



Growth and Betacyanin Content of Beetroots (*Beta vulgaris* L.) Under Water Deficit in A Tropical Condition

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ABSTRACT

The present study, a part of a series of studies carried out to explore the possibility of beetroot cultivation in the tropics on highlands, was designed to investigate the growth and betacyanin content of beetroot plants in response to water deficit in a tropical condition. The water deficit was executed through varied watering intervals consisting of once three (as control), four, five, six and seven days. A randomized block design with five replicates was used to impose the treatments. The effect of water deficit was found to reduce the leaf water content, the biomass production (total dry weight) and the root yields of beetroot plants. Root dry weight decreased by more than 20% and 50% with the watering interval once four and seven days respectively compared with that once three days. The betacyanin content of roots was not influenced by the water deficit when expressed per unit volume of fresh root extracts (340.5 ± 60.1 mg/l), and increased when expressed per unit root dry weight. It is suggested that an optimal supply of water, for instance watering once three days, is necessary to obtain beetroot plants growing optimally and producing a high root yield and betacyanin content of roots.

INTRODUCTION

Beetroot (*Beta vulgaris* L.), widely cultivated in European countries for hundred years (Nottingham, 2004), produces edible taproots that contain a high antioxidant betalains and inorganic nitrate. In recent years, natural products that contain compounds with high antioxidant activities such as betalains from beetroots received considerable attention. This is related to increasing awareness and knowledge of healthy foods and life in addition to a better understanding of antioxidants and various disease relations. It is well known that the oxidation of molecules may result in free radicals leading to chain reactions ended in molecule and cell damage. The defect of molecules and cells caused by reactive oxygen species (ROS) is considered widely as the main cause of aging and several diseases (Halliwell, 1997). The oxidation may also occur with reactive nitrogen species (RNS) (Clifford, Howatson, West, & Stevenson, 2015).

Betacyanins and betaxanthins are betalain pigment that contain nitrogen with colors ranging from red-violet (betacyanin) to yellow or yellow-orange (betaxanthins) (Ravichandran et al., 2013; Tanaka, Sasaki, & Ohmiya, 2008). Betanin is the most ample compound of betalains in beetroots, and belongs to the group of betacyanins (Pavokovic & Krsnik-Rasol, 2011). In a previous study using a modified DPPH* (1,1-diphenyl-2-picrylhydrazyl) method, it was found that the antioxidant activity of betacyanins and betaxanthins from *Amaranthaceae*, based on EC_{50} , reached > 3-4 fold higher than that of ascorbic acid (Cai, Sun, & Corke, 2003). This study also showed that antioxidant activity of betacyanins and betaxanthins was higher than that of typical natural antioxidants (polyphenols rutin and catechin). Other report showed that antioxidant activity was higher in dried beetroots than in boiled and pickled beetroots, and the lowest in beetroot juice (Guldiken et al., 2016). This is independent of methods used encompassing DPPH, ABTS ((2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic

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acid) diammonium salt), FRAP (Ferric Reducing Antioxidant Power) and CUPRAC (Cupric Ion Reducing Antioxidant Capacity).

The cultivation of beetroots as an excellent source of antioxidants has attracted much attention in Indonesia in the last few years. Results of a previous study, conducted at the same time and site as the present study (elevation $\geq 1,700$ m asl), suggests that beetroots with reasonable root yields and betacyanin content can be cultivated in the tropics on areas of high altitudes with sufficiently low temperature (Sitompul & Zulfati, 2019). In these areas, however, the cultivation of beetroots is hampered by water shortage due to the limited water resource which is generally dependent upon rainfall. In such conditions, plants may suffer from water stress particularly during mid-days in the tropics let alone in El Nino years with drought increasingly intense in later decades due to climate change.

The effect of water deficit on the growth of beetroots including roots (storage roots) would be implemented mainly through primary metabolism resulting from reduced carbohydrate production due to a decrease in the rate of photosynthesis as a consequence of decreasing stomatal conductance. Gago *et al.* (2016) showed a significantly positive correlation ($P < 0.001$) between stomatal conductance and net photosynthetic rate based on pooling data of eight species. In beetroots, watering at the level of 50% and 30% WHC (water holding capacity) reduced, on average, stomatal conductance by $> 50\%$ and almost 60% respectively (Stagnari, Galieni, Specca, & Pisante, 2014). A decrease in betacyanin content may occur under water deficit due to a reduction in nitrogen uptake following a decrease in water absorption and a reduction in the supply of carbohydrate to roots. This would limit the synthesis of tyrosine, the precursor of betacyanin (Chung *et al.*, 2015; Nakatsuka *et al.*, 2013; Polturak *et al.*, 2016), and a source of carbon skeleton and metabolic energy in the biosynthesis of betacyanin. The activity of tyrosinase, an essential enzyme in the biosynthesis of betacyanin, was significantly reduced in all tissues of the two genotypes of *Amaranthus hypochondriacus* under water stress resulting from withholding irrigation for 18 or 23 days except in roots of one genotype (Casique-Arroyo, Martínez-Gallardo, de la Vara, & Délano-Frier, 2014).

Other possibility is that the concentration of betacyanin may be maintained or even increased under water deficit for osmotic adjustment and protection of molecules and plant cells from oxidation. In *Amaranthus hypochondriacus*, a significant increase in betacyanin content was reported in leaves, stems and roots of two betacyanic genotypes (Casique-Arroyo, Martínez-Gallardo, de la Vara, & Délano-Frier, 2014). Lakshmi Sahitya, Krishna, Sri Deepthi, Shiva Prasad, & Peda Kasim (2018) reported a strong correlation, based on principal component analysis, between seed antioxidants (e.g. phenolics) and water stress tolerant indices (DPPH, total phenolics, proline, relative water content of leaves, chlorophyll contents, superoxide dismutase, and catalase activities) in seedlings of 20 Chilli (*Capsicum annum* L) genotypes. However, an increase in the betacyanin content of roots may also result from a decrease in the size of roots with a reduction in the supply of carbohydrate.

