collections of Indonesia Research Institute for Biotechnology and Bioindustry (PPBBI), isolated from soil root areas with a sand fraction of 60 – 80% in Central Kalimantan. Sources of insoluble silica used to test the dissolving activity of silica by SSB are magnesium trisilicate (Sigma 63148), quartz and feldspar minerals. Quartz mineral from Bangka, Sumatra, has a size of 325 mesh. The feldspar mineral was obtained from Sukabumi with a size of 60 mesh.

Silica dissolving activity

As much as 0.1 mL SSB isolates of *Pseudomonas fluorescence*-B41, KI-19, and *Pantoea dispersa*-IGD with a population of 10^8 were inoculated into 100 mL of liquid basalt medium with a composition of 10 gL^{-1} glucose, 1 g L^{-1} NH_4SO_4, 0.2 g L^{-1} KCl, 0.2 g L^{-1} $MgSO_4$, and 0.1 g L^{-1} K_2HPO_4 at pH 7.0 – 7.2 (Vasanthi *et al*., 2016) with 2.5 g/L insoluble silicate mineral sources in the form of Mg-trisilicate, quartz, and feldspar. Then the medium was shaken at 200 rpm at 28 °C. Dissolved silica concentration was measured at incubation times of 2, 4, 8, and 12 days. Silica concentration measurement in the filtrate medium was carried out using the modified standard method of 4500-SiO_2 D Heteropoly blue (Eaton *et al*., 2017) based on the formation of a blue silica-molybdate complex.

Drought stress treatment and SSB application

Controlled drought stress conditions were carried out based on the method of Snow and Tingey (1985) with a modification of Fernandez and Renolds (2000) using a dense column with low water permeability properties that separated the water surface from the root area. The level of drought intensity was set by filling the nutrient solution (Hoagland's 4x dilution) at 5, 15, and 25 cm from the bottom of the polybag for treatment without, moderate, and severe drought stress, respectively (Figure 2). Silica solubilizing bacteria application was carried out in a soil-quarts medium with 9 kg soil and 1 kg quartz composition. The medium had been sterilized through gamma radiation 25 kGy. As much as 5 mL of SSB filtrate with a cell density of 10^8 CFU/mL was sprayed evenly onto the surface of the soil-quarts medium. Drought stress conditions in maize with varieties Pertiwi were maintained from 45 to 75 DAP (days after planting). The experimental design used a completely randomized design factorial with irrigation conditions and SSB species as variables with 4 replications.

Measuring water use efficiency

The sap flow method is the most commonly used measurement application to determine plant transpiration directly and can calculate the estimated total plant water requirement, which correlates with water use efficiency. The principle of this method is to use heat to track the movement of sap flow in the plant vascular system, with two basic principles, namely the heat balance method and the heat pulse method (Smith and Allen, 1996). The SFM1x Sap flow meter instrument from ICT International was used in this study. This instrument has three types of needles. The middle needle located in the sapwood area generates heat pulses. The other two needles are equipped with sensitive thermocouples that measure the difference in heat over time. The three needles are attached to the maize plant stem at the height of 5-10 cm from the surface of the soil, with the middle needle in the sapwood area (Figure 3). Measurements were made in real-time recorded at intervals of 30 minutes for a cycle of 20 days in the three treatment groups (normal irrigation, moderate and severe drought) with SSB treatment and without SSB treatment.

Result and discussion

Silica dissolving activity

The three isolates showed good growth and were able to dissolve magnesium trisilicate. The *P. dispersa*-IGD produced the widest diameter of the silica dissolution zone compared to *P. fluoresces*-B41, and KI-19 after an incubation time of 7 days, clear zone diameter is 12.5>10.6>9.7 respectively. However, on a solid medium with quartz and feldspar as sources of insoluble silica, although the three isolates were still able to grow, after 7 days of incubation, the clear zone was only seen in isolate B41 and with a smaller clear zone area than when grown on medium with Mg-trisilicate as the substrate (Table 1). The dissolution process of silica also occurred faster in the medium with Mg-trisilicate substrate, because the clear zone started to appear after three days of incubation (Figure 4), while in the quartz and feldspar on day 5. Although clear zones were not formed in isolates KI-19 and IGD on a solid medium with quartz and feldspar as substrates, all isolates showed the potential for dissolving silica in a liquid medium.

