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Exogenous application of paclobutrazol promotes water-deficit tolerance in pepper (*Capsicum annuum*)

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Abstract. *Slichatun, Khasanah FU, Pitoyo A, Etikawati N, Mudyantini W. 2021. Exogenous application of paclobutrazol promotes water-deficit tolerance in pepper (*Capsicum annuum*). Cell Biol Dev 5: 1-6.* Pepper (*Capsicum annuum* L.) is a vegetable commodity with high economic value. However, pepper cultivation in Indonesia is still hampered by pests and drought, reducing its growth and production. The efforts to increase pepper productivity and its resistance to drought stress can be conducted by applying growth regulators (ZPT), one of which is paclobutrazol (PBZ). This study aims to determine the effect of PBZ on the growth and accumulation of proline, a plant osmoprotectant, in pepper seedlings grown in water-deficit soil. This study used a completely randomized design (CRD) with two factors, i.e., a variation of PBZ and soil water capacity. PBZ concentration was made in four levels, namely 0 ppm, 25 ppm, and 50 ppm. The variation of the water-capacity level was made in three levels, namely 100% FC (field capacity as well-watered treatment), 75% FC (mild water deficit), and 50% FC (severe water deficit). The treatment was given for three weeks. The quantitative data obtained were analyzed by ANOVA, and if there was a significant difference, a further test was carried out with DMRT at the 95% confidence level. In addition, qualitative data were analyzed descriptively. The results showed that applying PBZ to pepper (*Capsicum annuum* L.) seedlings that grew under water-deficit stress significantly affected proline levels, total chlorophyll content, and carotenoid levels; meanwhile, the parameters of plant dry weight and root shoot ratio had no significant effect. Furthermore, this indicates that PBZ is quite capable of inducing resistance to pepper seedlings in drought conditions.

Keywords: Drought, paclobutrazol, pepper, proline

INTRODUCTION

Pepper (*Capsicum annuum* L.) is a critical vegetable commodity from the family of Solanaceae and is widely cultivated by local farmers in Indonesia. The gross national production of fresh pepper in 2014 reached 0.8 million tons (BPS 2015), which means it increased in production by about 86.98 thousand tons (12.19 %) compared to the previous year. Moreover, this proves that the demand for Indonesian peppers is quite high.

Several factors still hamper the success of pepper cultivation in Indonesia. These factors include the presence of pests and diseases as well as environmental stress conditions. One of the environmental threats is drought. The water deficit condition seriously impacts the productivity of peppers planted by farmers. The previous research by Yusniwati et al. (2008) showed that drought stress on several pepper varieties could reduce their production (Tit Super 29.2%, Teak profit 47.72%, Hot Pepper 25.74%, Laris 52.63%, and Prabu 50.83%). According to Matiu et al. (2017), globally, drought has reduced the production of several other horticultural crops, including corn (11.6%), wheat (9.2%), and soybeans (33.1-12.2%).

Several attempts have been made to increase the productivity of peppers or other cultivated plants, one of which is the application of growth regulators (ZPT). According to Saglam et al. (2002), ZPT is widely applied to vegetable crops to increase seed germination and yield and induce disease tolerance and unfavorable growing conditions.ZPT treatment on seed germination has proved to support plant growth and its resistance to drought stress (Fletcher et al. 2000). One growth regulator prospectively used to improve the quality of seedling growth in drought stress is paclobutrazol (PBZ). PBZ is a derivative of triazole compounds that are known to protect some horticultural plants from environmental stresses such as drought stress, cold temperatures, heat, and ultraviolet radiation (UV) (Tesfahun 2018). PBZ was reported to have a positive impact on improving the quality of seedling growth of kenikir (*Tagetes patula* L.) and geranium (*Pelargonium hortorum* L.H. Bailey) (Pasian and Bennett 2001); kamelina (*Camelina sativa* L. Crantz) (Kumar et al. 2012); tomatoes (*Lycopersicon esculentum* Mill.) (Still and Pill 2004); potato (*Solanum tuberosum* L.) (Nuraini et al. 2018); rice (*Oryza sativa* L.) (Ningsih and Rahmawati 2017); black rice (*Oryza sativa* L. 'Cempo Ireng') (Dewi et al. 2016); cucumber (*Cucumis sativus* L., cv. Poinsett 76SR) and tomato (*Solanum lycopersicum* L., cv. Sun 6108) (Magnitskiy et al. 2006); and canola (*Brassica napus* L.) (Anwar et al. 2017).