It is therefore of great importance to study the growth and betacyanin content of beetroots in response to water deficit on areas of high altitudes in the tropics. The main objective was to appraise the adaptability of beetroots to limited water supply in an effort to increase water use efficiency in the cultivation of this crop in the tropics. The limited water supply was approached in the present study through increasing watering interval to imitate water supply from rainfall in natural conditions. Such a study so far has never been done particularly in the tropics.

MATERIALS AND METHODS

Study Sites

The present study, a part of a series of studies in 2016-2017, was carried out in a greenhouse at the Agrotechnopark of Universitas Brawijaya in Cangar ($7^{\circ}44'$ S & $112^{\circ}32'$ E) during dry season (March - June) in 2017. Cangar is located around 1,700 m asl with daily minimum and maximum temperature of about 10°C and 26°C respectively. During the day, the average air temperature at Cangar measured at 06.00 AM, 13.00 PM, and 18.00 PM for five successive days in February and May was 20°C corresponding with the results of previous study (Sitompul, Sitawati, & Sugito, 2013).

Sowing and Treatments

Seeds of beetroots (cv. Ayumi 04), purchased from a local agricultural shop, and were grown in plastic bags (20 cm in diameter) and 30 cm in height on 3 March 2017. In each pot (plastic bag), filled with top soil (Andisol, sandy clay loam, pH 6.4) mixed with compost (3:1), five seeds were sown to obtain more uniform seedlings and a healthy plant per pot selected on day 20 after sowing. Each pot was supplied with 0.54 g P₂O₅ (SP36) at sowing, and 1.47 g N/pot (Urea) and 0.96 K₂O (KCl) on day 30 after sowing. The treatment of watering intervals consisted of watering once in 3 days (as control), 4, 5, 6, and 7 days, at around 09.00 AM. A randomized block design with five replicates was used to arrange the imposition of treatments. Plants were freed from weeds controlled manually, and a fungicide (Benlate 0.2%) was used as necessary to control fungi (*Peronospora schachtii*).

Plant Growth

Plant growth in leaf area and total plant dry weight was observed with destructive samples on day 14, 28, 42, 56, 70, and 84 (harvest) after leaf number was recorded. Sample plants were cleaned from adherent soil as necessary and each plant was placed in a plastic bag to prevent water loss during the period between sampling and measurement. Fresh leaves were weighed to obtain fresh weight (FW) followed by the measurement of leaf area with leaf area meter (Licor LI 3100). The fresh weight of roots (taproots) and stems were also recorded. Sub-samples from the representative part of leaves, roots and stems were dried in an oven at 80°C for 48 h for the estimation of dry weight of roots, leaves and stems. RWC (relative water content) of leaves, observed on day 16 and 44 after the imposition of watering treatments, was calculated with the following equation:

$$RWC = \frac{FW - DW}{TW - DW} \dots\dots\dots 1)$$

where: FW = fresh weight; DW = dry weight; TW = turgid weight. The turgid weight of leaves was the weight of leaf samples after submersion in distilled water.

Betacyanin Determination

Betacyanin content of roots was determined by following the method developed by Stintzing, Schieber, & Carle (2003). All chemicals (Merck) used in the betacyanin analysis, purchased from a local chemical shop, were of analytical grade. The root of each treatment and replicate was cleaned, and sliced from the middle part to obtain 1 g sample (fresh weight). Each sample was ground with distilled water then filtered (Whatman 42), and the extract was diluted with deionized water. The absorbance of the diluted samples was measured with a mini UV/VIS spectrophotometer (Shimadzu-1240) at 536 nm which was corrected for impurities including browning substances with the absorbance at 600 nm as in a previous study (Sitompul & Zulfati, 2019). The concentrations of betacyanins (BC in mg/l) were calculated with the following equation:

$$BC = (A \times DF \times MW \times 1000) / (\epsilon \times i) \dots\dots\dots 2)$$

where: A = A_{536nm} - A_{600nm}; DF = dilution factor (100 in general); MW (molecular weight) = 550 g/mol; ε = 60,000 (molar extinction coefficient in L x mol⁻¹ x cm⁻¹ in H₂O); and i = path length (cm).

Statistical Analysis

Statistical data analyses including analysis of variance (Anova) were executed in excel manually or with Data Analysis Tool at the level of significance of P ≤ 0.05. Comparisons of mean values between treatments were analyzed with least significant difference (LSD) at P ≤ 0.05.

RESULTS AND DISCUSSION

Relative Water Content

Relative water content (RWC) of leaves, as an indicator of water stress level encountered by plants, was not significantly affected by the treatment of watering intervals on day 16 after the imposition of the watering treatments. A significant decrease in the RWC of leaves was observed on day 44 after the imposition of the watering treatments from 97% to 60% in plants watered once three and seven days respectively. A linear regression was found highly significant (P < 0.01) to describe the decrease of leaf RWC observed on day 44 with the increase of watering intervals (Fig. 1).

