



# Physiological performance and fruit quality of noni (*Morinda citrifolia* L.) cultivated in different agro-climatic zones of Fiji

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

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**Abstract:** Noni (*Morinda citrifolia*) fruit juice is widely used as a strong antioxidant nutritional supplement. With its demand for supplementary products globally, commercial noni farming is now increasing in the Pacific Islands. Information on its growth performance and fruit quality under variable climatic condition is limited. This study aimed to establish the climatic requirements and identify the agro-climatic zone in Fiji that provides for increased antioxidant levels in fruits in addition to optimal plant growth and physiological performance. The study investigated plant growth, photosynthetic performance, fruit yield and antioxidant properties of plants that were cultivated under rain fed conditions in the dry, wet and intermediate agro-climatic zones in Fiji Islands. The physiological performance was significantly influenced by the soil moisture, sunshine hours and soil nutrients. Physiological performance including fruit yields were the highest in the intermediate zone which was characterized by a moderate rainfall and fairly good soil properties while it was lowest in the dry zone. Highest fruit antioxidant properties occurred in the dry zone followed by wet zone. The study implies that under cultivation, moderate abiotic stress can enhance the antioxidant properties of noni.

## 1. Introduction

Noni (*Morinda citrifolia* L.) is a tropical evergreen perennial plant with large elliptical leaves and a compound fruit (Nelson and Elevitch, 2006). Noni is native to is native to Southeast Asia and Australia and has a pantropical distribution (Nelson, 2003; Pandiselvi *et al.*, 2019) The plants are significant source of traditional Polynesian medicine. The fruits range from 4-10 cm in length and about 3-4 cm in diameter and are the most commonly used parts. The fruit has a broad range of nutraceutical and therapeutic potentials (Chan-Blanco *et al.*, 2006; Mahantesh *et al.*, 2018; Almeida *et al.*, 2019; Inada *et al.*, 2020). Noni fruit products have become quite popular in the area of health care due to its biologically active com-

pounds and high antioxidant potential. Commercial noni farming occurs in most tropical countries and is now growing in the Pacific Islands.

Growing noni under rain fed conditions are highly beneficial in tropical countries where rainfall is abundant with good volcanic soil (Nelson and Elevitch, 2006). Most countries, however, have their own agro-climatic zones where certain crops perform well. Agro-climatic zone is the characterization of an area based on its climatic parameters that are suitable for agriculture (Parry *et al.*, 1988). Farming in agro-climatic zones ensures that a crop is ecologically viable together with being economically profitable. Amin *et al.* (2004), lists some agro-climatic parameters as rainfall, maximum and minimum temperature, humidity, evapotranspiration, maximum possible sunshine hours and wind speed. Crop growth and agricultural productivity are primarily affected by all climatic elements, whether its effects occur singly or in combination (Parry *et al.*, 1988; Mittler, 2006). Precipitation, temperature and solar radiation have a direct effect on key plant metabolic processes such as photosynthesis and respiration while humidity is crucial for regulating transpiration and plant water balance. Sub-optimal values of precipitation, temperature, light intensity and relative humidity can result in crop yield reduction and product quality (Ferrante and Mariani, 2018).

Noni is renowned for tolerating a wide range of climatic conditions in its habitats. According to Nelson (2003), noni is found from 1 m to 800 m above sea level growing in a wide range of soils (infertile soils, acidic and alkaline soils) in its natural habitats. The mean annual rainfall range is from 250-4000 mm, and mean annual temperature range is from 20°C to 35°C. Noni plants can survive dry seasons with less than 40 mm of rainfall for at least 3 - 4 months depending on plant size and age and its surrounding temperature and humidity (Nelson, 2003). According to Nelson and Elevitch (2006), in cultivation, about 500-1500 mm annual rainfall that evenly spreads over the year is ideal for obtaining high yields. In wet areas where the annual rainfall is up to 4000 mm/year, the yield of noni is high, but the fruits are usually very watery, less sweet and tend to have much slower and uneven ripening while in drier areas fruits are much sweeter with rapid and even ripening (Nelson and Elevitch, 2006). Under cultivation, noni has been found to be a very low-maintenance plant which requires low irrigation and fertilizer.