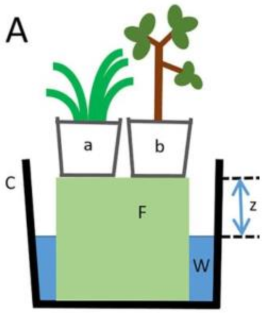


Figure 2. Controlled water deficit application scheme (Fernandes & Renolds, 2000). Plants in polybags (a & b) were placed on top of a dense column with low water permeability (f) in a plastic container (c) filled with nutrient solution (w). The intensity of water deficit is controlled through the height of the solution (z)

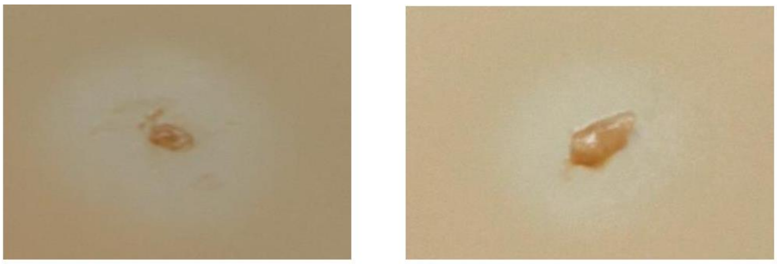
Gambar 2. Skema aplikasi defisir air di rumah kaca (Fernandes & Renolds 2000). Tanaman di polybag (a & b) ditempatkan di atas kolom padat dengan sifat permiabilitas rendah (f) di dalam kontainer plastik (c) berisi larutan nutrisi (w). Intensitas defisit air dikontrol melalui ketinggian larutan (z)

Figure 3. Maize daily water usage measurement with a Sap flow meter

Gambar 3. Pengukuran air harian yang digunakan tanaman jagung dengan Sap flow meter

Table 1. The ability of SSB in solubilizing silica with some silica minerals as substrate with three replications
Tabel 1. Pelarutan silika oleh BPS dengan beberapa mineral silika sebagai substrat dengan 3 ulangan

SSB isolate *Isolat BPS*	Clear zone diameter (mm) *Rataan Diameter zona bening (mm)*					
	Mg-trisilicate	SD	Quartz	SD	Feldspar	SD
B41	10.6	0.43	3.4	0.36	4.1	0.1
IG-D	12.5	0.26	-		-	
KI-19	9.7	0.3	-		-	



(a) (b)

Figure 4. The ability of SSB to solubilizing Si indicated by the formation of a clear zone in solid media with Mg trisilicate as substrate after 3 days incubation. (a) SSB B41, (b) SSB IGD

Gambar 4. Kemampuan BPS melarutkan silika ditandai dengan pembentukan zona bening setelah 3 hari inkubasi (a) BPS B41, (b) BPS IGD

Figure 5 shows the silica dissolving activity of isolates tested on several silicate minerals as substrates. The highest activity among all tested substrates is Mg-trisilicate, with the greatest activity 81.93 ppm after eight days of incubation was produced by isolate B41. However, the IGD clear zone formation had a larger area than B41. These results support the statement of Santi and Goenadi (2017) that there is no correlation between silica dissolving activity and the dissolving zone. The substrate of quartz and feldspar isolate B41 also had the highest dissolution activity, but the concentration was much lower than Mg-trisilicate, at 2.67 ppm for quartz and 4.85 ppm for feldspar. For Mg-trisilicate as the substrate source, the dissolving activity of B41 is higher than that of the isolate reported by Vasanti *et al*. (2016), but the value is lower for quartz and feldspar substrates. The KI-19 isolate had the lowest silica dissolving activity among the three isolates, and on quartz, it was not seen that the concentration of dissolving silica was too significant.