An attempt was made to estimate leaf water potential as a function of leaf RWC based on Höfler diagram (Slatyer, 1969) to envisage the level of water stress ensued in plants. The estimated water potential of leaves varied from -0.1 MPa to -2.3 MPa suggesting that most plants have experienced water stress after 16 days of the treatment imposition (Fig. 2). Leaf water potential at wilting point is commonly considered at -1.5 MPa (de Oliveira, Ramos, & de

Aquino, 2015; Slatyer, 1969), but was reported to vary between tree species and growth stages in a recent study (Maréchaux, Bartlett, Gaucher, Sack, & Chave, 2016). In potato grown in pots in a growth chamber, leaf RWC of plants after three weeks varied considerably between cultivars in the range of 64.4-86.7% (Soltys-Kalina, Plich, Strzelczyk-Żyta, Śliwka, & Marczewski, 2016).

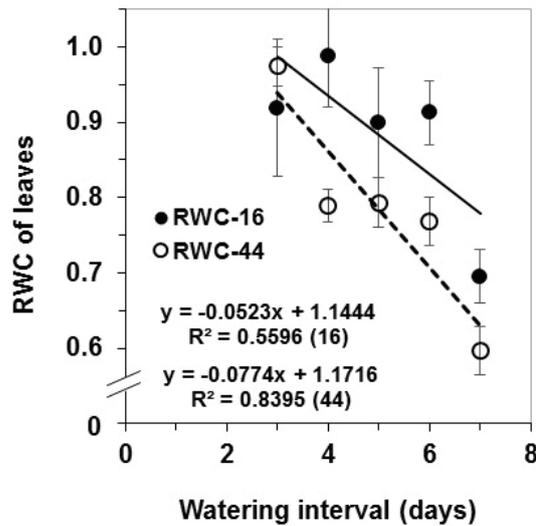


Fig. 1. Relative water content (RWC) of leaves as a function of watering interval. The RWC of leaves was observed on day 16 and 44 after the imposition of watering treatments as indicated by the legend in the figure. Each point represents the mean of five replicates, and vertical bars represent the standard error of the mean

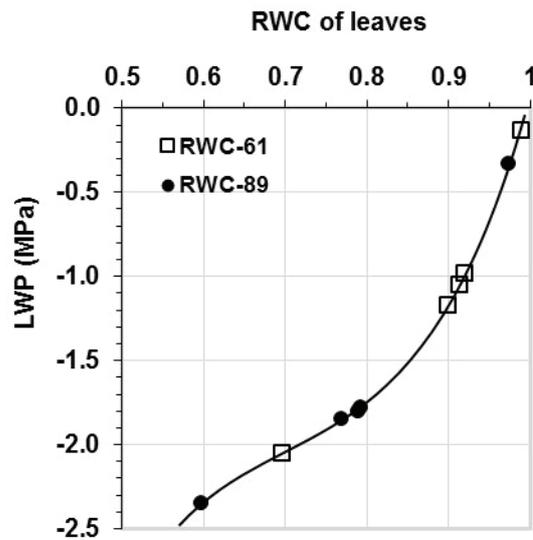


Fig. 2. The estimated leaf water potential (LWP) as a function of RWC of leaves. The graph is a hand-made graph based on Höfler digram (Slatyer, 1969)

Leaf Number and Area

Leaf number and leaf area per plant, not significantly different between plants before the imposition of the watering treatments, were not significantly affected by the treatment of watering intervals. On average, the number of leaves increased rapidly since the initial growth from 2.7 ± 0.98 (sd) on day 14 up to 10.3 ± 1.34 on day 76 after sowing (Fig. 3). This number of leaves corresponds with that observed in Serra Talhada-PE, Brazil ($7^{\circ}57'15''$ S, $38^{\circ}17'41''$ W, and 461 m asl.) grown in the fall and spring-summer and harvested

at 85 and 80 days after sowing respectively (dos Santos *et al.*, 2017). Leaf area per plant, showing a different growth pattern, increased slowly during the first two weeks, rapidly thereafter from 9.8 ± 3.54 cm² on day 14 up to 539.9 ± 114.48 cm² on day 56 (Fig. 4). Both leaf number and area were relatively constant or decreased slightly during the last two weeks of growth. A good relationship ($P < 0.01$) was found between leaf area and leaf number independent of growing stages (Fig. 5). The pattern of leaf number and leaf area development with time can be described by a modified beta model.

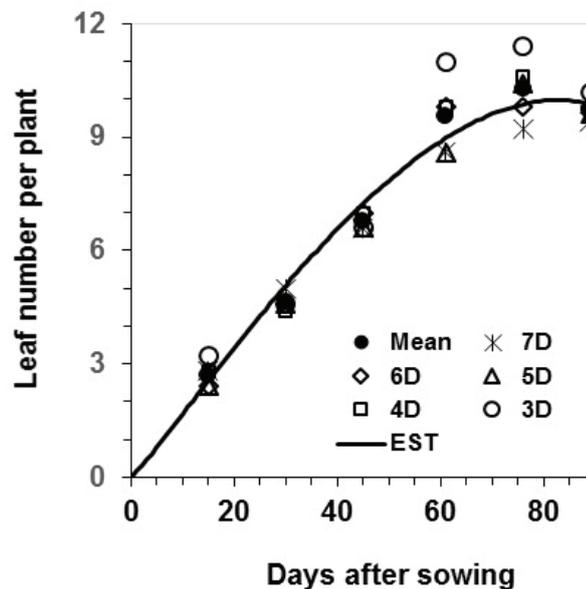


Fig. 3. Development with time in leaf number of plants. Each point represents the mean of five replicates. 3D-7D is the watering interval once 3-7 days, respectively. Mean is the average of treatments, and EST is the graph generated by a modified beta model: $LN = \exp(\mu)(t-t_0)^\alpha(t_m-t)^\beta$ where LN = leaf number, t = time (days after sowing), t_0 = time at $t = 0$, t_m = time at LN = maximum, and μ , α & β are model parameters (Yin, Kropff, McLaren, & Visperas, 1995)