This study aims to determine the effect of the paclobutrazol application on pepper seedling growth and its ability to enhance proline accumulation under suffering water deficit conditions. In addition, this research is expected to be a reference to improve the quality of pepper seedlings to increase the quality of pepper production in dry land.

MATERIALS AND METHODS

Plant materials and seed sowing preparation

This research was done from March to September 2019 at the Laboratory and greenhouse facilities of the Department of Biology, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Indonesia. The materials needed for this research are 540 pepper seeds (CF-1999 cultivar produced by PT. Seed Citra Asia, Jember, Indonesia), as well as paclobutrazol (PBZ), 3% sulfosalicylic acid, ninhydrin acid, toluene, glacial acetic acid (AAG), acetone 80%, DMSO, Whatman Filter paper, FAA solution, alcohol, xylol,5% NaOCl, safranin 1 %, paraffin, etc., and planting medium in the form of a mixture of soil: compost (1: 1).

Before sowing, uniform and vigorous seeds are selected and soaked in warm water (± 50°C) for about one night and drained on tissue paper. Soaking is intended to select seeds and speed up germination. The float seeds are discarded, and the seeds that sink are used. Furthermore, a tetrazolium test is performed to determine the seeds' viability or vigor. The tetrazolium test was carried out by immersing 30 seeds in distilled water for 24 hours. Then, the seeds were split into two parts and soaked again in 1% tetrazolium solution for 24 hours at room temperature (28-30°C). Red seeds are counted as viable seeds. The tetrazolium test was carried out on the seeds before treatment to determine their initial viability. The tetrazolium test was carried out in 3 replications. The percentage of seed viability is calculated using the following formula (Subantoro 2014):

The percentage of viable seed = number of viable seed (red)/ total seed tested x 100%.

The sowing media used a mixture, soil: compost (1: 1). The soil and fertilizer were separately sieved and then mixed and stirred evenly. The media is filled into a polybag (25x30 cm) until it reaches ¾ of its parts. Seeds soaked in distilled water for 24 hours are then sown in polybags filled with the medium inside. Seed sowing is carried out in a greenhouse. Each treatment was made 3 with replications.

Paclobutrazol application and water stress treatment

The paclobutrazol solution was set in 3 variations of concentration, namely 0 ppm (control), 25 ppm, and 50 ppm, referring to the research of Brigard et al. (2006) and Magnitskiy et al. (2006). Next, adding the PBZ weighed as much as 25 mg and 50 mg, then each was added a little DMSO and stirred until dissolved. Finally, each homogeneous mixture of PBZ and DMSO powder was added with distilled water until the volume reached 1000 ml.

The paclobutrazol application was carried out directly on the soil medium in the root area (soil treatment) following the procedure performed by Berova and Zlatev (2000) with modification. First, PBZ is applied once when the pepper seeds are four weeks old. Next, 100 ml of PBZ solution was poured directly into the plant's root area. Finally, watering is done in the morning at around 6 am.

Drought stress treatment was carried out three days after PBZ application. The variations in drought stress used were 100% field capacity (KL) as a control, 75% KL, and 50% KL. The determination of field capacity is determined by the gravimetric method. Drought stress was treated for three weeks with watering intervals every two days. The volume of water added follows the calculation of the media's field capacity according to the treatment. Variations of PBZ application and drought stress were made into nine treatment combinations. Each treatment consisted of 3 replications, each consisting of 3 seedlings, so the total seedlings used in this study were 81.