Since noni's commercial market firmly bases its

advertises on high antioxidant properties, noni growers must ensure that good plant growth and high fruit yield also accompanies fruits with high antioxidant properties. Antioxidants can be enhanced in fruits by changing some cultivation practices once the specific environmental effects on fruits are known (Dumas *et al.*, 2003; Wang, 2006). Major antioxidants, both enzymatic and non-enzymatic protect higher plant cells from oxidative stress damage that usually occurs when the plants undergo environmental stresses. Antioxidant properties of noni are highly associated with the non-enzymatic phenolic compounds (Dussosoy *et al.*, 2011). The non-enzymatic antioxidants in plants are mainly comprised of ascorbic acid, glutathione,  $\alpha$ -tocopherol, carotenoids, phenolics, flavonoids, and amino acid cum osmolyte proline. Due to their essential role in protection and development, high levels of non-enzymatic antioxidants are usually expected in plants undergoing adverse environmental stress hence noni's antioxidants may be elevated under stressful conditions.

Growers often make the cultivation environments ideal and stress-free for plants which are likely to lower the antioxidant capacity of the fruits. Hence apart from increasing the plant growth and fruit yield, it is also important to know what kind of environmental conditions would enhance the antioxidant levels in the noni fruits. This study examined the growth and physiological performance of noni plants cultivated in Fiji's three different agro-climatic zones and compared its growth, fruit production and yield together with its antioxidant properties. The study implicates how noni's cultivation environment can be altered to enhance the antioxidant production together with maintaining the overall productivity.

## 2. Materials and Methods

Plant growth and physiological performance of noni cultivated in dry, intermediate and wet climate zones of Viti Levu, Fiji were studied for two years from January 2016 to January 2018.

### *Experimental sites*

The main centers of dry (Nadi - 17°45'15"S, 177°28'3"E), wet (Suva - 18°14'80" S, 178°44'76"E) and intermediate (Sigatoka - 18°06'05"S, 177°32'13"E) agro-climatic zones on the island of Viti Levu, Fiji were chosen as the experimental sites (Fig. 1).

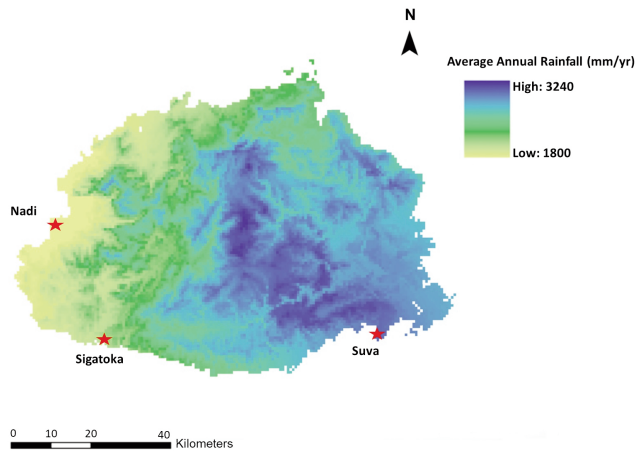


Fig. 1 - Average annual rainfall distribution in Viti Levu and the locations of the study sites in dry zone (Nadi), intermediate zone (Sigatoka) and wet zone (Suva) (Adapted and modified from Printemps, 2008).

Data on rainfall and temperature for 2016 and 2017 was obtained from the Fiji Meteorological Service office for the three sites (Table 1). Soil samples were collected randomly three times during the experiment duration, and the nutrient content was analyzed for the three cultivation sites.

#### Cultivation of noni

Four-month old noni plants were established in ground plots located in the dry, intermediate and wet agro-climatic zones of the island of Viti Levu, Fiji (Fig. 1). A total of 20 plants were transplanted with a spacing of 2 m. The transplanted plants were watered for the first month on a 3-day interval and on a weekly interval for the second and third month. Starting from the third month of establishment, the plants were left to be rain-fed. Balanced nitrogen, phospho-

Table 1 - Weather and soil attributes of the experimental sites for the two-year growth period (2016 and 2017)