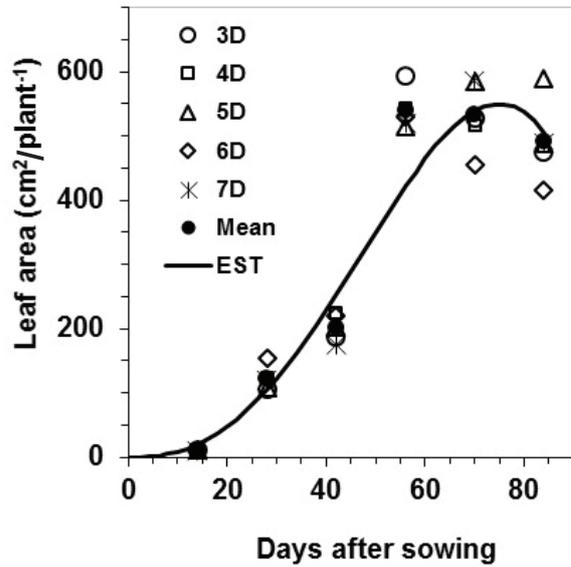


Fig. 4. Development with time in leaf area of plants. Mean is the average of treatments, EST is the graph generated by a modified beta model: $LA = L_{max}[1 + (t_e - t)/(t_e - t_m)](t/t_e)[t_e/(t_e - t_m)]$ where LA = leaf area; L_{max} = maximum leaf area, t = time (days after sowing), $t_e = t$ at L_{max} , and $t_m = t$ at the mid between $t = 0$ and t_m (Yin, Goudriaan, Lantinga, Vos, & Spiertz, 2003). Each point, other than Mean, represents the mean of five replicates.

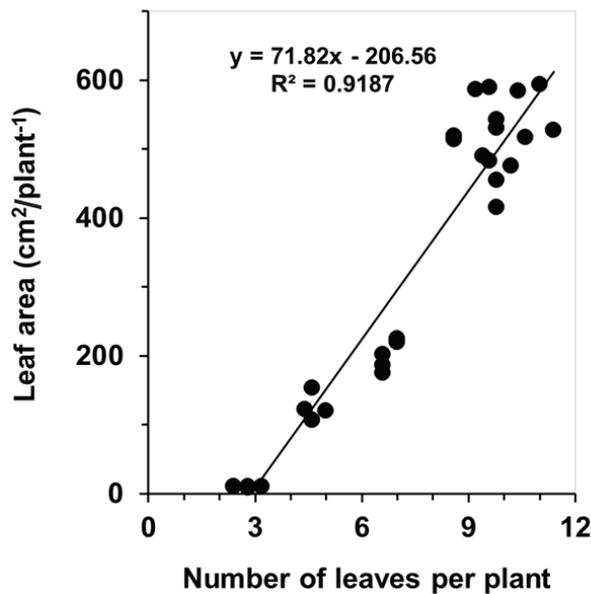


Fig. 5. Relationship between leaf area and leaf number for all growing stages. Each point represents the mean of five replicates

Total and Root Weight

The accumulation of total dry weight with time increased slowly during the first two weeks, and rapidly thereafter from, on average, 1.71 g per plant up to 7.51-10.6 g per plant at the end of growth observation (Fig. 6). Before the imposition of treatments, the total dry weight (TDW) showed no significant difference between plants intended to different watering intervals. The effect of watering intervals on TDW was not significant on day 56 and 70 which was 11 and 25 days after the imposition of

treatments respectively. At harvest on day 84 or 38 days after the imposition of treatments, TDW was significantly different between treatments (Table 1). A reduction in watering from once three to four days had no significant effect on TDW which was significantly reduced only after watering interval increased to once five days or more. There was, however, a close relationship ($P < 0.01$) between watering intervals and TDW observed on day 70 and 84 (Fig. 7).

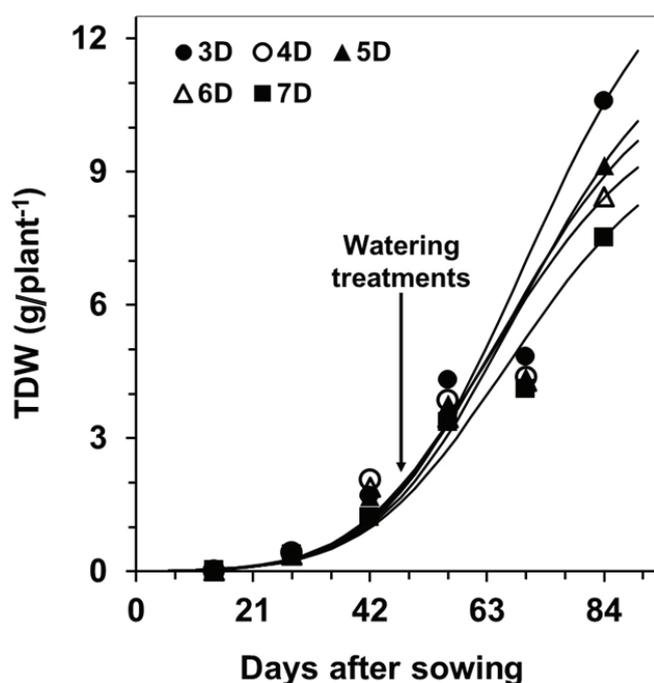


Fig. 6. Development with time in total dry weight of plants (TDW). Watering interval of 3-7 days is indicated by 3D-7D, and the initial imposition of watering treatments is indicated by an arrow. Each point represents the mean of five replicates. The graphs were generated by the Richards model (France & Thornley, 1984)

