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Partial root-zone irrigation effects on growth, metabolism and calcium status of Mangosteen seedling (*Garcinia mangostana* L.)

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Efficient irrigation technique for mangosteen seedling was evaluated, from October 2016 to May 2017, in order to determine the growth and morphophysiology of both the conventional deficit irrigation (CD) and partial root-zone irrigation (PR). A set of randomized block design, with 4 replicates each, was applied on 100% field capacity (control), 50% field capacity (CD1), 30% field capacity (CD2), and ratios of 100:50% field capacity (PR1), 100%:30% field capacity (PR2) and 50:30% field capacity (PR3). The results showed a restriction in mangosteen growth, except control, as indicated by decrease in total dry mass, which due to decrement in leaf number, photosynthetic rate and root growth. Malondialdehyde (MDA) level and glutathione peroxidase (GPX) activity was higher while proline accumulation was lower in PR compared to those of both CD and control treatments. Secondary metabolites content changes in treatments, such as octacosane, cysteamine sulfonic acid, propyl oleate, 1-nanodecene, and 2-butyn-1-ol-4metoxy were synthesized in the low soil moisture conditions. Leaf Ca-pectate, Ca-phosphate and dissolved Ca tended to increase in low soil moisture. The PR1 treated plant had the highest water use efficiency. Therefore, PR technique could be applied when the soil moisture level represents 50% (or more) of the field capacity.

1. Introduction

Mangosteen (*Garcinia mangostana* L.) is a tropical perennial crop that plays an ecologically important role in agroforestry system (Wijayanto and Hartoyo, 2015). It produces an exotic fruit with high antioxidant level (Kurniawati *et al.,* 2010). However, many producing countries, such Indonesia, Malaysia, Thailand and India (Osman and Milan, 2006), are facing both fluctuations in production and yellow latex matter in mangosteen fruits production (Sdoodee and Limpun-Udom, 2002; Poerwanto *et al.,* 2010). Matra *et al.* (2016) reported that mangosteen exhibits moderate genetic variation within a population. On the other hand, according to

Martias and Mansyah (2014), the variations in mangosteen quality were affected by seasonal variation, water availability, and cultivation techniques. Furthermore, Poerwanto *et al.* (2010) stated that high water fluctuation in the soil will affect the turgor pressure so that the duct secretory of yellow latex will break out and contaminate the fruit.

As other tropical fruits, mangosteen requires low soil moisture root zone to promote flowering (Paull and Nakasone, 1998). However, extended low water status might adversely affect the plant growth (Mustaha, 2012). Preventing water fluctuation on mangosteen root zone is not easily carried out due to the fact that mangosteen is usually planted in an arid area (and depend on rainfall). On the other hand, mangosteen was spread in hills area with agroforestry system, making the irrigation setting difficult. Thus, the management of irrigation becomes an important factor.

However, mangosteen is allegedly non-responsive to irrigation due to the uniqueness of its root morphology (Wiebel *et al.,* 1994). Indeed, irrigation was observed to be ineffective in decreasing the water fluctuation (Sdoodee and Chiarawipa, 2005). Besides, the root growth of mangosteen is both slow and seasonal and it grows faster before the appearance of new leaves, steadily decreases during the leaves development, and stops post-dormancy period (Hidayat, 2005). Thus, an efficient irrigation technique is needed to decrease water fluctuation. Partial root-zone irrigation (PRI), often referred to as partial root-zone drying (PRD), is a well-known irrigation method which alternately irrigates the root zone (Sepaskhah and Ahmadi, 2010). Adwirman (2006) has already applied the PRD technique on mangosteen but further studies are still needed on both physiological responses and nutrient status of the plant. In present study, the calcium status was also analyzed, either in the form of dissolved, pectate, phosphate and oxalate.

In fact, calcium is one of important mineral in mangosteen, especially in relation to yellow latex (Dorly *et al.,* 2011; Kurniadinata, 2015) and plays an important role in the mechanism of adaptation to stress condition (Liu *et al.,* 1998; Chen *et al.,* 2002), especially on signal transduction in the responses to water deficit (Hong-Bo *et al.,* 2008). Its status was analyzed in the present research to determine the correlation between the plant root water status and the occurrence of yellow latex, especially Ca-complex such as Ca-oxalate (Korth *et al.,* 2006; Setyaningrum, 2011), Ca-dissolved, phosphate, and pectate (Saure, 2005). Therefore, this study aims to: (1) determine the growth and morpho-physiological responses of mangosteen seedlings, (2) determine the role of calcium, and (3) evaluate the level of secondary metabolites in different water status conditions.