Data	Nadi (Dry zone)	Sigatoka (Intermediate zone)	Suva (Wet zone)
Total rainfall (mm)	2237.3 ± 92.9	1645.5 ± 107.9	3102 ± 299.1
Total rainfall wet months (mm)	1936.2 ± 184.1	1091.7 ± 34.8	2119 ± 128.3
Rainfall dry months (mm)	301.3 ± 90.8	553.5 ± 72.8	982.8 ± 427.4
Mean monthly rainfall (mm)	186.4 ± 41.6 ab	139.3 ± 24.4 b	258.6 ± 37.5 a
Mean rainfall wet months (mm)	322.7 ± 59.5 ab	201.4 ± 38.76 b	353.3 ± 56.55 a
Mean rainfall dry months (mm)	50.2 ± 18.4 a	77.2 ± 16.8 a	163.8 ± 32.3 b
Mean temperature (°C)	26.3 ± 1.4 a	25.8 ± 1.7 a	26.4 ± 1.6 a
Mean max temperature (°C)	30.5 ± 1.2 a	30.7 ± 1.7 b	29.2 ± 1.8 c
Mean Min temperature (°C)	22.0 ± 1.7 a	20.9 ± 1.9 b	23.5 ± 1.4 c
Mean sunshine hours	7.0 ± 0.4 a	5.9 ± 0.4 b	4.9 ± 0.7 c
Mean soil pH	5.7 ± 0.1 a	6.8 ± 0.1 b	7.2 ± 0.1 c
Mean electrical conductivity (mS cm <sup>-1</sup> )	0.01 ± 0 a	0.09 ± 0.01 b	0.27 ± 0.06 c
Mean total nitrogen (N) (%)	0.13 ± 0.03 a	0.19 ± 0.01 b	0.20 ± 0.02 c
Mean available phosphorous (P) (mg/kg)	3.7 ± 0.6 a	36.3 ± 0.6 b	10.6 ± 2.9 c
Mean potassium (K) (mg/kg)	66.5 ± 39.1 a	518.7 ± 19.3 b	520.0 ± 92.2 c
Mean calcium (Ca) (mg/kg)	221.3 ± 23.4 a	5607.3 ± 89.5 b	7568.7 ± 401.1 c
Mean magnesium (Mg) (mg/kg)	35.4 ± 4.4 a	872.7 ± 28.9 b	619.8 ± 7.4 c

Mean ± SE are shown, n=24 (weather attributes) and n= 6 (soil attributes). According to Kruskal-Wallis test at p<0.05, there was a significant difference in mean rainfall per month (p= 0.0332, H= 6.810), rainfall per wet month (p=0.0664, H= 5.423), rainfall per dry month (p=0.0030, H= 11.63), maximum temperature (p=0.0032, H=11.50), minimum temperature (p= <0.000, H= 20.82), sunshine hours per month (n=24, p=<0.0001, H=23.63). There is no significant difference in monthly temperature (p =0.4951, H= 1.406). Soil attributes were also significantly different using Kruskal-Wallis test at p<0.05, soil pH (p<0.0001, H=15.51), electrical conductivity (p=<0.0001, H=16.11), total nitrogen (p=0.0001, H=12.48), phosphorus (p=<0.0001, H=15.3), potassium (p=0.0004, H= 11.69), calcium (p=<0.0001, H= 15.25) and magnesium (p<0.0001, H=15.25). Means not sharing the same letter are significantly different using the Mann-Whitney test at p<0.05.

Note: Ideal ranges for Fiji soil mineral nutrient content include 0.3-0.6% nitrogen, 20-30 mg/kg phosphorous, 117-234 mg/kg potassium, 400-2000 mg/kg calcium, 122-366 mg/kg magnesium. Source: Koronivia Research Station, Fiji.

rus and potassium (N.P.K) fertilizer was applied in equal amounts (2 g) to each of the plants at the end of the 2-month period. After that, N.P.K fertilizer in a 13:13:21 ratio was applied in equal amounts (2 g) at three months' interval up to 8 months (the end of 5 months and 8 months). Fertilizer treatment was then stopped, and the plants were left to grow in its cultivated environment.

#### *Determination of plant survival and growth rate*

Plant survival rates were calculated by counting the number of noni plants that had survived until the end of the two-year period divided by the number of plants that initially planted (20 plants). The growth rates of plants at the three sites were determined using the plant height. Initial plant height was measured on the day of transplanting, and the final height was measured at the end of the experiment. The plant growth rate in cm per day was calculated by dividing the change in height by the number of days.