Root fresh weight, measured at harvest, was significantly affected by the treatment of watering intervals and was reduced as the watering interval increased from once in three days to five days (Table 1). The fresh weight of roots was slightly higher than that reported in Urutai-GO, Brazil (17°29'10"S, 48°12'38"W and 697 m asl.) with a tropical climate (da Silva Curvêlo, Diniz, de Azevedo

Pereira, & Ferreira, 2018) and close to that reported in other study (dos Santos *et al.*, 2017). Root dry weight decreased by more than 20% and 50% with the watering interval once in four and seven days respectively compared to that once three days. A good relationship ($P < 0.01$) was found between the root dry weight and the watering intervals (Fig. 8).

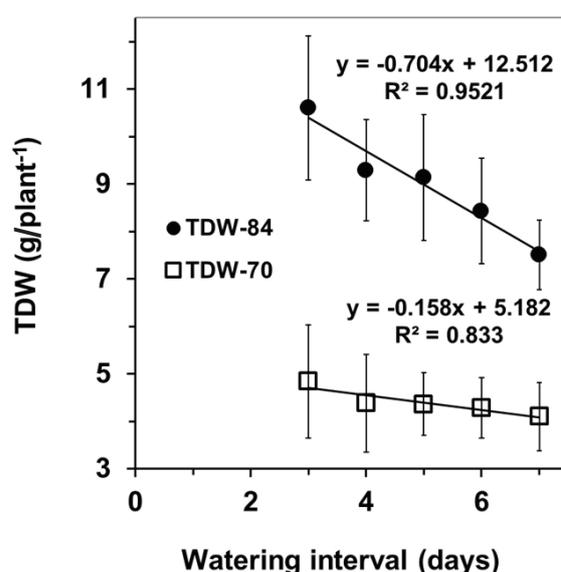


Fig. 7. Relationship between total dry weight and watering intervals. TDW-70 and TDW-84 is the TDW observed on day 70 and 84 respectively. Each point represents the mean of five replicates, and vertical bars represent the standard error of the mean

Table 1. Total dry weight (TDW), root fresh weight (RFW), root dry weight (RDW), harvest index (HI) and betacyanin (BC) content of roots under different watering intervals (WI)

WI (days)	Weight (g/plant)			HI	BC	
	TDW	RFW	RDW		(mg/l)*	mg/g**
3	10.6c	87.5b	5.2d	0.50c	322.7	54.9a
4	9.29bc	85.1b	4.1c	0.44bc	329.1	69.8a
5	9.13b	78.0a	3.9bc	0.43bc	310.6	62.0a
6	8.43ab	77.1a	3.3b	0.39ab	385.6	91.9b
7	7.51a	76.8a	2.4a	0.33a	354.6	112.0c
LSD (0.05)	1.42	4.7	0.7	0.07	ns	17.1

Remarks: *mg/l root extract, and ** mg/g root dry weight (root extract, fluid obtained from fresh roots; root dry weight, the weight of tubers after drying in an oven at 80°C for 48 hours); ns = not significant at $P \leq 0.05$ and values followed by the same letters are not significantly different at LSD (0.05)

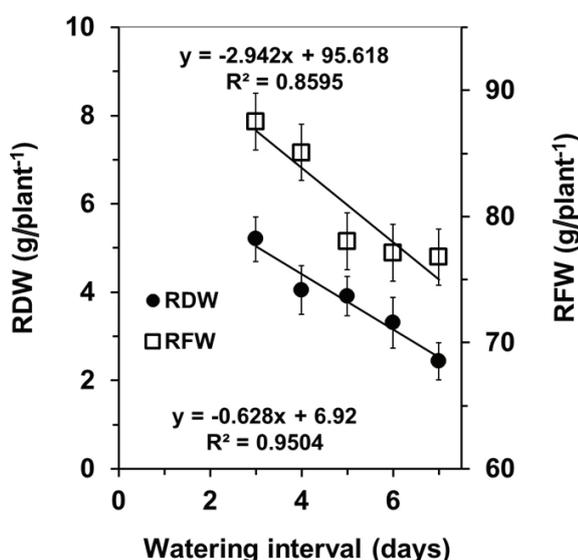


Fig. 8. Root dry weight (RDW) and fresh weight (RFW) in relation to watering interval. Each point represents the mean of five replicates, and vertical bars represent the standard error of the mean

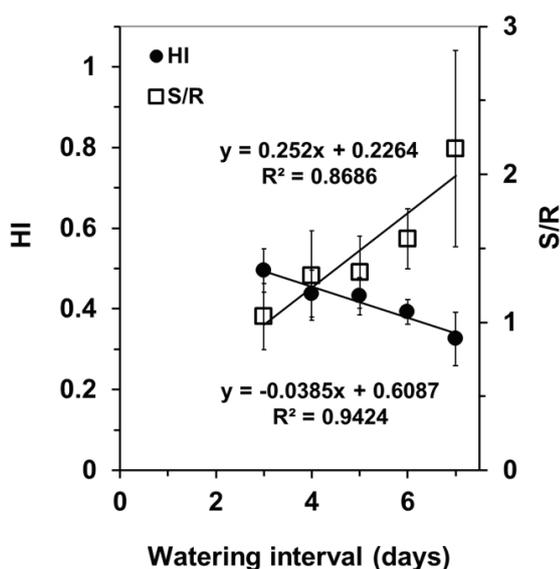


Fig. 9. Harvest index (HI) and shoot/root ratio (S/R) in relation to watering interval. Each point represents the mean of five replicates, and vertical bars are standard deviation of the mean