2. Materials and Methods

Orchard and plants

Two-years old mangosteen seedlings at averages height of 30±2.02 cm and leaves number, ranged from 17 to 20, were planted on *Pasir Kuda* experimental field (±260 m asl), Bogor, Indonesia between October 2016 and May 2017. A set of randomized block design, with 4 replicates, was used for field capacities of both conventional deficit irrigation (CD) and partial root-zone irrigation (PR) methods, comprised of 100% (control), 50% (CD1), 30% (CD2), 100% A: 50% B (PR1), 100% A: 30% B (PR2) and 50% A: 30% B (PR3). Mangosteen seedlings were planted in root-boxes (50 x 40 x 20 cm) in accordance with each treatment. One side of the root-box was made of glass and covered with a black thick cloth to observe the root growth, in a non-destructive way. The root-box was divided into two parts (Fig. 1) in the PR treatment and the partitions were layered by hydrophobic plastic material, in order to avoid water flow from one side to the other side. Both sides of the root-box were filled with soil and compost at a ratio of 1:1 (w/w). Mangosteen roots were carefully cleaned and divided into two symmetrical parts and planted on both side A and side B of the root-box, respectively. The planted mangosteen seedlings were acclimatized and watered within the field capacity conditions for four weeks. In the last week of the acclimatization period 100 g dolomite [$CaMg(CO_3)_2$; ±30% CaO] was applied in each root-box.

Fig. 1 - Root-box illustration in conventional deficit (A) and partial root-zone (B) irrigation treatment. In the PR treatment, the root was planted on 2 sides, side A and side B.

Field capacity determination

Soil water content (WC) and humidity (RH) were measured to quickly determine the field capacity (FC). Soil water content was determined by gravimetric method (Abdurachman *et al.,* 2006). A hundred grams of soil was weighed (FW) and heated at 105°C for 24 hours (DW) and the soil water content calculated with the following equation:

$$FC = (FW - DW) / DW \times 100\%$$

Soil RH was measured by means of a soil moisture meter (HT5213, China). Soil water content and RH were measured every 3 days for the optimization (Table 1). Water treatment was applied based on Table 1. The 100% field capacity occurred when the condition was 64.39% for the soil water content and 10 for the RH. The 50% and 30% field capacity treatments were obtained at a soil water content of 28.16% (day 13) and 20.06% (day 25), respectively. In order to simplify the water availability application, the watering syllabus was set for every 2 days, 2 weeks and 3 weeks for the 100%, 50% and 30% field capacity treatments. Soil moisture meter was installed on root-box to control the soil condition.

Measurements

Leaf water potential and relative water content. Healthy mature leave samples were taken at 7.00 A.M., placed into sealed plastic, and kept in a cooler box for further observation in laboratories. The leaves were cut in a cup and measured in a WP4 chamber and leaf water potential was measured using a WP4 *Dewpoint Potential Meter* (Decagon Devices Inc, USA). The relative water content (RWC) measurement of leaves was carried out in accordance with the leaf water potential. The samples used in the leaf water potential measurement (the fresh weight/FW measured) were soaked in the cup containing distilled water. The surface of the cup was covered with filtering paper so that the leaves do not float. Afterwards, the cup was kept in a cool storage for 24 hours, then drained and weighed to determine the turgid weight (TW). Leaves samples were dried at 70°C for 3 days and weighed to determine the dry weight (DW). The RWC was calculated by means of the following equation:

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

Photosynthesis and transpiration rates. Photosynthesis and transpiration rate were measured prior to treatment and 2 months post-treatment using LI-COR 6400 (LI-COR Inc, USA).

Calcium content. Calcium content in dissolved form was determined following the method developed by Suwwan and Poovaiah (1978), while calcium in complexed forms was measured gradually according to Chen and Uetomo (1976) procedure.