#### *Gas exchange measurements*

Photosynthesis rate ( $A_n$ ), transpiration (E) and stomatal conductance ( $g_s$ ) were measured using the portable photosynthesis meter (LCpro-SD by ADC Bioscientific) connected to a broad leaf chamber. At the cultivation plot, mature leaf (fifth fully expanded leaf) from each plant was selected for gas exchange and transpiration measurements. Over the two-year period, gas exchange measurements were done 17 times at random intervals starting from the 5<sup>th</sup> month of growth at the dry, intermediate and wet sites. The instantaneous leaf water use efficiency ( $WUE_i$ ) was calculated as the  $A_n/E$  ratio.

#### *Determination of changes in fruiting, fruit yield and total soluble solids*

The plants started flowering and fruiting by eighth month of growth. Once all plants at the three sites had fully developed fruits, number of fruits (both young and mature) on each plant were counted randomly on eight occasions. Any mature fruit (hard and whitish in colour) present at the time of observation was collected weighed using a top pan balance. The weight of the fruit was recorded as yield in grams. The total soluble solids (TSS) in °Brix was measured using the hand-held refractometer (ATAGO). A total of 10 fruits were randomly collected and sampled.

#### *Determination of antioxidant capacity of fruits*

The total antioxidant activity of fruits from differ-

ent locations was estimated by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method, as described by Yang *et al.* (2011). A total of 10 fruits from each site were used to find out antioxidant capacity where 1 fruit was used as an individual sample. A solution of DPPH was made in methanol using 0.025 g DPPH in 1 L of methanol. Noni fruit was ground, and its juice was extracted using a muslin cloth. Diluted noni fruit extracts (2  $\mu$ L, 5  $\mu$ L, 10  $\mu$ L, 20  $\mu$ L, 30  $\mu$ L and 40  $\mu$ L) were added to the 3 mL DPPH solution and incubated at room temperature for about 40 minutes. After 40 minutes, the absorbance of the mixture was measured at 515 nm with a spectrophotometer (CE1021 UV-VIS). Inhibitions of DPPH radicals in percentage were calculated as follows:

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where:  $A_{\text{control}}$  is the absorbance value of the control reaction (containing all reagents except for the tested fruit extracts) and  $A_{\text{sample}}$  is the absorbance value of fruit extracts.

The 50% radical scavenging activity was determined by calculating the half-maximal inhibitory concentration ( $IC_{50}$ ). The  $IC_{50}$  value was calculated by plotting the percentage inhibition against the concentrations of fruit extracts. The concentration that provided 50% inhibition was noted as the  $IC_{50}$  value. The noni fruit extract concentration at  $IC_{50}$  was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg/ 100 g fresh weight (FW) of fruits. To find out the AEAC, a standard curve was prepared using ascorbic acid at 1mg/mL concentration at various concentrations of 2  $\mu$ g/mL, 3  $\mu$ g/mL, 5  $\mu$ g/mL, 10  $\mu$ g/mL, 20  $\mu$ g/mL, 30  $\mu$ g/mL, 40  $\mu$ g/mL and 50  $\mu$ g/mL. The antioxidant activity of noni fruits was expressed as AEAC per 100 g of fresh weight (AEAC/100 g FW):

$$AEAC = IC_{50} (\text{ascorbic acid}) / IC_{50} (\text{sample}) \times 10^5 (\text{Velde } et al., 2013)$$

#### *Determination of total phenol content in fruits*

Total phenol content (TPC) of noni fruits were determined with Folin-Ciocalteu reagent as described by Yang *et al.* (2011). From each site, 10 ripe fruits were collected, ground, and the juice was extracted using a muslin cloth. For each individual sample, 1 fruit was used. Exactly 20  $\mu$ l of noni fruit extract was mixed with 1.58 mL of distilled water. To this mixture, 100  $\mu$ l of Folin-Ciocalteu reagent and 300  $\mu$ l of 20%  $Na_2CO_3$  was added. The mixture was incubated



at 40°C for 30 minutes. After 30 minutes, the absorbance was measured at 765 nm with the spectrophotometer. A standard curve of total phenols was prepared using gallic acid at various concentrations (1 mg/mL, 2 mg/mL, 4 mg/mL, 6 mg/mL, 7 mg/mL, 10 mg/mL and 20 mg/mL). The equation of the standard curve was used to determine the TPC and it was expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight (mg GAE/100 g of FW).