The main structure of silicate minerals is a silica tetrahedron, consisting of a silicon atom in the center and surrounded by four oxygen atoms ($SiO4^{4-}$). Each oxygen atom in the tetrahedron shares one electron with the Si atom in a covalent bond, forming a strong bond. In silicate minerals, tetrahedron bonds are arranged and linked in various ways, from single units to forming complex frameworks. Magnesium trisilicate ($Mg_2O_8Si_3$) is naturally found as the mineral forsterite, also known as white olivine. Olivine is a nesosilicate, a group with the simplest structure in which silica tetrahedrons are surrounded from all sides by other ions and not in contact with each other tetrahedrons. The tetrahedron is bound to each other only by ionic bonds from interstitial cations like Mg or Fe atoms which are divalent cations so that it can balance the -4 charge of the tetrahedron (Earle, 2019). This explains the ability of SSB to dissolve silica in Mg-trisilicate substrate much higher than feldspar and quartz. In feldspar silica tetrahedron bonded as a complex three-dimensional shape and as a charge balancer, there are cations such as Al^{3+}, K^+, Na^+, Ca^{2+} between the bonds of the tetrahedron, but quartz is only composed of tetrahedron bonds in a perfect three-dimensional form where each tetrahedron is bonded to four silicas in other tetrahedrons, the sharing of oxygen at each corner of the tetrahedron results in a balanced charge between the silica and oxygen and no other cations are needed in the bonds, this forms a very strong covalent bond between the tetrahedrons in the quartz mineral which is difficult to remove. Because of that, the dissolving activity of SSB silica in quartz is smaller than that of feldspar, the difference is not as significant as in Mg-trisilicate because the bond structure of the two minerals has the same complexity. SSB takes a longer incubation time to produce a significant concentration of silica dissolving from feldspar and quartz, namely on 4-day incubation, while on the Mg-trisilicate on the second-day incubation, a fairly high silica dissolving activity has started to appear. The optimum incubation time for Si solubilizing activity is slower than research by Santi & Goenadi (2017), with an optimum incubation time of 24 hours for *A. punctata* and *B. vietnaminesis*.

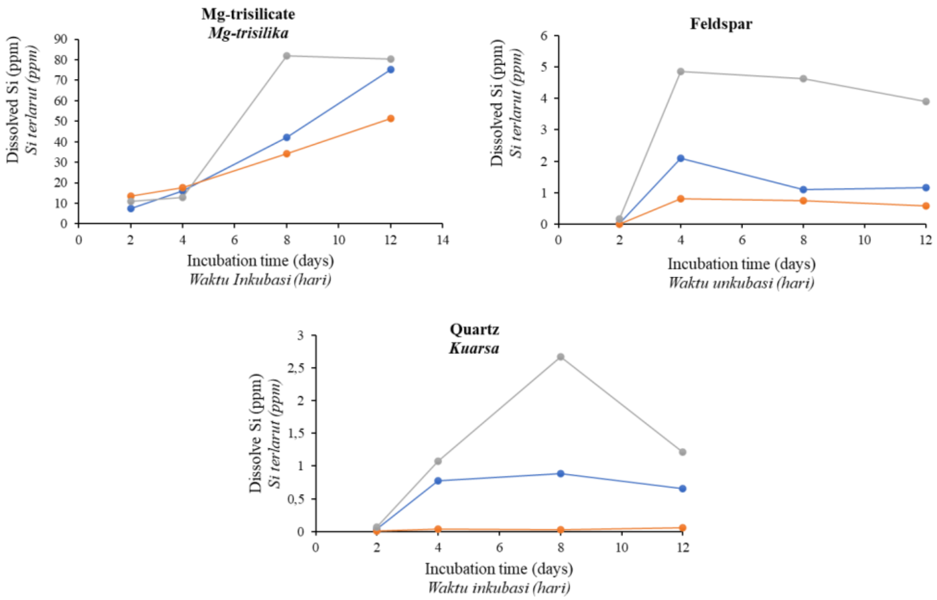


Figure 5. Silica solubilizing activity of SSB on silicate mineral as substrat

Gambar 5. Aktivitas pelarutan silika oleh BPS di beberapa mineral silika

(B.41; IG-D; KI-19)

The water uses efficiency and vegetative growth of maize in the greenhouse with drought treatment

The potential application of SSB in increasing maize resistance to drought stress was analyzed directly using a sap flow meter on maize plants with three irrigation conditions (normal, moderate, and severe drought). Based on its dissolving activity, the selected SSB in this application is *Pseudomonas fluoresce*-B41 which better dissolves complex-structured silicate minerals. As a source of insoluble silica, quartz is mixed in the soil by comparison 9 soil:1 quartz. The same irrigation conditions were used without the SSB application as a control. Measurements with a sap flow meter can monitor the movement of sap flow in the xylem expansion of a certain area (sapwood). Experiments from Uddin *et al.* (2014) showed that sap flow and transpiration showed the same pattern or flux. Thus, the sap flow meter readings can be used to observe the conditions of the sap flow rate and transpiration in maize in real-time. Transpiration is sensitive to the water status of plants with effects mediated by stomata opening and the movement of sap flow is also driven by water evaporating through the leaves, so these two factors can be used as indicators of plant water status.