Malondialdehyde (MDA) level. Lipid peroxidation activity was determined from MDA content that was measured by mean of Wang *et al.* (2013) procedure. Briefly, 0.4 g leaves sample was homogenized with 10 ml TCA. Homogenate was centrifuged at 4°C for 10 min at 3000 *g*. Then, 2.5 ml supernatant was added to the reagent which is containing of 0.5% TBA and 20% TCA to be incubated at 80°C for 25 min. The absorbance was read at 440, 532 and 600 nm. MDA level was calculated with the following equation:

$$MDA = 6.45 (A532-A600) - (0.56 \times A440)$$

Proline content. Proline content was measured following the method developed by Bates (1973). In brief, 0.5 g leaves sample was homogenized with 3% sulfosalicylic acid. The homogenate was centrifuged at 12.000 *g* for 10 min. The supernatant was mixed with reagent which contains ninhydrin and glacial acetic acid. The mixture was incubated at 100°C for 60 min and transferred into an ice bath immediately. Afterwards, sample was extracted with 4 ml toluene and stirred in vortex. The absorbance was read at 520 nm. Proline concentration was calculated using proline standard curve.

Glutathione peroxidase (GPX) activity. GPX activity was analyzed following the method developed by Urbanek *et al.* (1991). In brief, leaf were extracted in phosphate buffer. The reaction mixture containing

Table 1 - Optimization of soil water content and humidity of mangosteen root zone during drought stress to determine the watering syllabus

Variables	Period of drought stress (days)								
	1	4	7	10	13	16	19	22	25
Water content (%)	**64.39 a**	43.06 b	44.24b	41.37 b	**28.16 c**	27.43 c	24.19 cd	21.12 d	**20.06 d**
Relative humidity (1-10)	**10.00 a**	8.68 b	8.41 bc	8.04 bc	**7.60 c**	7.60 c	5.71 d	5.71 d	**5.53 d**

Numbers, within the same row, followed by the same letters showed no significant differences based on DMRT at a probability level of 5%. Bold numbers indicating 100%, 50% and 30% field capacity, consecutively.

phosphate buffer (pH 7.0), EDTA, guaiacol, H_2O_2 and 50 l enzyme extract. The enzymatic reaction was initiated by addition of extract and the increase in absorbance recorded at 470 nm for 1 min. Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient (26.6 mM^{-1} cm^{-1}).

Secondary metabolites content. Secondary metabolites was analyzed in Jakarta Regional Health Laboratory using GCMS on fresh mature leaves from control and the most severe (PR3) treatment.

Plant growth and biomass. The length and volume of the root, the fresh and dry weight of plant were observed 2 months post treatments. Plants were cleaned prior to observation. The root variable observation, in PR treatment, was carried out by merging both side A and side B. The length of root was measured from the boundary between root and main stem. The volume of root was measured based on Archimedes principle. Plant was weighed to measure the fresh weight then heated at 80°C for 72 h to determine the dry weight.

Statistical analysis

Data were analyzed with F test and Duncan Multiple Range Test (DMRT) at a probability level of 5% using Statistical Analysis System 9.4 (SAS 9.4M4) software.

3. Results

The leaves in all treatments, except control, withered on three weeks post-treatment (Fig. 2). However, the plants became fresh again after rewatered, except in CD2 and PR3 treatments. Besides, abortion and drying leaves were also observed in mangosteen seedlings. Abortion of old leaves was more severe in PR2 and PR3 treatments. In fact, stressed mangosteen leaf has a unique dried pattern. The whole leaves of the stressed mangosteen seedling did not dry entirely, indeed, it dried step by step starting from both the edge and tip of the leaf blade (Fig. 2G).

The reduction in leaves number was observed since the first month of the treatment; the decrease was more severe during the second month, especially in CD2, PR2, and PR3 treatments (average 9.1, 8.7, 7.2 leaves per plant, respectively) compared to control which had 14.9 leaves (Table 2). PR1 treatment did not indicate any severe leaves abortion. Thus, there were no significant differences in terms of leaves number between control and PR1 treatments from the beginning until the end of the treatment period (Fig. 2 and Table 2).

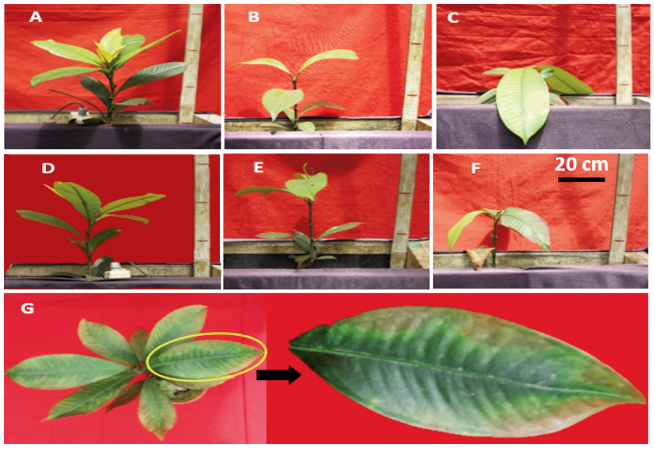

Fig. 2 - Mangosteen seedling canopy in different water availability treatments (2 months post treatment). A= control, B= conventional deficit irrigation, 50%, C =conventional deficit irrigation, 30%, D= partial root-zone irrigation 100% side A: 50% side B, E= partial root-zone irrigation 100% side A: 30% side B, F= partial root-zone irrigation 50% side A: 30% side B, G= dried pattern on mangosteen leaf).