#### Statistical analysis

Analysis of data collected was performed by using Graph Pad Prism version 7.0 (GraphPad Software Inc, San Diego, California, USA). Shapiro-Wilk test was used to determine the normality of the data. The assumption of homogeneity of variances was tested using Bartlett's test and Brown-Forsythe test at 95% significance level. One-way ANOVA and Kruskal-Wallis test at 95% significance level were used to test the significant differences among the results obtained for the three sites. Significant differences among the treatments were compared using Tukey's test and Mann-Whitney test at  $p < 0.05$ .

### 3. Results

#### Weather and soil attributes of dry, intermediate and wet cultivation sites

The total amount of rainfall for the two-year period was highest for Suva (wet zone) and lowest for Sigatoka (intermediate zone) (Table 1). A distinct difference in rainfall was observed during the dry months (May to October). Highest rainfall level was recorded for Suva area and the lowest level was for Nadi area while Sigatoka area received rainfall in moderate amounts (Table 1). The average temperature and humidity were comparable for the three sites during the two-year study period. Considerable differences were seen in the average sunshine hours for the three sites. Lowest sunshine hours were recorded for the wet zone, followed by the intermediate zone while highest sunshine hours occurred in the dry zone. Considerable differences were also observed for the soil quality among the three sites (Table 1). Soil moisture was highest in the wet site, while it was lowest for the dry site ranging. The wet site had the highest average soil pH and highest EC while the dry site had the lowest. Major mineral nutrition content levels were also different for the

three sites. The dry zone site had considerably low nutrient content, average soil total N, available P and K were lowest. For the intermediate site, nutrient levels were adequate. For the wet zone site, N and K levels were adequate. However, available P, however, was quite low.

#### Plant growth

Survival rate was high in the wet zone and the intermediate zone while it was lowest in the dry zone (Table 2). Decrease in survival rate was due to death of plants that occurred by the end of 9 months. Plant growth rate was highest in the intermediate zone and lowest in the dry zone (Table 2).

Table 2 - The survivability and growth attributes in noni plants in the dry, intermediate and wet cultivation zone

Cultivation zones studied	Survival rate (%)	Growth rate (cm/day)
Dry	45%	0.09 ± 0.009 a
Intermediate	85%	0.23 ± 0.006 b
Wet	80%	0.15 ± 0.011 c

Mean ± SE are shown, n=9 (dry zone), n=15 (intermediate and wet zones). Mean growth rate is significantly different (Kruskal-Wallis test ( $P < 0.0001$ )). For the given cultivation zones, means not sharing the same letter are significantly different using the Mann-Whitney test.

#### Gas exchange attributes

Net photosynthesis ( $A_n$ ), transpiration (E), instantaneous water use efficiency ( $WUE_i$ ) and stomatal conductance ( $g_s$ ) (Fig. 2) were significantly different among the cultivation zone. All three physiological parameters were significantly higher in plants grown in intermediate and wet zones compared to the plants that grew in the dry zone. Dry zone plants had the lowest mean  $A_n$  (Fig. 2A) while plants in the intermediate zone had the highest  $A_n$ . Wet zone plants had comparable mean  $A_n$  rate to intermediate zone. Similar patterns were also observed for E,  $g_s$  and  $WUE_i$ . Mean E recorded was highest for plants growing in the wet zone (Fig. 2B). Plants in the dry area had the lowest mean E. For plants growing in the intermediate E was comparable to the wet zone. Mean  $g_s$  of plants was also significantly different among the three zones (Fig. 2D). Lowest mean  $g_s$  was recorded for the plants in the dry zone while plants in the intermediate and wet zone plants had comparable mean  $g_s$ .  $WUE_i$  was also lowest for plants in the

