INTRODUCTION

The cultivation of beetroots (*Beta vulgaris* L.) has attracted increasing attention in recent years in Indonesia in order to obtain fresh root yield (taproots). Air temperature is the main limiting factor of beetroots, a subtropical species by the origin and known as a cool-weather crop, to grow in the tropics. For optimum growth, this crop requires temperatures in the range of 12-19°C (Nottingham, 2004; Starke Ayres, 2014) or 15-20°C (ARC, 2013). A reduction in yield quantity and quality (flavor and texture) was observed to occur in addition to growth retardation especially during prolonged high temperature (ARC, 2013). As a consequence, from the biological and economic point of view, this crop is only likely to be cultivated on areas of high elevations in the tropics. Based on the results of a previous study on air temperatures during the day at several elevations in the region of Malang, East Java (Sitompul, Sitawati, & Sugito, 2013), the optimal elevation for beetroot cultivation in this region is in the range of > 1800 - ≤ 2900 m asl.

The results of an experiment, conducted in the region of Malang at an elevation of 1700 m asl, led to a conclusion that beetroots can be cultivated in the tropics on highlands with good growth and reasonable yields (Sitompul & Zulfati, 2019). The highest yield of 150.5 g RFW (root fresh weight) per plant was obtained from the plants fertilized with 0.6 g N/plant (~ 150 kg N/ha). This root yield, with 250,000 plants/ha (20 cm x 20 cm spacing), is equivalent to 37.6 t/ha. In other experiment designed to study watering intervals (once 3-7 days) in the objective of water use efficiency, the highest yield (RFW) of 87.5 g per plant (~ 21.9 t/ha) was observed in plants with the watering interval of once three days (Sitompul, Roviq, & Riedo, 2019). The root yields of both experiments are higher than the root yield of beetroots (cv. Early Wonder) from an experiment at Urutai-GO, Brazil (17°29'10" S, 48°12'38" W) at the elevation of about 800 m asl with an average annual temperature of 230C (da Silva Curvêlo, Diniz, de Azevedo Pereira, & Ferreira, 2018). The RFW of this latter experiment was 16.25 t/ha and 15.5 t/ha with conventional fertilization and organic fertilizer respectively. High root yields were reported in other experiments in a
tropical region of Brazil (Serra Talhada-PE, 7°57’15” LS) at an elevation of 429 m asl (dos Santos et al., 2017). The RFW of 46.48 t/ha was reported from an experiment conducted during autumn (March-June) with the application of 15.6 t/ha of green manure (Calotropis procera). A lower root yield (41.48 t/ha) was found from an experiment carried out during spring-summer (October-January) with the same application of green manure.

The cultivation of beetroots on a large scale in the tropics is hampered by limited areas of high elevations suitable for beetroots. At suboptimal elevations, beetroots may suffer from heat stress which is the main environmental stress limiting the productivity of crops worldwide (Hasanuzzaman, Nahar, Alam, Roychowdhury, & Fujita, 2013). This could be attributed to the disturbance of many biochemical reactions sensitive to high temperatures and oxidative stress due to ROS (reactive oxygen species) formation in large quantities (Hasanuzzaman, Hossain, da Silva, & Fujita, 2012; Hasanuzzaman, Nahar, Alam, Roychowdhury, & Fujita, 2013). The results of studies in Brazil as mentioned previously suggest that beetroots adapted well to suboptimal elevations down to 429 m asl. In the experiment at Serra Talhada-PE mentioned above, the annual average temperature was reported to be 24.7°C, and the maximum and minimum of monthly average temperature varied successively in the range of > 31 - < 36°C and ≥ 20 - ≤ 23°C (dos Santos et al., 2017). A review showed that the negative effect of heat stress on plants, in general could be alleviated with various means including the supply of nitrogen (N), phosphorus (P) or potassium (K) nutrients (Hasanuzzaman, Nahar, Alam, Roychowdhury, & Fujita, 2013).

The present study was designed to study the adaption of beetroots to suboptimal elevations in a tropical region with the growth performance of plants used as an indicator of adaptation. The potency of nitrogen nutrients to increase the adaptability of beetroots to suboptimal elevations was also explored. A very high rate of N fertilizer (300 kg N/ha) was involved in the present study as the results of the previous experiment showed a linear increase in root yield with an increase in N fertilizer up to 150 kg N/ha (Sitompul & Zulfati, 2019). This is also supported by the results of a pot experiment in a greenhouse with a day/night temperature maintained at 22/15°C in Bloemfontein, South Africa (Rantao, 2013). This latter experiment showed an increase in root yields to 14.47 t/ha with 200 kg N/ha compared with 2.99 t/ha without N fertilizer. Better performance of beetroot growth was also reported in plant height, leaf number, leaf area, leaf fresh weight, total fresh weight and the diameter and volume of tuberous roots.