Harvest Index and Shoot/Root Ratio

Harvest index (HI), the ratio of RDW and TDW (RDW/TDW), was affected significantly by the treatment of watering intervals. A significant decrease in HI by > 20% ensued as the watering intervals increased from once tree to once six and seven days (Table 1). A close relationship ($P < 0.01$), however, was found between HI and the watering intervals which means that the partitioning of biomass to

roots (storage organ) was reduced with a decrease in the supply of water (Fig. 9). This can be seen also in the shoot/root ratio (SDW/RDW) that increased with an increase in the watering intervals. This suggests that the maintenance of shoot growth is accentuated more than the accumulation of biomass in the storage organ of roots as the total production of biomass decreased with a decrease in the supply of water.

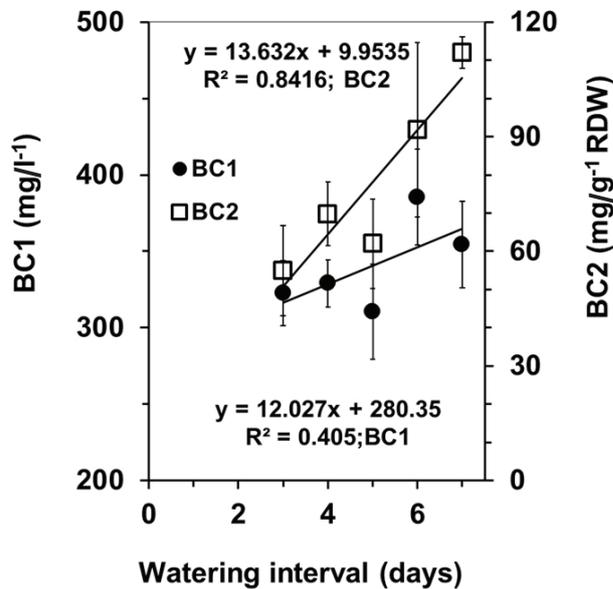


Fig. 10. Betacyanin content of roots in relation to watering interval. Each point represents the mean of five replicates, and vertical bars are standard deviation of the mean

Betacyanin

Betacyanin content of roots was not significantly affected by the treatment of watering intervals and varied in the range of 310.6-385.6 mg/l (Table 1) which is close to that of cactus fruits (*Hylocereus polyrhizus*) (Stintzing, Schieber, & Carle, 2003). The betacyanin content in the present study is also plausible compared to betacyanin content of beetroots reported by Wruss et al. (2015) showing a high variation between cultivars. When expressed per unit of root dry weight, a significant increase was found in betacyanin as the watering interval increased from once in three days to six or seven days. There was no, however, significant effect of the watering treatments on total betacyanin content per plant which was, on average, 274.6 ± 47.6 mg. A positively good relationship ($P < 0.01$) was found between betacyanin content per unit root dry weight and the interval of watering (Fig. 10).

Water stress is a phenomenon generally encountered by plants during the mid-days in the tropics despite water is supplied sufficiently everyday due to a higher water loss than water uptake resulting from a high resistance of roots to water uptake. Therefore, a more severe water stress would be expected with a reduction in water supply. The effect of water deficit, undertaken by an increase in watering interval in the present

study, was found to implicate a change not only in primary metabolism (biomass production) but also secondary metabolism (betacyanin biosynthesis).

A reduction in the biomass production (plant dry weight) in the present study could be attributed to a decrease in the leaf water content as a consequence of an increase in the watering intervals. This brought likely about a decrease in stomatal conductance, to reduce water loss, leading to a decrease in the rate of photosynthesis resulting from a decrease in the internal concentration of CO₂. Stagnari, Galieni, Specca, & Pisante (2014) reported a decrease in both RWC of leaves and stomatal conductance in beetroot plants with a decrease in water supply from the level of 100% water holding capacity (WHC) to 50% and 30% WHC. A study with 1-year-old poplar plant (*Populus simonii*) exposed to a high temperature (42°C) showed a significant decrease in the rate of photosynthesis (Pn) after three hours of heat stress (Song, Chen, Ci, Shao, & Zhang, 2014). This decrease was concluded due mainly to stomatal conductance (Gs) which decreased concurrently with intercellular CO₂ concentration (Ci). It was argued that, if Ci was not affected and Gs remained constant or descended, the decrease of Pn would be due to factors other than Gs.

S.M. Sitompul et al.: *Betacyanin of Beetroots Under Water Deficit*.....

A possible increase in the quantity of secondary metabolites has been reported by several studies in a number of plant species under water stress (Yang et al., 2018). In the present study, the effect of water deficit on the betacyanin content of roots was absent when expressed per unit of root extrat volume, but it increased significantly when expressed per unit of RDW. This suggests that this pigment may play an important role in the process of beetroot plants to withstand water stress. This is a typical response of secondary metabolites in plants subjected to stresses such as water stress (Ramakrishna & Ravishankar, 2011). For instance, plant tissues somewhat tolerant to water stress contain anthocyanins increasing under water stress (Chalker-Scott, 1999). A recent review showed that a number of secondary metabolites including phenols (Salidroside, Chlorogenic acid, Catechins, (-)-epicatechins, Tanshinone, Cryptotanshinone, Cryptotanshinone, and Abietic acid) and Alkaloids (Camptothecin, Morphine, Codeine, Glycine betaine) was reported to increase under water stress (Yang et al., 2018). Stagnari, Galieni, Specca, & Pisante (2014) reported an increase of betacyanin by > 90% and > 70% in plants watered at 50% and 30% field capacity respectively compared to that of control plants (100% field capacity). The betacyanins in plants under water stress, as secondary metabolites, may function as scavengers of ROS, photoprotectants, and stress signals (Yang et al., 2018) and even as osmoregulants (Miguel, 2018).