Sap flow meter readings showed that the sap flow flux in maize that did not receive SSB application was higher under normal irrigation treatment and moderate drought treatment than that applied by SSB (Figure 6). In P1B0 the sap flow average reached 0.242 kg/day while P1B1 was only 0.151 kg/day, even in the P2B1 treatment, the sap flow average was much lower at only 0.011 kg/day while P2B0 reached 0.060 kg/day. For the severe drought treatment (P3), although the average sap flow in the SSB treatment was still lower, it was not too much different (Table 2). When compared, the average sap flow measured in P1 is much higher than P2 and P3. This is because drought stress conditions at P2 and P3 limit the availability of water that can be absorbed by maize. Although the sap flow rate and transpiration are different things, they are correlated. From the above data, it can be concluded that SSB administration can reduce the transpiration process in maize. This happened because SSB dissolved the silica contained in the growing media into mono silicic acid (H_4SiO_4), increasing the availability of Si for maize. Maize as Si accumulator will actively absorb H_4SiO_4 from the soil to the roots. This compound will be transported to the root symplast by the action of Lsi1 aquaporin on the plasma membrane, then H_4SiO_4 will diffuse to the root endodermis, and then be transported to the stellar apoplast by the Lsi2 transporter. The H_4SiO_4 is distributed by xylem then by specific transporter Lsi6 transport and accumulated in the cell wall, lumen, and intercellular spaces. The H_4SiO_4 then undergoes concentration through water loss due to the transpiration process and is polymerized into amorphous silica. The deposition and polymerization of silica will replicate the cell structure, coat the walls of the epidermis and vessel elements in the xylem, thereby reducing transpiration. And on the leaf epidermal cell wall, a hydrated amorphous polymer can form a silica cuticle double layer, effectively reducing transpiration on the leaf surface (Ali *et al.*, 2020; Chen *et al.*, 2018).

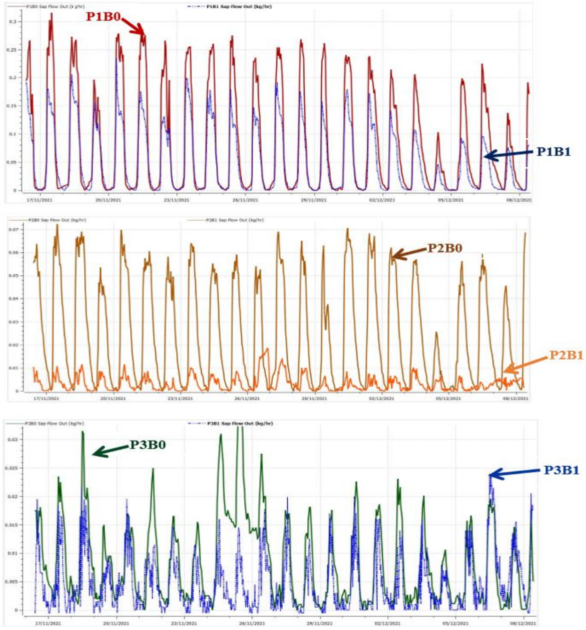


Figure 6. Sap flow out (kg/days) in normal irrigation (P1), moderate drought (P2), and severe drought (P3) with SSB B.41 inoculation (B1) and without inoculation (B0)

Gambar 6. Sap flow out (kg/hr) pada perlakuan irigasi normal (P1), kekeringan sedang (P2), dan kekeringan parah (P3) dengan inokulasi BPS B.41 (B1) dan tanpa inokulasi (B0)

Status of total water consumption in maize plants that were given SSB application for 20 days of observation with a sap flow meter was smaller than those that were not applied. Except for the severe drought treatment, which gave the opposite result (Table 2). In normal irrigation conditions, the use of SSB can increase the efficiency of water use by 46%, in moderate drought treatment, the application of SSB can increase the efficiency of water use by up to 84%. The percentage is based on comparing reduced total water use in control and treatment with total water use in control. But in the severe drought condition, treatment with SSB (P3B1) uses total water more than control (P3B0) which SSB did not apply, this may have happened because the drought intensity was very high in the P3 treatment, so the water content in soil was not conducive enough to support bacteria growth to solubilizing quartz in severe drought treatment. Water content is important to regulate oxygen diffusion, and aerobic microbes need humidity between 50-70% of water holding capacity to maintain their activity (Franzlubbers, 1999).

The role of SSB in increasing maize's drought tolerance is indirectly done by mediating the availability of dissolved silica (H_4SiO_4) in the growing media. When the availability of Si is sufficient for maize, Chen *et al.* (2018) stated that there are several key possible mechanisms for silica to maintain water balance in plants, including (1) increasing aquaporin activity through regulation of the expression plasma membrane intrinsic protein (PIP) gene aquaporins and reduces reactive oxygen species that induce aquaporin inhibition. (2) Silica increases the accumulation of dissolved sugars or amino acids in the xylem sap through osmoregulation, the accumulation of osmolytes in the xylem sap will increase the osmotic driving force, and (3) Silica increases the root/shoot ratio, collaborates with increasing aquaporin activity and the osmotic driving force increases hydraulic conductance roots, absorption of nutrients, and maintaining the rate of photosynthesis thereby increasing plant tolerance to drought stress.