**MATERIALS AND METHODS**

**Study Sites**

The study consisted of four experiments carried out in the field during the dry season (June-September) in 2019 within the region of Malang (7°57’59” S, 112°37’57” E). Experiment 1 (as a control), 3 and 4 was undertaken at the Experimental Station of the Universitas Brawijaya in Cangar at 1700 m asl, Jatimulyo (520 m asl) and in Jatikerto (320 m asl), respectively. Experiment 2 was conducted at a farmer’s field in Batu at 850 m asl. The average air temperature at Cangar during the day, based on measurements at 06.00 AM, 13.00 PM and 18.00 PM for five successive days in February and May 2016, was 20°C corresponding with the results of the previous study (Sitompul, Sitawati, & Sugito, 2013). The air temperature during the day at the site of experiments 2, 3 and 4, based on the previous study, was estimated to be 26.0, 28.3 and 29.6°C respectively.

**Sowing and Treatments**

Seeds of beetroot (cv. Ayumi 04), purchased from a local agricultural shop, and were grown in plastic bags (20 cm in diameter and 30 cm in height) in June 2019. In each pot (plastic bag), filled with topsoil (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 20 das (days after sowing). Each pot was supplied with 0.6 g P₂O₅ (SP36) at sowing and 0.8 K₂O (KCl) on 20 das for experiments not involving phosphorus (P) or potassium (K) fertilizer as a treatment.

The imposition of treatments in the field was arranged according to a randomized block design with four replicates. The treatment of nitrogen (N) consisting of 0, 0.4, 0.8 and 1.2 g N/plant (urea) was involved in all experiments and applied on 5 das. Other treatments were organic manure (chicken manure): 0 and 80 g/plant (~ 20 t/ha) in experiment 1 and 4 applied at sowing, phosphorus (SP36): 0 and 0.6 g P₂O₅/plant (~ 150 kg P₂O₅/ha) in experiment 2 applied at sowing, and potassium (KCl): 0 and 0.8 g K₂O/plant (~ 200 kg P₂O₅/ha) in experiment 3 applied
on 15 das. Plants were free from weeds controlled manually, and a fungicide (benlate 0.2%) was used as necessary to control fungi (*Peronospora schachtii*).

**Plant Growth Observation**

Plant growth in leaf area and total plant dry weight was observed with destructive samples on days 20, 40, 60 and 90 (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) was observed on 60 and 90 das. Sub-samples from the representative part of leaves, stems, and roots were dried in an oven at 80°C for 48 hours for the estimation of total and root dry weight.

**Data Analysis**

Statistical analyses of data including analysis of variance (ANOVA) were executed in the MS excel manually. Least significant difference (LSD for n ≤ 6) or Duncan multiple range tests (DMRT for n > 6) at P ≤ 0.05 was used to compare mean values between treatments. The present paper is focused on the growth adaptability of beetroots on suboptimal elevations with the supply of nitrogen fertilizer. For this purpose, data of all experiments were reorganized so that a Split Plot Design could be used to analyze the effect of site elevation as the main factor and nitrogen as a sub factor.

The growth pattern of plants with time was analyzed with a Richards’s model (France & Thornley, 1984) as follows:

\[
W = \frac{W_m W_0}{W_m + \left(W_0 - W_m\right) e^{-k t}}
\]

Where: \(W\) is the growth parameter (biomass or leaf area) at a particular time \(t\) (day), \(W_0\) and \(W_m\) is \(W\) at \(t = 0\) and the end of growth period respectively, and \(n, k, W_0\) and \(W_m\) are constants. A significant difference in the growth patterns, analyzed with the Richards model, between plants at the optimal elevation (1700 m asl) and plants at other elevations was analyzed by Kolmogorov-Smirnov (KS) test (Zaiontz, 2014) at P ≤ 0.05. The application of an exponential model available in the MS excel was also explored to analyze the growth pattern of plants.