Growth parameters, physiological and biochemical characteristics

Seedling morphological observations were carried out when the pepper plants were seven weeks old. The observed morphology of seedlings included plant dry weight (grams) and shoot: root ratio based on dry weight (Sinaga 2008). Physiological observations of pepper seedlings were carried out by measuring proline, chlorophyll, and carotenoid content. The analysis of total leaf proline levels was carried out using Bates et al. (1973). Measurement of the total chlorophyll and carotenoid content of pepper leaves was carried out at the end of the observation by referring to the research of Kurniawan et al. (2010) and Sayyari & Ghanbari (2012). DL-proline (Sigma) dissolved in sulfosalicylic acid (3% w / v) is used as standard. Quantitative data were analyzed by ANOVA (Analysis of Variance). If there was a significant difference between the treatment groups, a further test was carried out with Duncan's Multiple Range Test (DMRT) at the 95% confidence level.

RESULTS AND DISCUSSION

Seedling morphological characters in water deficit treatments

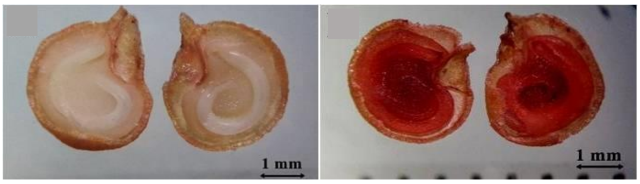
Vigor or viability test

The viability test of pepper seeds was carried out with a 1% tetrazolium solution. The seeds were categorized as having good vigor, marked by a change in color from yellowish-white (Figure 1A) to red (Figure 1B) after immersion in a 1% tetrazolium solution for 24 hours. The tetrazolium test results on three replications resulted in a seed viability percentage value of 100%.

Dry weight and shoot: root ratio of pepper seedling

Table 1 revealed that drought stress only significantly affects the dry biomass of seedlings in conditions without PBZ. Moreover, giving PBZ concentrations of 25 ppm or 50 ppm under all soil water levels (100%, 75%, and 50% of field capacity) had no significant effect. The pepper seedlings are shown in Figure 2.

Drought stress can affect plant growth and production, including physiological, biochemical, anatomical, and morphological processes. Table 1 shows that drought stress significantly affects the dry biomass of seedlings without PBZ application. That is in line with Khan et al. (2012) research on pepper (*Capsicum annuum* L.), which showed that drought stress caused a decrease in dry crown weight and total dry weight. The most significant difference between dry biomass reduction and drought stress occurred in the 0 ppm PBZ treatment, which was ±44.8%. In comparison, the difference in reduction was smaller in the 25 and 50 ppm PBZ treatments.



A B

Figure 1. The morphology of the median-longitudinal section of pepper (*Capsicum annum* L) seed was observed under a stereomicroscope at 20x optical magnification. A. Seed before tetrazolium 1% test. B. Seed after tetrazolium 1% test

The decrease in dry biomass is a plant response to drought stress. Overall dry biomass of pepper in the three PBZ treatments decreased due to drought stress. However, the results of this study indicated that the addition of PBZ to the seedlings was thought to cause a more tolerant response to drought stress. That can be seen from the difference in the reduction of dry biomass from normal conditions is lower than the treatment without PBZ.

Table 1. Dry weight (gram) and shoot: root ratio based on seedling dry-weight after applying various paclobutrazol (PBZ) concentrations in different soil water capacities levels treatments

Parameters	PBZ conc. (ppm)	Soil water capacity levels		
		100%FC	75%FC	50%FC
Dry weight (gram)	0	0.29^{c}±0.037	0.19^{b}±0.039	0.16^{ab}±0.007
	25	0.14^{ab}±0.035	0.13^{ab}±0.038^{s}	0.13^{a}±0.053
	50	0.14^{ab}±0.015	0.13^{ab}±0.012	0.12^{a}±0.016
Shoot: root ratio	0	11.86^{b} ±1.413	9.85^{ab}±1.040	5.35^{a} ±1.111
	25	9.29^{ab}±5.531	9.5^{ab} ±4.611	6.72^{ab}±2.414
	50	8.45^{ab}±0.438	5.84^{a}±1.407	9.36^{ab}±3.991