Table 2 - Decreasing in mangosteen leaves number in different water availability treatments

Field capacity	Number of leaves		
	0 MAT	1 MAT	2 MAT
Control (100%)	18.2	16.6 a	14.9 a
CD1 (50%)	18.2	15.2 abc	10.8 b
CD2 (30%)	19.2	14.7 bc	9.1 c
PR1 (100%A:50%B)	19	16.0 ab	13.6 a
PR2 (100%A:30%B)	18.3	14.0 c	8.7 c
PR3 (50%A:30%B)	18.4	14.6 bc	7.2 d
F-test	NS	*	*

Numbers, in the same column, followed by the same letter indicates no significant differences based on DMRT at a probability level of 5%. MAT= month after treatment.

Mangosteen leaf water potential ranged from -2.95 to -3.59 MPa before the treatments (Table 3). In present research, the leaf water potential above -3.59 MPa in two months after treatment was classified as a stress condition for seedlings, coincide with the leaf morphological characteristics which showed withered condition (Fig. 2). Leaf water content decreased post-drought treatment in both CD and PR conditions (Table 3). The lowest water potential was observed in PR3 treatment (-4.72 MPa) which had a field capacity of 50% on side A and 30% on side B. On the other hand, leaf water content also decreased in

all treatment compared to control, although the decreasing was not significant (α 5%), except for PR3 treatment which had the lowest leaf water content (Table 3).

Table 3 - Water potential and content of mangosteen seedling leaf in different water availability treatments at the beginning (0 months) and 2 months post-treatment

Treatment (FC)	Leaf water potential (Mpa)		Leaf water content (%)	
	0 MAT	2 MAT	0 MAT	2 MAT
Control (100%)	-2.95	-3.09 a	81.71	97.57 a
CD1 (50%)	-2.96	-4.00 b	77.5	71.62 ab
CD2 (30%)	-3.00	-3.99 b	73.93	53.55 ab
PR1 (100%A:50%B)	-3.59	-4.12 b	69.51	56.89 ab
PR2 (100%A:30%B)	-3.37	-4.51 b	68.08	58.81 ab
PR3 (50%A:30%B)	-3.53	-4.72 b	68.04	31.68 b
F-test	NS	*	NS	*

Numbers, in the same column, followed by the same letter indicates no significant differences based on DMRT at a probability level of 5%. MAT= month after treatment.

Drought treatment decreased both photosynthesis and transpiration rates of mangosteen seedlings two months after treatment (Table 4). PR treatments showed the lowest photosynthesis and transpiration rate and, among PR treatments, PR3 recorded the worst performances in terms of photosynthesis and transpiration rate (6.06 µmol CO_2 m^{-2} s^{-1} and 0.02 Mmol H_2O m^{-2} s^{-1}, respectively).

The dry weights of canopies and roots were significantly lower in CD and PR treated plants than control (Table 5). Unexpectedly, PR1 treatment tended to have the lowest decrement in dry weights of the canopy, roots and total plant. On the other hand, the highest decrements were observed in CD2 and PR2 treatments on both canopy and total plant dry weight, although statistically similiar. Control and PR1 treatments had the longest root apparatus (Fig. 3), although they did not differ significantly (Table 6). Drought stress significantly restricted the root growth in CD1, CD2 and PR3, excepted PR1 and PR2 treatments. Meanwhile the root volume did not show any differences among treatments.

MDA content in CD1, CD2 and PR1 treatments did not differ significantly to that of the control treatment (Fig. 4). PR2 and PR3 treatments had the high-

Table 4 - Photosynthesis and transpiration rates of mangosteen seedling in different water availability treatments at the beginning (0 months) and 2 months post-treatment

Treatment	Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})		Transpiration rate (Mmol H_2O m^{-2} s^{-1})	
	0 MAT	2 MAT	0 MAT	2 MAT
Control (100%)	16.23	16.20 a	0.52	0.21 a
CD1 (50%)	15.49	11.76 ab	0.49	0.11 ab
CD2 (30%)	18.27	12.05 b	0.56	0.05 ab
PR1 (100%A:50%B)	15.51	8.35 cd	0.62	0.07 ab
PR2 (100%A:30%B)	15.56	10.42 bc	0.55	0.05 ab
PR3 (50%A:30%B)	15.33	6.06 d	0.61	0.02 b
F-test	NS	*	NS	*

Numbers, in the same column, followed by the same letter indicates no significant differences based on DMRT at a probability level of 5%. MAT= month after treatment.