**RESULTS AND DISCUSSION**

**Total Dry Weight**

The plant growth of beetroots, the accumulation of total dry weight (TDW) with time, was significantly influenced by the site elevation on 20, 60 and 90 das (days after sowing), but not by nitrogen (N) fertilizer on all days of observation. A significant interaction between site elevation and N fertilizer was observed on 40, 60 and 90 das prompting an interest to analyze the pattern of beetroot growth with time for all treatment combinations. It appears that the growth of beetroots up to 90 das showed a similar pattern that increased slowly at the beginning up to 40 das, then rapidly thereafter (Fig. 1).

The use of an exponential model to analyze the pattern of increases in the average TDW of plants for each treatment with time resulted in high coefficients of determination (R\(^2\) ≥ 0.824). The coefficients of determination were not, however, significant for R\(^2\) < 0.903 due to a limited sample number (n = 4). A much better description of TDW development with time was found with the use of a Richards model with R\(^2\) ≥ 0.994 (P < 0.003) as drawn for all treatments (Fig. 1). The R values can be seen in the relationship between R2 values of the Richards model and those of the exponential equations (Fig. 2A). No significant difference in the growth pattern was found between plants at the optimal elevation and others based on the KS test. A linear relationship between the observed TDW and the estimated TDW with the Richards model for all treatments is highly significant (Fig. 2B).

It is clear, however, that the biomass production (TDW) of plants was lower at suboptimal elevations than at the optimal elevation (1700 m asl) particularly at later stages of growth when N nutrient was supplied sufficiently. The interaction of site elevation and N fertilizer on TDW at harvest (90 das) showed, in general, a decrease in the TDW of N-fertilized plants with a decrease in the site elevation with a rate of decrease depending on the rate of N fertilizer (Fig. 3A). A significant, linear relationship between TDW and the site elevation (P ≤ 0.05) was found in the plants with 1.2 g N/plant that showed a reduction of 39.8 g/plant in TDW with a decrease of 1000 m in the site elevation. Similar trends, but not significant, were also found in plants with 0.8 g N/plant (N2, P > 0.10) and plants with 0.4 g N/plant (N1, P > 0.14).
Fig. 1. The progress with time in total dry weight (TDW) of beetroots grown at different site elevations (E) with different rates of N fertilizer. E1, E2, E3 and E4 is 1700, 850, 520 and 320 m asl respectively; A. N0 and N1 is 0 and 0.4 g N/plant, respectively; B. N2 and N3 is 0.8 and 1.2 g N/plant, respectively. The graph for each treatment combination was generated by a Richards model. The $R^2$ of all graphs is highly significant ($P \leq 0.003$).
Fig. 2. Richards $R^2$ was plotted as a function of exponential $R^2$. $R^2 = \text{Coefficient determination}$, and Richards and exponential refers to Richards and exponential equation respectively. The dashed line (horizontal) and dotted line (vertical) is the border between not significant and significant $R^2$ of exponential equations at $P = 0.05$ and of Richards equation at $P = 0.01$ respectively.

Fig. 3. Relationship between total dry weight (TDW) at harvest (90 das) and site elevation. The linear equation for N0, N1, N2 and N3 is $y = 3E-05x + 22.57$ ($R^2 = 4E-05^{*}$), $y = 0.0256x + 7.8741$ ($R^2 = 0.809$, ns), $y = 0.0101x + 22.662$ ($R^2 = 0.7253$, ns) and $y = 0.01x + 19.198$ ($R^2 = 0.9683^{*}$) respectively (A), ns = not significant and * = significant at $P \leq 0.05$. Each point is the average of 4 replicates (A), and the relationship between the mean TDW of N1, N2 and N3 of four replicates ($n = 16$) and the site elevation was analyzed with linear and exponential model (B).
When the TDW of N1, N2 and N3 treatment was averaged for each elevation (n = 16), a good relationship ($R^2 = 0.719; P < 0.05$) was found between the TDW and site elevation with the linear model and exponential model (Fig. 3B). The linear relationship suggests in general that a decrease of 1000 m in the elevation led to a decrease of about 30% in the TDW compared with that at the optimal elevation. The TDW of plants without N fertilizer showed no significant difference between site elevations and was much lower than that with the supply of N nutrient at high elevations (> 800 m) particularly at 1700 m asl (Fig. 3A).