Note: Mean values± standard deviation followed by the same letter in the same column for each parameter indicated no significant differences at P: 0.05 with the DMRT post hoc test.FC: field capacity

Figure 2. The morphology of pepper seedling at 21 days after applying various paclobutrazol (PBZ) concentrations in different soil water capacities levels (FC). (A-C) Treatments of 0 ppm PBZ combined with (A) 100% FC; (B) 75% FC; (C) 50% FC. (D-F) Treatments of 25 ppm PBZ combined with (D) 100% FC; (E) 75% FC; (F) 50% FC. (G-I) Treatments of 50 ppm PBZ combined with (G) 100% FC; (H) 75% FC; (I) 50% FC. Bar = 2 cm

Drought conditions cause a decrease in water content in the plant growing media, which causes a reduction in water uptake by the roots. This condition causes insufficient water supply for growth; consequently, plant growth is stunted. According to Hidayati et al. (2017), reduced dry biomass in plants experiencing drought stress is a plant response closely related to decreased photosynthesis. Likewise, Subantoro (2014) states that a decrease in photosynthesis rate causes photosynthate to be translocated for limited root and canopy growth, resulting in low biomass.

Paclobutrazol is a derivative of triazole compounds known to protect plants from environmental stresses such as drought stress (Tesfahun 2018). PBZ is a synthetic growth regulator that inhibits the biosynthesis of sterols and gibberellins (GA) in plants (Khan et al. 2012) and induces various morphological and biochemical responses. Furthermore, the PBZ compounds could inhibit shoot elongation, reduce leaf area, stimulate root growth, increase cytokinin and ABA synthesis, and provide protection from various environmental stresses (Fletcher et al. 2000).

The drought conditions in the vegetative phase also inhibit the growth of leaves and roots, but the magnitude of this effect is not the same. A decrease in the crown-root ratio occurs when the decrease in leaf growth is greater than that of the roots. Based on Table 1, drought stress only significantly affects the root canopy ratio in the 0 ppm PBZ treatment. In the 25 and 50 ppm PBZ treatments, drought stress did not have a significant effect even though it caused a decrease in the average root canopy ratio. This decline is thought to be an attempt by the pepper to increase water uptake by expanding the root system, while the increase is thought to be an effort to utilize water extraction to support crown growth.

In the treatment without PBZ, the condition of 50% soil water capacity reduced the average ratio of pepper seedlings significantly compared to normal conditions. Moreover, it is agreed with Nejad et al. (2010), which state that drought conditions affect the ratio of the canopy root of maize plants. The difference in the reduction in the average crown-root ratio in the treatment without PBZ was greater, i.e., 54.9% than the treatment with the addition of PBZ at the same level of water capacity. According to the research results of Navarro et al. (2008), the root canopy ratio decreased by 0.25 g compared to control under stress conditions, while the addition of PBZ decreased the root crown ratio by 0.38 g.

The percentage reduction in root canopy ratio due to drought stress treatment was greater than the percentage reduction in root canopy ratio due to the addition of PBZ. The addition of PBZ in drought stress conditions caused the root crown ratio to increase. Possibly, PBZ inhibited crown growth, so plants treated with 25 and 50 ppm PBZ tended to allocate more assimilates aimed at the crown section.

In general, the character of the root canopy ratio of pepper shows a negative response to drought conditions. According to Sinaga (2008), the condition of the lower availability of water encourages pepper seedlings to distribute photosynthate, which tends to be aimed more at the roots. As a result, biomass allocation to the roots increases to expand the root system, thereby reducing the root canopy ratio (Zlatev and Lidon 2012).

Drought stress conditions trigger plants to increase root growth further and suppress crown growth so that the root canopy ratio becomes smaller. This response is a form of plant adaptation in dealing with drought stress conditions related to leaf area parameters and seedling height. According to Wu and Cosgrove (2000), adaptation is carried out by suppressing crown growth to reduce water loss during transpiration and stimulating root growth to absorb more water by reaching deeper soil layers. According to Bayat and Sepehri (2012), the decrease in root and canopy biomass in PBZ treatment is closely related to the inhibition of gibberellic acid (GA) biosynthesis induced by PBZ causes a decrease in shoot growth.