CONCLUSION

The effect of water deficit, executed through an increase in the watering intervals, was found to reduce the leaf water content, the production of biomass (total dry weight), the root yields of beetroot plants and harvest index (HI). The reduction in the dry weight of root yield increased by > 20% to > 50% with an increase in the watering interval from once four to seven days respectively. In turn, the leaf number and area per plant were not influenced by the water deficit which reached, on average, a maximum of 539.9 ± 114.5 cm² on day 56 and 10.3 ± 1.34 leaves on day 76 per plant respectively. The water deficit has no also significant effect on the betacyanin content of roots and total betacyanin per plant which was, on average, 340.5 ± 60.1 mg/l and 274.8 ± 47.6 mg per plant respectively. The

betacyanin content of roots expressed per unit root dry weight increased significantly as the watering intervals increased. It is suggested that an optimal supply of water (i.e. watering once three days), is necessary to obtain beetroot plants growing optimally and producing a high root yield.

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REFERENCES

- Cai, Y., Sun, M., & Corke, H. (2003). Antioxidant activity of betalains from plants of the Amaranthaceae. *Journal of Agricultural and Food Chemistry*, 51(8), 2288–2294. <https://doi.org/10.1021/jf030045u>
- Casique-Arroyo, G., Martínez-Gallardo, N., de la Vara, L. G., & Délano-Frier, J. P. (2014). Betacyanin biosynthetic genes and enzymes are differentially induced by (a)biotic stress in *Amaranthus hypochondriacus*. *PLoS ONE*, 9(6), e99012. <https://doi.org/10.1371/journal.pone.0099012>
- Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology*, 70(1), 1–9. <https://doi.org/10.1111/j.1751-1097.1999.tb01944.x>
- Chung, H. H., Schwinn, K. E., Ngo, H. M., Lewis, D. H., Massey, B., Calcott, K. E., ... Harrison, D. K. (2015). Characterisation of betalain biosynthesis in *Parakeelya* flowers identifies the key biosynthetic gene DOD as belonging to an expanded LigB gene family that is conserved in betalain-producing species. *Frontiers in Plant Science*, 6, 499. <https://doi.org/10.3389/fpls.2015.00499>
- Clifford, T., Howatson, G., West, D. J., & Stevenson, E. J. (2015). The potential benefits of red beetroot supplementation in health and disease. *Nutrients*, 7(4), 2801–2822. <https://doi.org/10.3390/nu7042801>
- da Silva Curvêlo, C. R., Diniz, L. H. B., de Azevedo Pereira, A. I., & Ferreira, L. L. (2018). Influence of fertilizer type on beet production and post-harvest quality