**Leaf Area**

Leaf area (LA) per plant was mainly influenced by the site elevation as observed significantly on 20, 60 and 90 das. No significant effect of N fertilizer on the LA was found at any time (day) of observation, and a significant interaction between site elevation and N fertilizer was observed only on 40 das. The response of LA to the site elevation on day 20, different from that of TDW, showed a tendency of increase as the site elevation increased from 320 m up to 850 m, but then decrease at a higher elevation (Fig. 4). On day 60 and 90, LA showed no clear-cut responses of LA that tended to decrease with an increase in the site elevation from 320 m to 850, then relatively constant or to increase slightly at a higher elevation (Fig. 4).

The development of leaf area (LA) per plant with time was similar to that of TDW and independent of site elevation and the rate of nitrogen fertilizer. The LA of plants grown at the optimal elevation (E1, 1700 m asl) increased slowly during the first 20 das, and rapidly thereafter (Fig. 5). At other elevations, the LA increased slowly during about the last 30 days of growth following slow and rapid growth in the previous stages as observed also in the plants at the optimal elevation. The use of a Richards's model was good to capture the development of the LA with time with a high coefficient of determination for each site elevation ($P < 0.01$). The development of LA with time is closely related to that of leaf number (LN) with time independent of site elevations (Fig. 6). This relationship suggests that the development of LA with time was due mainly to an increase in LN ($R^2 = 0.827$), and the contribution of individual LA (leaf area per leaf) was < 20%. The relationship between TDW and LA, in general, was significant for each site elevation particularly during the first 60 days of growth (Fig. 7).
Fig. 5. The development with time in leaf area of plants (beetroots) grown at optimal (E1: 1700 m asl) and suboptimal elevations (E2: 850 m, E3: 520 m and E4: 320 m asl). Graphs in the figure were generated by a Richards with $R^2 > 0.985$ ($P \leq 0.01$) for all graphs.

Fig. 6. Relationship between leaf area (LA) and leaf number (LN) of plants grown at 1700, 850, 520 and 320 m asl is shown with different symbols (A). The data of all elevations were pooled and analyzed with a linear equation (B) as shown in the figure for the line and the linear equation (B, $n = 16$). Each point is the average value of four replicates and four rates of nitrogen fertilizer.
The present study designed to explore the adaptability of beetroots to suboptimal elevations (< 1700 m asl) for the purpose of large scale cultivation in the tropics. As this crop, known as a cool-weather crop, requires a temperature range of 12-20°C for optimum growth (ARC, 2013; Starke Ayres, 2014), heat stress is likely to be the major factor limiting its cultivation in tropical regions. The effect of heat stress could be increasingly significant with time in the tropics due to the phenomenon of global warming (Stocker et al., 2014). The frequency and duration of high temperatures are also considered to increase with dramatic effects on agriculture, economy, and ecology (Easterling et al., 2000; Tripathi, Tripathi, Chauhan, Kumar, & Singh, 2016; Wang, Heckathorn, Mainali, & Tripathee, 2016). On the other hand, the potential impact of climate change (higher temperatures) is not sufficiently understood in most agriculture systems including vegetable crops (Leisner, 2020).

The key to beetroot adaptability is the level of the growth process to tolerate heat stress at the suboptimal elevations where air temperatures are higher than the optimal temperatures. The growth process encompasses all processes leading to the synthesis of plant biomass consisting mainly of photosynthetic products through photosynthesis process. Photosynthesis, the most fundamental physiological process in plants, on the other hand, is highly sensitive to heat stress (Mathur, Agrawal, & Jajoo, 2014; Song, Chen, Ci, Shao, & Zhang, 2014).

It was found in the present study that the growth of beetroots is quite adaptable to suboptimal elevations (< 1700 m asl) down to about 300 m in the tropical areas of study. The growth development of beetroots, the accumulation of TDW with time, showed a normal pattern as observed in the plants at the optimal elevation (1700 m) that can be captured by a Richards's model (Fig. 1). No such information has been found in the literature so far. A similar pattern was also reported in previous studies carried out at the elevation of 1700 m (Sitompul & Zulfati, 2019; Sitompul, Roviq, & Riedo, 2019).