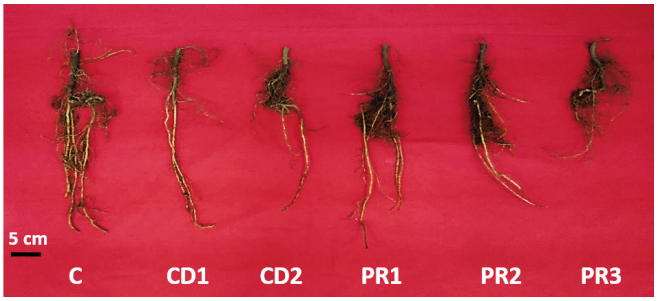

Fig. 3 - The root system of mangosteen seedling in different water availability treatments (2 months post treatment). C= control), CD1= conventional deficit irrigation 50%, CD2= conventional deficit irrigation 30%, PR1= partial root-zone irrigation 100% side A:50% side B, PR2= partial root-zone irrigation 100% side A:30% side B, PR3= partial root-zone irrigation 50% side A:30% side B.

Table 5 - Dry weight of mangosteen seedling canopy, root and total plant in different water availability treatments at 2 months post-treatment

Treatment	Dry weight (g)			Relative decrease to control (%)		
	Canopy	Root	Total	Canopy dry weight	Root dry weight	Total dry weight
Control (100%)	12.84 a	5.15 a	16.43 a	-	-	-
CD1 (50%)	9.20 b	3.08 b	12.28 b	28.35± 0.15	40.19±1.02	39.80±0.11
CD2 (30%)	7.44 b	3.33 b	10.77 b	42.06±0.11	35.34±1.00	47.21±0.10
PR1 (100%A:50%B)	9.65 b	4.83 ab	13.98 ab	24.84±0.11	6.21±0.73	31.47±0.13
PR2 (100%A:30%B)	6.99 b	3.87 ab	10.49 b	45.56±0.07	24.85±1.14	48.58±0.01
PR3 (50%A:30%B)	8.30 b	3.49 b	12.17 b	35.36±0.08	32.23±2.05	40.34±0.03
F-test	*	*	*	-	-	-

Numbers, in the same column, followed by the same letter indicates no significant differences based on DMRT at a probability level of 5%.

est MDA content, being 1.033 and 1.501 µmol/ml respectively, which were significantly different to that of the control treatment. Proline accumulation was in accordance with that of MDA content with the highest value in PR3 treatment followed by CD1 and CD2 (Fig. 4). Proline content in PR1 and PR2 treatments did not show any significant different to that of the control treatment. Glutathione peroxidase (GPX) activity showed different result compared to proline and MDA. In fact, the control treatment had the highest GPX activity showed not significantly differences among treatments, except PR3 treatment (Fig. 4), as a result of severe stress.

Table 6 - Root length and volume of mangosteen seedling in different water availability treatments at 2 months post-treatment

Treatment	Root length (cm)	Root volume (ml)
Control (100%)	35.00 ab	11.67
CD1 (50%)	32.70 bc	8.33
CD2 (30%)	29.45 cd	6.33
PR1 (100%A:50%B)	37.75 ab	10.33
PR2 (100%A:30%B)	33.30 abc	7.67
PR3 (50%A:30%B)	28.00 d	7.5
F-test	*	NS

Numbers, in the same column, followed by the same letter indicates no significant differences based on DMRT at probability level of 5%.