Biochemical changes in seedlings under water deficit treatment

Proline levels

Plants with low soil water availability accumulate proline, which plays a vital role in osmotic adjustment. According to Kurniawati et al. (2014), proline is an osmoregulator that protects membranes and plant enzymes dealing with drought stress. In drought stress conditions, an increase in leaf proline content was higher than in the optimum environmental conditions. Based on Yusniwati et al. (2008), the leaf proline content in several pepper genotypes increased by 12.62% to 646.31% under stressful conditions.

Table 2 shows that the application of 50 ppm PBZ significantly affects pepper seedlings' proline content grown at the lowest water capacity level. Meanwhile, seedlings at 0 ppm and 25 ppm PBZ had no significant difference in leaf proline levels. Based on the results of this study, the pepper seedlings treated with PBZ were thought to have a better tolerance to drought stress than those without PBZ. PBZ affects in increasing plant-free proline levels to protect against drought stress. Mohamed et al. (2011) reported that the free proline levels in tomatoes given 50 ppm PBZ grown under 60% KL increased 1.52 times compared to controls. Seedlings that are more tolerant of drought respond to drought stress by increasing levels of leaf proline.

Table 2. Leaves proline content (mg/g fresh weight) of pepper seedling in various paclobutrazol applications at different water regimes (field capacities)

PBZ conc. (ppm)	Soil water capacity levels		
	100%FC	75%FC	50%FC
0	$3.98^{a}\pm 1.202$	$4.36^{a} \pm 1.245$	$4.66^{a}\pm 1.741$
25	$3.02^{a}\pm 0.777$	$4.6^{a}\pm 0.314$	$4.93^{a}\pm 0.121$
50	$3.6^{a\pm}0.702$	$4.81^{a\pm}1.461$	$6.91^{b}\pm 0.496$

Note: Mean values followed by the same letter in the same column for each parameter indicate no significant differences at P: 0.05 with the DMRT post hoc test. Sd: deviation standard; FC: field capacity

Table 3. Total leaf chlorophyll and carotenoid levels (mg/g fresh weight) pepper seedling in various paclobutrazol applications at different water regimes (field capacities)

Parameters	PBZ conc. (ppm)	Soil water capacity levels		
		100%FC	75%FC	50%FC
Total leaf chlorophyll (mg/g fresh weight)	0	36.05[ab]±0.962	35.5[a] ±2.073	35.26[a] ±2.905
	25	40.77[c] ±1.521	40.41[bc]± 1.814	39.54[abc]±3.571
	50	39.69[abc]± 1.602	38.81[abc]±2.482	38.58[abc]±2.714
Leaf carotenoid (mg/g fresh weight)	0	0.82[a]±0.041	0.85[ab]±0.040	0.89[abc]±0.075
	25	0.95[bc]±0.075	0.97[c] ±0.070	0.98[c] ±0.049
	50	0.94[bc]± 0.020	0.95[bc]± 0.060	0.96[c] ±0.057

Note: Mean values followed by the same letter in the same column for each parameter indicate no significant differences at P: 0.05 with the DMRT post hoc test. Sd: deviation standard; FC: field capacity

According to Alcazar et al. (2011), water deficiency can stimulate a chemical signal in the form of abscisic acid (ABA) from the roots, which is translocated into the canopy inducing the accumulation of proline in leaves. Proline accumulates in leaves, is partly used as a protective compound against macromolecular compounds and enzymes from damage due to drought stress, and is partially transferred to roots for root growth (Tuasamu 2009). Increased proline levels are a characteristic of plant tolerance to drought stress because proline acts as an N storage compound and protector of certain enzymes (Maestri et al. 1995).

Total chlorophyll and carotenoids content

Besides the accumulation of proline, water deficit also challenges plants with a physiological change in their metabolic activity. Plants with low water potential have decreased chlorophyll and an increase in secondary metabolites, one of which is carotenoids. This study indicated that the water deficit and PBZ treatment could induce changes in the content of photosynthetic pigments. The two PBZ concentrations significantly increased seedlings' chlorophyll and carotenoid content under normal and stressful conditions. Drought stress conditions also showed an insignificant effect on total chlorophyll content and carotenoids.