The vegetative growth, i.e., plant height, number of leaves, and diameter of rod at 70 DAP of maize var. Pertiwi was presented in Table 3. The data show that the application of SSB tends to improve the vegetative growth of plants compared to control without SSB. In the condition of normal irrigation and moderate drought, the plant height significantly increases with the application of SSB. For the number of leaves and diameter of rod application SSB, just slightly more height than without SSB, except for P2B0 treatment. In general, the application of SSB tends to improve the vegetative growth of maize var. Pertiwi because the deposition of Si in the cell wall could decrease plant transpiration, so daily water use and plant growth were more optimum. This finding was similar to (Yuvakkumar *et al.*, 2011). In the soil-quartz medium available Si dissolves by SSB is present as mono-silicic acid. This form, which is referred to as plant-available silica (PAS), is taken up by the plant and has a direct influence on crop growth (Rao & Susmitha, 2017).

Table 2. Daily water consumption and sap flow out in maize
Tabel 2. Konsumsi air harian dan rataan sapflow out tanaman jagung

Treatment *Perlakuan*	Code *Kode*	Sapflow average (Kg/days) *Rataan sapflow (Kg/hari)*	Total water consumption (mL) *Total konsumsi air (mL)*	Daily water consumption (mL/days) *Konsumsi air harian (mL/hari)*
Normal irrigation–SSB	P1B0	0.242	4580	229
Normal irrigation+SSB	P1B1	0.151	2470	123.50
moderate drought–SSB	P2B0	0.060	957	47.85
moderate drought+SSB	P2B1	0.011	146	7.30
Severe drought–SSB	P3B0	0.019	202	10.10
Severe drought+SSB	P3B1	0.016	267	13.35

Table 3. Vegetative growth of maize var. Pertiwi at 70 DAP
Tabel 3. Pertumbuhan vegetatif jagung var. Pertiwi pada 70 HST

Treatment *Perlakuan*	Plant height (cm) *Tinggi tanaman (cm)*	Number of leaves (strand) *Jumlah daun (helai)*	Diameter of rod (mm) *Diameter batang (mm)*
P1B1 (normal irrigation + SSB)	152.6 a*)	10.0 ab	12.72 ab
P2B1 (moderate drought + SSB)	152.4 a	10.0 ab	13.12 a
P3B1 (severe drought + SSB)	135.2 b	11.0 a	9.91 bc
P1B0 (normal irrigation without SSB)	136.1 b	10.0 ab	12.56 ab
P2B0 (moderate drought without SSB)	136.4 b	9.5 b	13.28 a
P3B0 (severe drought without SSB)	135.0 b	9.5 b	10.90 abc

*) Means in the same column followed by the same letter are not significantly different according to Duncan's multiple range test at $\alpha = 0.05$.
**) Angka dalam kolom yang sama diikuti oleh huruf yang sama berarti tidak berbeda nyata menurut uji jarak berganda Duncan pada $\alpha = 0.05$.*

Conclusion

Silica solubilizing bacteria *P. fluoresces* B41, KI-19, and *P. dispersa* IGD showed silica dissolving activity both on magnesium trisilicate, feldspar, and quartz substrates. The highest activity produced by B41 was 81.93 ppm for Mg trisilicate, 4.85 ppm for feldspar, and 2.67 ppm for quartz. Optimum silica dissolution occurred on day 8 for Mg trisilicate and quartz, and on day 4 for feldspar. The application of SSB *P. fluoresces*-B41 to maize effectively improves water use efficiency under normal irrigation conditions by 46% and moderate drought stress by 84%. Still, it was not yet effective in severe drought stress conditions. In general application of SSB tends to improve vegetative growth in maize var. Pertiwi. Silica solubilizing bacteria can also reduce the transpiration rate in maize plants in all treatment conditions to maintain the plant's water balance and increase drought resistance.

Suggestion

Further research is needed on the mechanism of silica dissolution by SSB *P.* fluoresces-B41, KI-19, and *P. dispersa*-IGD, by identifying pH, organic acids produced during the incubation period in each silica source, and the correlation of silica dissolution by SSB with the availability of other micro and macronutrients that contained in silicate minerals and their correlation to plant growth.

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