- S.M. Sitompul *et al.*: *Betacyanin of Beetroots Under Water Deficit*.....
- characteristic. *Agricultural Sciences*, 09(05), 557–565. <https://doi.org/10.4236/as.2018.95038>
- de Oliveira, R. A., Ramos, M. M., & de Aquino, L. A. (2015). Irrigation management. In F. Santos, A. Borém, & C. Caldas (Eds.), *Sugarcane: Agricultural Production, Bioenergy and Ethanol* (pp. 161–183). Academic Press. <https://doi.org/10.1016/B978-0-12-802239-9.00008-6>
- dos Santos, M. G., Souza, Ê. G. F., da Silva, A. F. A., Barboza, M., Soares, E. B., Lins, H. A., ... da Silveira, L. M. (2017). Beetroot production using *Calotropis procera* as green manure in the Brazilian Northeast semiarid. *Australian Journal of Crop Science*, 11(10), 1268–1276. Retrieved from <https://search.informit.com.au/document-summary;dn=404719526734825;res=IELHSS;-type=pdf>
- France, J., & Thornley, J. H. M. (1984). *Mathematical models in agriculture: A quantitative approach to problems in agriculture and related sciences*. London: Butterworth. Retrieved from https://books.google.co.id/books/about/Mathematical_models_in_agriculture.html?id=HsrwAAAAMAAJ&redir_esc=y
- Gago, J., de Menezes Daloso, D., Figueroa, C. M., Flexas, J., Fernie, A. R., & Nikoloski, Z. (2016). Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: A multispecies meta-analysis approach. *Plant Physiology*, 171(1), 265–279. <https://doi.org/10.1104/pp.15.01660>
- Guldiken, B., Toydemir, G., Nur Memis, K., Okur, S., Boyacioglu, D., & Capanoglu, E. (2016). Home-processed red beetroot (*Beta vulgaris* L.) products: Changes in antioxidant properties and bioaccessibility. *International Journal of Molecular Sciences*, 17(6), 858. <https://doi.org/10.3390/ijms17060858>
- Halliwell, B. (1997). Antioxidants and human disease: A general introduction. *Nutrition Reviews*, 55(1), S44–S49. <https://doi.org/10.1111/j.1753-4887.1997.tb06100.x>
- Lakshmi Sahitya, U., Krishna, M. S. R., Sri Deepthi, R., Shiva Prasad, G., & Peda Kasim, D. (2018). Seed antioxidants interplay with drought stress tolerance indices in chilli (*Capsicum annum* L) seedlings. *BioMed Research International*, 2018, 1605096. <https://doi.org/10.1155/2018/1605096>
- Maréchaux, I., Bartlett, M. K., Gaucher, P., Sack, L., & Chave, J. (2016). Causes of variation in leaf-level drought tolerance within an Amazonian forest. *Journal of Plant Hydraulics*, 3, e004. <https://doi.org/10.20870/jph.2016.e004>
- Miguel, M. G. (2018). Betalains in some species of the Amaranthaceae family: A review. *Antioxidants*, 7(4), 53. <https://doi.org/10.3390/antiox7040053>
- Nakatsuka, T., Yamada, E., Takahashi, H., Imamura, T., Suzuki, M., Ozeki, Y., ... Nishihara, M. (2013). Genetic engineering of yellow betalain pigments beyond the species barrier. *Scientific Reports*, 3, 1970. <https://doi.org/10.1038/srep01970>
- Nottingham, S. (2004). *Beetroot*. Retrieved from <https://www.academia.edu/21542519/Beetroot>
- Pavokovic, D., & Krsnik-Rasol, M. (2011). Complex biochemistry and biotechnological production of betalains. *Food Technology and Biotechnology*, 49(2), 145–155. Retrieved from <https://hrcaak.srce.hr/file/103483>
- Polturak, G., Breitel, D., Grossman, N., Sarrion-Perdigones, A., Weithorn, E., Pliner, M., ... Aharoni, A. (2016). Elucidation of the first committed step in betalain biosynthesis enables the heterologous engineering of betalain pigments in plants. *New Phytologist*, 210(1), 269–283. <https://doi.org/10.1111/nph.13796>
- Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, 6(11), 1720–1731. <https://doi.org/10.4161/psb.6.11.17613>
- Ravichandran, K., Saw, N. M. M. T., Mohdaly, A. A. A., Gabr, A. M. M., Kastell, A., Riedel, H., ... Smetanska, I. (2013). Impact of processing of red beet on betalain content and antioxidant activity. *Food Research International*, 50(2), 670–675. <https://doi.org/10.1016/j.foodres.2011.07.002>
- Sitompul, S. M., & Zulfati, A. P. (2019). Betacyanin and growth of beetroot (*Beta vulgaris* L.) in response to nitrogen fertilization in a tropical condition. *AGRIVITA Journal of Agricultural Science*, 41(1), 40–47. <https://doi.org/10.17503/agrivita.v41i1.2050>
- Sitompul, S. M., Sitawati, & Sugito, Y. (2013). Spatial productivity analysis of tropical apple (*Malus sylvestris* Mill) in relation to temperature with PCRaster. *Journal of Agricultural Science and Technology A*, 3, 183–192. Retrieved from <http://www.davidpublisher.org/index.php/Home/Article/index?id=14564.html>
- Slatyer, R. O. (1969). Physiological significance of internal water relations to crop yield. In J. D. Eastin, F. A. Haskins, C. Y. Sullivan, C. H. M. van Bavel, & R. C. Dinauer (Eds.), *Physiological aspects of crop yield: Proceedings of A Symposium*

- S.M. Sitompul *et al.*: *Betacyanin of Beetroots Under Water Deficit*.....
- (p. 186). Madison, Wisconsin: American Society of Agronomy & Crop Science Society of America. <https://doi.org/10.2135/1969.physiologicalaspects.c6>
- Soltys-Kalina, D., Plich, J., Strzelczyk-Żyta, D., Śliwka, J., & Marczewski, W. (2016). The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-derived potato cultivars. *Breeding Science*, *66*, 328–331. <https://doi.org/10.1270/jsbbs.66.328>
- Song, Y., Chen, Q., Ci, D., Shao, X., & Zhang, D. (2014). Effects of high temperature on photosynthesis and related gene expression in poplar. *BMC Plant Biology*, *14*, 111. <https://doi.org/10.1186/1471-2229-14-111>
- Stagnari, F., Galieni, A., Speca, S., & Pisante, M. (2014). Water stress effects on growth, yield and quality traits of red beet. *Scientia Horticulturae*, *165*, 13–22. <https://doi.org/10.1016/j.scienta.2013.10.026>
- Stintzing, F. C., Schieber, A., & Carle, R. (2003). Evaluation of colour properties and chemical quality parameters of cactus juices. *European Food Research and Technology*, *216*(4), 303–311. <https://doi.org/10.1007/s00217-002-0657-0>
- Tanaka, Y., Sasaki, N., & Ohmiya, A. (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*, *54*(4), 733–749. <https://doi.org/10.1111/j.1365-313X.2008.03447.x>
- Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P., Müller, U., ... Weghuber, J. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis*, *42*, 46–55. <https://doi.org/10.1016/j.jfca.2015.03.005>
- Yang, L., Wen, K. S., Ruan, X., Zhao, Y. X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules*, *23*(4), 762. <https://doi.org/10.3390/molecules23040762>
- Yin, X., Goudriaan, J., Lantinga, E. A., Vos, J., & Spiertz, H. J. (2003). A flexible sigmoid function of determinate growth. *Annals of Botany*, *91*(3), 361–371. <https://doi.org/10.1093/aob/mcg029>
- Yin, X., Kropff, M. J., McLaren, G., & Visperas, R. M. (1995). A nonlinear model for crop development as a function of temperature. *Agricultural and Forest Meteorology*, *77*(1–2), 1–16. [https://doi.org/10.1016/0168-1923\(95\)02236-Q](https://doi.org/10.1016/0168-1923(95)02236-Q)