Based on the data from Table 3, adding PBZ to soil media leads to an increase in total leaf chlorophyll. That indicates that the presence of PBZ provides a better tolerance response to drought stress than the treatment without PBZ. Kumar et al. (2012) and Tsegaw et al. (2005) reported that the chlorophyll content was higher in plants treated with PBZ than in controls. The increase in total chlorophyll content in pepper leaves with PBZ treatment is thought to be closely related to the effect of PBZ on endogenous cytokinin concentrations. Fletcher et al. (1982) stated that PBZ could stimulate cytokinin biosynthesis, which induced chloroplast differentiation and chlorophyll biosynthesis and prevented chlorophyll degradation.

Carotenoids are additional photosynthetic pigments that plants generally need only in small quantities alongside chlorophylls. However, to maintain the photosynthesis process, their presence is more needed when plants suffer a water deficit that is terminating to chlorophyll degradation. Table 3 shows that PBZ supplementation has significantly affected carotenoid content. This result follows a previously reported by Gopi et al. (2007) that PBZ can increase carrot root carotenoids. According to Fletcher et al. (2000), carotenoid production triggered by PBZ is a beneficial antioxidant in plants to combat oxidative stress. Under drought stress conditions, the average carotenoid leaves of pepper were higher than normal. Following Armita et al. (2017) research, the carotenoid content in plants experiencing severe drought stress (30% KL) was significantly higher than in control plants.

Based on the results of this study, giving 25 and 50 ppm PBZ to pepper seedlings was thought to induce better tolerance to water deficit treatments than seedlings without PBZ. Plants more tolerant of drought stress showed a significant increase in total chlorophyll and leaf carotenoids. A decrease in total chlorophyll and an increase in carotenoids in pepper peppers indicated that drought conditions caused changes in plant metabolic activity. According to Oukarroum et al. (2007), one of the physiological characteristics of plant resistance to drought is to increase photosynthesis by increasing the chlorophyll content. The increase in carotenoids in plants indicates plants' ability to adapt to new environmental conditions (Othman et al., 2014).

In summary, paclobutrazol (PBZ) application to pepper seedlings (*Capsicum annuum* L.) growing in drought stress significantly affected the accumulation of proline levels, total chlorophyll content, and carotenoid levels; Meanwhile, the parameters of plant dry weight and root shoot ratio had no significant effect.

REFERENCES

Alcazar R, Bitrián M, Bartels D, Koncz C, Altabella T, Tiburcio AF. 2011. Polyamine metabolic canalization in response to drought stress in Arabidopsis and the resurrection plant *Craterostigma plantagineum*. Plant Signal Behav 6: 243-250.

Anwar S, Kuai J, Khan S, Kausar A, Rehman M, Shah N, Zhou G. 2017. Soaking seeds with paclobutrazol enhances winter survival and yield of rapeseed in a rice-rapeseed relay cropping system. Intl J Plant Prod11 (4): 491-504.

Armita D, Arumyngtyas EL, Mastuti R. 2017. Tolerance level of three genotypes of pepper (*Capsicum frutescens* L.) toward drought stress of vegetative phase based on morphological and physiological responses. Intl J Plant Prod 10 (2): 183-192.

Badan Pusat Statistik. 2015.Produksi Cabai Besar 1,075 Juta Ton, Cabai Rawit 0,8 Juta Ton, dan Bawang Merah 1,234 Juta Ton. Berita Resmi Statistik. Badan Pusat Statistik, Jakarta. [Indonesian]. https://www.bps.go.id/pressrelease/2015/08/03/1168/produksi-cabai-besar-1-075-juta-ton--cabai-rawit-0-8-juta-ton--dan-bawang-merah-1-234-juta-ton.html. Diakses 13 November 2018.

Bates IS, Waldren RP, Tare ID. 1973. Rapid Determination of Free Proline for Water Stress Studies. Plant Soil 39: 205-207.