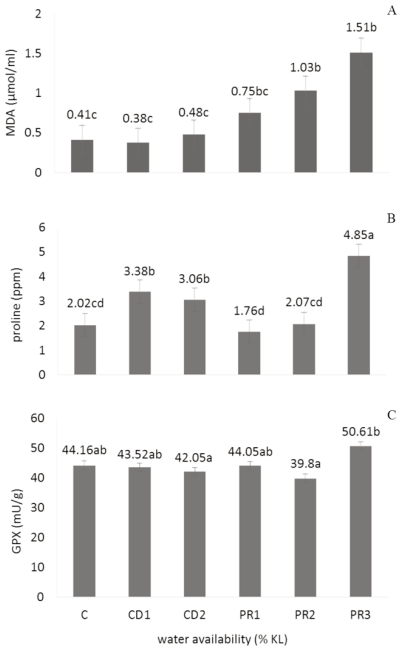

Fig. 4 - MDA (A), proline (B) content and GPX activity (C) of mangosteen seedling in different water availability treatments (2 months post-treatment). Numbers followed by the same letter indicates no significant differences based on DMRT at a probability level of 5%.

Mangosteen seedling, on limited water condition, produced more diverse secondary metabolites than the control, especially terpenoid and fatty acid (Table 7). Five secondary metabolites were found in the leaves of control plant, i.e. oleic and hexadecenoic acid (from the fatty acid group), squalene, vitamin E and neophytadiene (from terpenoid group). Vitamin E and squalene content increased in stressed mangosteen seedling by 28% and 62% respectively, while oleic acid content decreased by 15%. On the other hand, hexadecenoic acid and neophytadiene were not detected in stress plant. There were 2 kinds of unknown compounds produced in severe stress, i.e. 1-nonadecene and 2-butyn-1-ol 4 metoxy. Dissolved Ca was mostly found in mature leaves, young leaves, roots, and branch, while Ca-pectate and Ca-phosphate were mostly found in mature leaves, roots, young leaves and branch (Table 8). Table 8 shows that both dissolved and pectate calcium contents were high in severely stressed plants, especially in young and mature leaves. Furthermore, the leaves calcium content in dissolved and pectate form of CD1 treatment were significantly higher than that of control. In CD2 treatment, calcium, in dissolved and pectate form, was tended to be lower compared to CD1 treatment but not significantly different from the control. A similar result was observed in dissolved calcium content of mangosteen seedling roots, while no differences in calcium content in branch, was observed. Table 8 also shows that Ca-phosphate and Ca-oxalate in branch were significantly higher in all PR treatments compared to control, while there were no differences in Ca-oxalate content in leaves and roots.

Table 7 - Secondary metabolites content in control and PR3 treatments of mangosteen seedling 2 months post-treatment

Secondary metabolite compound	Group of compounds	The existence	
		Control	PR3
Oleic acid	Unsaturated fatty acid	++	++
Iliadic acid	Unsaturated fatty acid	-	+
Hexadecenoic acid	Saturated fatty acid	+	-
Squalene	Terpenoid	+	+
Vitamin E	Terpenoid	+	+
Neophytadiene	Terpenoid	+	-
Octacaine	Acyclic hydrocarbon	-	+
Cysteaminesulfonic acid	Amino acid	-	+
Propyl oleate	Ester fatty acid	-	+
1-nonadecene	Unknown	-	+
2-butyn-1-ol, 4 methoxy	Unknown	-	+

- not detected, + peak area below 20%, ++ peak area between 20-50%, PR3= partial root-zone irrigation 50% side A:30% side B.

Table 8 - Calcium content of mangosteen seedling in different water availability treatments at 2 months post-treatment

Treatment	Calcium (%)				
	Dissolved	Pectate	Phosphate	Oxalate	Total
Young leaves					
Control (100%)	0.133 c	0.134 c	0.084 c	0.405	0.756 c
CD (50%)	0.396 bc	0.247 bc	0.096 bc	0.547	1.287 bc
CD (30%)	0.349 c	0.227 bc	0.091 c	0.428	1.096 bc
PR1 (100%A:50%B)	0.473 bc	0.290 abc	0.135 ab	0.668	1.568 ab
PR2 (100%A:30%B)	0.826 ab	0.429 ab	0.135 ab	0.372	1.763 ab
PR3 (50%A:30%B)	1.065 a	0.488 a	0.157 a	0.425	2.136 a
Mature leaves					
Control (100%)	0.322 b	0.267 b	0.124 b	0.611	1.326 b
CD (50%)	0.741 a	0.462 a	0.319 a	0.814	2.337 a
CD (30%)	0.600 ab	0.362 ab	0.187 ab	0.501	1.651 ab
PR1 (100%A:50%B)	0.690 a	0.428 a	0.185 ab	0.743	2.047 ab
PR2 (100%A:30%B)	0.814 a	0.346 ab	0.209 ab	0.68	2.051 ab
PR3 (50%A:30%B)	0.763 a	0.441 a	0.174 ab	0.473	1.851 ab
Branch					
Control (100%)	0.078	0.171	0.086 b	0.763 bc	1.099 bc
CD (50%)	0.134	0.198	0.107 ab	0.923 abc	1.364 abc
CD (30%)	0.144	0.159	0.104 ab	0.555 c	0.963 c
PR1 (100%A:50%B)	0.156	0.247	0.127 ab	1.266 a	1.798 a
PR2 (100%A:30%B)	0.142	0.236	0.139 a	1.108 ab	1.627 ab
PR3 (50%A:30%B)	0.148	0.227	0.123 ab	1.335 a	1.835 a
Root					
Control (100%)	0.202 b	0.373	0.148	0.675	1.399
CD (50%)	0.342 a	0.307	0.219	0.971	1.84
CD (30%)	0.231 ab	0.317	0.192	0.862	1.604
PR1 (100%A:50%B)	0.185 b	0.232	0.167	1.443	2.052
PR2 (100%A:30%B)	0.208 b	0.247	0.17	1.296	1.904
PR3 (50%A:30%B)	0.273 ab	0.287	0.198	1.316	2.075