The analysis of TDW observed at harvest (90 das) in relation to the site elevation with a linear regression in the plants with 1.2 g N/ha showed a decrease of only 10 g/plant with a decrease of 1000 m in the site elevation in the range of 1700-320 m.
asl (Fig. 3). This is equivalent to 2.5 t/ha or 27.6% of TDW at the optimal elevation (1700 m asl), and the TDW of beetroots at 320 m asl may reach 22.4 g/plant (~5.6 t/ha) or 61.9% of TDW at the optimal elevation. A relatively similar trend was shown by the TDW of plants with 0.4 g N/plant. The TDW of plants without N fertilization was low and showed no response to the site elevation as expected suggesting that nitrogen nutrient has an important role in the adaptability of beetroots to high temperatures. The TDW response of plants with 0.8 g N/plant to a decrease in LA was not significant and could be an artifact that needs further investigation. The decrease in the biomass production of plants at suboptimal elevations could be related to a decrease in LA as a consequence of LN decrease (Fig. 6). This is based on a close relationship between TDW and LA with R² ≥ 0.787 (Fig. 7) suggesting that the contribution of factors other than LA including the efficiency of photosynthesis (TDW/LA) to TDW was quite small (21.3%). This is in agreement with the previous studies showing photosynthesis as the main process determining the adaptability of plants to heat stress.

In contrast, for instance, apple trees are very sensitive to high temperature (heat stress). Based on the results of a previous study (Sitompul, Sitawati, & Sugito, 2013). The analysis of the linear relationship between fruit yield (fruit fresh weight) and elevation in the study showed a yield decrease of >10 kg/tree with a decrease of 100 m in elevation, and the apple trees produced no fruit at the elevation of <800 m asl. This is consistent with the reality of the cultivated apple trees in the region of Malang even with the varieties have adapted to the local condition that fruits are produced only at elevations above a certain limits.

The adaptability of beetroots to lower elevations found in the present study in is in agreement with the results of a study in Brazil (Serra Talhada-PE, 7°57’15” S) that showed a high fresh root yield (46.48 t/ha-6 t/ha of root dry weight) of beetroots at an elevation of 429 m asl (dos Santos et al., 2017). The beetroot crop was grown during the autumn season (March-June) with the supply of 15.6 t/ha green manure of Calotropis procera. With the same application of green manure at sowing, a lower root yield (41.48 t/ha) was obtained from a crop grown during the spring-summer season (October-January). These yields were much higher than that of an experiment (~17.0 t/ha) conducted in the field of Urutai-GO, Brazil (17029’10”S) at 697 m elevation ((da Silva Curvêlo, Diniz, de Azevedo Pereira, & Ferreira, 2018). This experiment was carried out during the period of October-December 2016 with an average temperature of 23°C.

The present study also showed that a sufficient N fertilizer is required to support the adaptability of beetroots to the suboptimal elevations. The results of several studies previously suggest an important role of plant nutrients in the adaptability of plants to heat stress (Liu et al., 2019). It was found, for instance, that the application of N fertilizer (e.g. 300 kg N/ha) may sustain a sufficiently high yield of rice under high temperature (Liu et al., 2019). The role of nitrogen on plant adaptation to high temperatures (heat stress) could be associated with photosynthesis, the most sensitive function of plants (Wang, Heckathorn, Mainali, & Tripathee, 2016). Leaves situated aboveground are exposed directly to air temperature and the main site of the photosynthetic process that determines the production of carbohydrates. The primary target of heat stress on photosynthesis is considered to be photosystem II and Ribulose bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) (Mathur, Agrawal, & Jajoo, 2014). It is therefore that photosynthesis is likely the main process responsible for the negative effect of high temperature on TDW or plant growth (Wang, Heckathorn, Mainali, & Tripathee, 2016). In natural conditions (380-400 ppm CO2), the main factor limiting photosynthesis of C3 plants is the formation of RuBP (ribulose 1,5-bisphosphate) and the carboxylation of Rubisco (RuBP carboxylase/oxygenase) (Farquhar, von Caemmerer, & Berry, 1980; von Caemmerer & Evans, 2015). Yamori, Nagai, & Makino (2011) found that CO2 assimilation at 380 µmol/mol CO2 was limited by RuBP carboxylation at low leaf N and by RuBP regeneration at high leaf N. In the present study, the positive effect of N could be associated with an increase in the RuBP carboxylation that needs further study.

CONCLUSION

The plant growth of beetroots is quite adaptable to suboptimal elevations (<1700 m asl) in the tropics. A decrease of 1000 m in the site elevation below the optimal elevation (1700 m asl) caused a reduction of only about 30% in TDW. The normality of growth patterns of plants at the suboptimal elevations similar to that at the optimal elevation was observed. The adaptability of
beetroots to heat stress could be enhanced by the application of nitrogen fertilizer in a sufficient amount (e.g. 1.2 g/plant). The leaf area of plants associated mainly with the leaf number is the major factor of plants supporting the adaptability of beetroot growth at the suboptimal elevations.

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