Bayat S, Sepehri A. 2012. Paclobutrazol and salicylic acid application ameliorate the negative effect of water stress on growth and yield of maize plants. J Res Agric Sci 8: 127-139.

Berova M, Zlatev Z. 2000. Physiological response and yield of paclobutrazol treated tomato plant (*Lycopersicon esculentum* Mill). Plant Growth Regul 30 (2): 117-123.

Brigard JP, Harkess RL, Baldwin BS. 2006. Tomato early seedling height control using a paclobutrazol seed soak. HortScience 41 (3): 768-772.

Cahyono B. 2003. Cabai Rawit: Teknik Budidaya dan Analisis Usaha Tani. Kanisius, Yogyakarta. [Indonesian]

Dewi K, Agustina RZ, Nurmalika F. 2016. Effects of blue light and paclobutrazol on seed germination, vegetative growth and yield of black rice (*Oryza sativa* L. 'Cempo Ireng'). Biotropia 23 (2): 84-95.

Fletcher RA, Gilley A, Davis TD, Sankhla N. 2000. Triazoles as plant growth regulators and stress protectants. Horticult Rev 24: 55-138.

Fletcher RA, Kallidumbil V, Steele P. 1982. An Improved bioassay for cytokinin using cucumber cotyledons. Plant Physiol 69: 675-677.

Gopi R, Jaleel CA, Sairam R, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R. 2007. Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of *Daucus carota L*. Colloids Surfaces B Biointerfaces 60: 180-186.

Hidayati N, Hendrati RL, Triani A, Sudjino. 2017. Pengaruh kekeringan terhadap pertumbuhan dan perkembangan tanaman nyamplung (*Callophylum inophyllum* L.) dan johar (*Cassia florida* Vahl.) dari provenan yang berbeda. Jurnal Pemuliaan Tanaman Hutan. 11 (2): 99-111. [Indonesian]

Irwan AW, Wicaksono FY. 2017. Perbandingan pengukuran luas daun kedelai dengan metode gravimetri, regresi dan scanner. Jurnal Kultivasi 16 (3): 425-429. [Indonesian]

Khan MAI, Hoque MA, Farooque AM, Habiba U, Rahim MA. 2012. Physio-morphological features of pepper accessions under moisture stress conditions. Bangladesh J Agric Res 37: 263-269.

Kumar S, Ghatty S, Satyanarayana J, Guha A, Chaitanya BSK, Reddy AR. 2012. Paclobutrazol treatment as a potential strategy for higher seed and oil yield in field-grown *Camelina sativa* L. Crantz. BMC Res Not 5 (137): 1-13.

Kurniawan M, Izzati M, Nurchayati Y. 2010. Chlorophyll, carotenoid and vitamin C in some species of aquatic plants. Bull Anat Physiol 18: 28-40.

Kurniawati S, Khumaida N, Ardie SW, Hartati NS, Sudarmonowati E. 2014. Pola akumulasi prolin dan poliamin beberapa aksesi tanaman terung pada cekaman kekeringan. Jurnal Agronomi Indonesia 42 (2): 136-141. [Indonesain]

Maestri M, Da Matta FM, Regazzi AJ, Barros RS. 1995. Accumulation of proline and quaternary ammonium compounds in mature leaves of water stressed coffee plants (*Coffea arabica* and *C. canephora*). J Horticult Sci 70 (2): 229-233.

Magnitsky SV, Pasian CC, Bennett MA, Metzger JD. 2006. Effects of soaking cucumber and tomato seeds in paclobutrazol solutions on fruit weight, fruit size, and paclobutrazol level in fruits. HortScience 41 (6): 1446-1448.

Matiu M, Ankerst DP, Menzel A. 2017. Interactions between temperature and drought in global and regional crop yield variability during 1961-2014. PLoS ONE 12 (5): e0178339. DOI: 10.1371/journal.pone.0178339.

Mohamed GF, Agamy RA, Rady MM. 2011. Ameliorative effects of some antioxidants on water-stressed tomato (*Lycopersicon esculentum* Mill.) plants. J Appl Sci Res 7: 24-70.