Numbers, in the same column, followed by the same letter indicates no significant differences based on DMRT at a probability level of 5%.

4. Discussion and Conclusions

In the present study, mangosteen leaves changes its morphology and aborts as a consequence of drought stress in different water treatments. Leaves abortion was more marked in CD2, PR2 and PR3 treatments, a common symptom of plants under drought stress (Munne-Bosch and Alegre, 2004). It was expected that the leaves of CD2, PR2 and PR3 treatments would accumulate more ABA and trigger the abscission process (Wingler and Roitsch, 2008; Peleg and Blumewald, 2011). On the other hand, leaves abortion in control and PR1 treatments were not significantly different from the beginning until the end of the treatment period, indicating that PR1 were not severely water stressed. Morphological changes and leaves abortion suggest that mangosteen seedlings were less tolerant to drought stress. However, mangosteen showed a low response to drought stress since the withered leaves occurred on 3 weeks post-treatment. The low response in mangosteen was expected to be a consequence of mangosteen seedling low growth as stated by Ramlan *et al.* (1992).

An high relation between leaf morphology and changes in water potential due to the variations in terms of water treatments was observed. It is expected to be the mechanism of mangosteen adjustment by decreasing water potential in tissues in order to absorb the water in the soil. Zimmerman (1978) stated that turgor potential is partially or fully maintained by osmoregulation during water stress by a reduction in the outflow of water from the cell. In previous study, the decrement of the leaf water

potential and relative water content were occurred in stressed wheat (Siddique *et al*., 2001) and *Hibiscus rosa-sinensis* (Egilla *et al.*, 2005).

The decreasing of photosynthesis and transpiration rate in stress mangosteen seedlings indicated that both moderate (50% FC) and severe (30% FC) stress conditions greatly affect mangosteen gas exchange. Purwanto and Agustono (2010) reported that photosynthesis rate in soybean, which was watered at a condition of 60% FC, decreased by 50%, while no significant fall in transpiration rate was noticed. As response to drought stress, stomata respond by reducing aperture, thereby restricting water loss, however, an inevitable consequence is the photosynthesis and canopy transpiration (Loveys *et al.*, 1999) reduction.

As mentioned in Table 6, control and PR1 treatments showed the longest roots compared to the other treatments, while the root volume did not show any differences among treatments. Hidayat (2005) reported in his research that mangosteen root has a seasonal growth where roots alternately grow with shoots. It expected lead to the slow response of mangosteen roots to drought stress. On the other hand, no significant differences were noticed in PR1 treatment in terms of root dry weight compared to control (Table 5). This was in agreement with Liu *et al.* (2006) which reported that PRD increased biomass allocation to roots. Promoting root growth under PRD has been reported in grapevine (Dry *et al.,* 2000), therefore this has been considered as an advantage of PRD irrigation.

Drought stress condition lead to high production of MDA which used as a stress indicator in the plant. High MDA content indicated that the lipid peroxidation rate, as the main effect of oxidative damage (Gill and Tuteja, 2010), was also high. Sofo *et al.* (2005) conclude that there is a direct correlation between MDA and drought stress, particularly at severe degrees of stress. In present research, the most severe case was observed in PR3 treatment which had highly MDA level that coincided with decrements in leaf water potential, photosynthesis and transpiration rates. Besides mangosteen, lipid peroxidation was also noticed in cucumber (Kubis *et al*., 2014), bean (Svetleva *et al*., 2012) and maize (Ti-da *et al*., 2006) within a stress condition.

The accumulation of proline is a common response in plants to abiotic stress. Increasing proline is a plant response to adjust its osmotic potential (Slama *et al.,* 2006) which has a strong relation with plant water potential. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). Present research was in agreement with Omidi (2010) which stated that in canola plants, proline content increased twofold as a result of drought stress treatment.

In the present study, the control treatment had the high GPX activity but did not show significantly differences among treatments, except PR3. It indicates that in normal condition, mangosteen seedlings have the high antioxidant activity, then became higher when the stress condition occurs, such as PR3. Halušková *et al.* (2009) stated that different abiotic stresses may cause differences in the GPX activation. Miller *et al.* (2010) noticed that GPX is plant protector against free radical. Previous studies by Sofo *et al.* (2005) and Aganchich *et al.* (2007) have reported up-regulation of the antioxidant defense system in young olive plants subjected to different degrees of water stress.

Mangosteen seedlings in limited water media produced secondary metabolites more than control, especially terpenoid and fatty acid groups. Varied antioxidant profile in different plant species is one of the principle reasons for the different adaptability and abiotic stress tolerance in plants (Jamali *et al*., 2016). Changes in composition and synthesis in drought stress condition were reported for group of terpenoid such as vitamin E (Gershenzon *et al.,* 1978), an important antioxidant that protects the cell from free radical effects (Serbinova and Packer, 1994) and photosynthetic apparatus (Fryer, 1992) from oxidative damages. In the present research, the increasing in vitamin E content by 3.2%, in stress mangosteen seedlings, was a response to the stress condition. Abiotic stress-induced changes in the fatty acid composition of plant membrane lipids mainly occur through the regulated activities of fatty acid desaturases (Upchurch, 2008). *Arabidopsis thaliana* shows remarkable tolerance to drought stress and has capacities to maintain polar lipid content and stable lipid composition, and increase the fatty acid unsaturation (Gigon *et al*., 2004).

Mangosteen seedlings under PR1 treatment had the highest calcium level. According to Bell and Biddulph (1963) some plants absorb calcium based on their physiological demand which is sometimes not comparable to the transpiration rate. As men-

tioned in Table 8, calcium contents were higher in drought stress plants in treatments of CD1, PR1, PR2 and PR3. Allegedly, there was a relationship between the response of mangosteen seedlings and both long watering interval time and calcium metabolism. CD2 treatment had a longer watering interval time compared to CD1 so that when the drought signaling occurred the water was not available, leading to a fail in calcium absorption. The increment in calcium levels in CD1, PR1, PR2 and PR3 treatments occurred in the forms of Ca-pectate and Ca-dissolved, both of which play a role in cell wall component (Peaucelle *et al.*, 2012) and cytoplasmic transduction signaling (Klimecka and Muszyńska, 2007), respectively. This was in agreement with Jin *et al.* (2016) which reported that increment in calcium levels in a salinity stress condition occurred in *Ziziphus jujuba* species. Mangosteen seedlings in PR1 treatment had higher water use efficiency (WUE) leading the possibility of calcium absorption in stress condition. In PR treatment, improvement in WUE was a results from partial stomatal closure. However, an inevitable consequence is the photosynthesis and canopy transpiration reduction (McCarthy *et al*., 2002). Hu *et al.* (2008) showed that PR method in maize able to preserve 29.5-33% water and increased WUE. The use of partial root-zone or deficit irrigation in grapevine (*Vitis vinifera*) increased WUE by about 40% while only decreasing yield by 15% when compared with full irrigation (Dos Santos *et al.,* 2003).

Mangosteen seedlings had a low response and different mechanisms in facing drought, by increasing MDA, proline, Ca-pectate and Ca-dissolved, GPX activity and synthetizing various secondary metabolites in mature leaves. In the present study, there were no significant differences between control and PR1 treatments in leaf morphology, proline, MDA and GPX activity, indicating that PR1 treatments were not severely water stressed. Therefore, present research concluded that irrigation of mangosteen through PR method was a promising method for implementation, especially in limited water areas, when the soil moisture level represents 50% (or more) of the field capacity. The implementation of PR method can reduce water requirement without significantly affecting the plant growth. It can reduce both time and labor requirements. However, improvements in the technical application to develop an effective procedure for field implementation and its relation to flowering and yellow latex of mangosteen, are still needed as further studies.

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