Navarro A, Vicente MJ, Martínez-Sánchez JJ, Franco JA, Fernández JA, Bañón S. 2008. Influence of deficit irrigation and paclobutrazol on plant growth and water status in *Lonicera implexa* seedlings. Acta Horticultural 782: 299-304.

Nejad TS, Bakhshande A, Nasab SB, Payande K. 2010. Effect of drought on corn root growth. Report and Opinion 2 (2): 47-53.

Ningsih R., Rahmawati D. 2017. Application of paclobutrazol and macro inorganic fertilizer on the yield and seed quality of rice (*Oryza sativa* L.). Agriprima J Appl Agric Sci 1 (1): 22-34.

Nuraini A, Sumadi, Mubarok S, Hamdani JS. 2018. Effects of application time and concentration of paclobutrazol on the growth and yield of potato seed of G2 cultivar medians at medium altitude. J Agronomy 17 (3): 169-173.

Othman R, Zaifuddin FAM, Hassan NM. 2014. Carotenoid biosynthesis regulatory mechanisms in plants. J OleoScience 63: 753-760.

Oukarroum A, Madidi SE, Schansker G, Strasser RJ. 2007. Probing the response of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and rewatering. Environ Exp Bot 60 (3): 438-446.

Pasian CC, Bennett M. 2001. Paclobutrazol-soaked marigold, geranium, and tomato seeds produce short seedlings. HortScience 36: 721-731.

Ruzin SE. 1999. Plant Microtechnique and Microscopy. Oxford University Press, New York.

Saglam N, Gebologlu N, Yilmaz E, Brohi A. 2002. The effects of different plant growth regulators and foliar fertilizers on yield and quality of crisp lettuce, spinach and pole bean. Acta Horticulturae 579: 619-623.

Sayyari M, Ghanbari F. 2012. Effects of super absorbent polymer A200 on the growth, yield and some physiological responses in sweet pepper (*Capsicum annuum L.*) under various irrigation regimes. Intl J Agric Food Res 1: 1-11.

Sinaga R. 2008. Keterkaitan nisbah tajuk akar dan efisiensi penggunaanair pada rumput gajah dan rumput raja akibat penurunanketersediaan air tanah. Jurnal Biologi Sumatera 3 (1): 29-35. [Indonesian]

Still JR., Pill WG. 2003. Germination, emergence, and seedling growth of tomato and impatiens in response to seed treatment with paclobutrazol. HortScience 38 (6): 1201-1204.

Subantoro, R. 2014. Pengaruh cekaman kekeringan terhadap respon fisiologis perkecambahan benih kacang tanah (*Arachis hypogaea L*). Mediagro 10 (2): 32-44. [Indonesian]

Tesfahun W. 2018. A review on: Response of crops to paclobutrazol application. Cogent Food Agric 4: 1-9.

Tsegaw T, Hammes S, Robbertse J. 2005. Paclobutrazol-induced leaf, stem, and root anatomical modifications in potato. Horticult Sci 40 (5): 1343-1346.

Tuasamu Y. 2009. Toleransi hotong (S*etaria italica* l. Beauv) pada berbagai cekaman kekeringan: pendekatan anatomi dan fisiologi. [Tesis]. Sekolah Pascasarjana. Institut Pertanian Bogor. Bogor. [Indonesian]

Willmer CM. 1983. Stomata. Longman Inc., New York.

Wu Y, Cosgrove DJ. 2000. Adaptation of root to low water potentials by changes in cell wall extensibility and cell wall proteins. J Exp Bot 51: 1543-1553.

Yusniwati, Sudarsono, Aswidinnoor H, Hendrastuti S, Santoso D. 2008. Pengaruh cekaman kekeringan terhadap pertumbuhan, hasil dan kandungan prolina daun cabai. Jurnal Agrista 12 (1): 19-27. [Indonesian]

Zlatev Z, Lidon FC. 2012. An overview on drought-induced changes in plant growth, water relations and photosynthesis. Emirates J Food Agric 24 (1): 57-72.