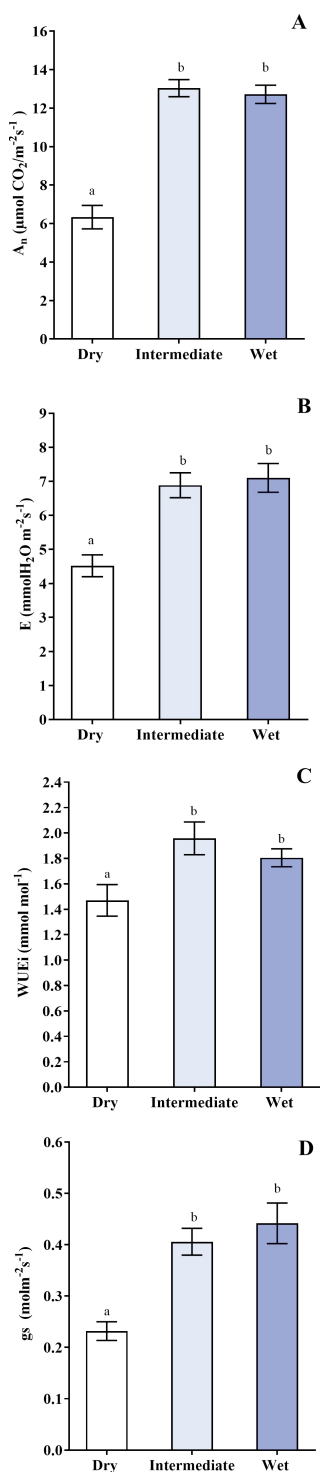


Fig. 2 - Net photosynthesis rate (A), transpiration rate (B), water use efficiency (C) and stomatal conductance (D) of noni plants growing in dry, intermediate and wet cultivation zones. Mean  $\pm$  SE is shown, n=17. There is a significant difference in  $A_n$  ANOVA (F df (2, 48) = 53.95)  $p < 0.0001$ , E ANOVA (F df (2, 48) = 14.77)  $p < 0.0001$ , WUE<sub>i</sub> ANOVA (F df (2, 45) = 5.05)  $p < 0.0104$  and g<sub>s</sub> ANOVA (F df (2, 48) = 5.807)  $p < 0.0001$ . For given cultivation zones, means not sharing the same letters are significantly different using Tukey's test.

dry zone (Fig. 2C). Plants in the intermediate zone had the highest WUE<sub>i</sub>.

### Fruit attributes

Fruit numbers were comparable in the intermediate and wet zone while plants in the dry zone had significantly lower number of fruits (Table 3). Fruit weights were also comparable in wet and intermediate zone while it was lowest in the dry zone (Table 1). Fruit TSS was significantly higher in the dry zone when compared to intermediate and wet zone (Table 3). There was no significant difference in the fruit TSS between intermediate and wet zones.

Table 3 - Mean number, weight and total soluble solids (TSS) of fruits from noni plants in dry, intermediate and wet cultivation zones

Cultivation zones studied	Fruit numbers per plant	Fruit weight (g)	TSS °Brix
Dry	3 $\pm$ 0.25 a	74.7 $\pm$ 3.8 a	8.4 $\pm$ 0.1 a
Intermediate	8 $\pm$ 0.28 b	299.8 $\pm$ 41.5	7.9 $\pm$ 0.1 b
Wet	7 $\pm$ 0.30 b	271 $\pm$ 19.7 b	7.7 $\pm$ 0.1 b

Mean  $\pm$  SE is shown, n=9 (dry zone), n=15 (intermediate and dry zones). There is significant difference in fruit numbers per plant ANOVA (F df (2, 33) = 0.6480, ( $p < 0.0001$ ), fruit weight ANOVA (F df (2, 42) = 11.75, ( $p < 0.0001$ ) and fruit sweetness ANOVA (F df (2, 36) = 0.7563, ( $p < 0.0001$ )). For given cultivation zones, means not sharing the same letters are significantly different using Tukey's test.

### Fruit antioxidant properties

Antioxidant activity (AEAC) in fruits was significantly higher in dry and wet zones compared to the intermediate zone (Table 4). AEAC was comparable for dry and wet zones AEAC while it lowest in the intermediate zone. There was with no significant difference in the mean IC<sub>50</sub> value between the wet and the dry zone as well (Table 4). TPC in fruits was significantly different at the three sites (Table 4). Highest mean TPC was obtained for fruits in dry location followed by fruits in the wet zone. TPC was lowest for fruits from the intermediate zone.

## 4. Discussion and Conclusions

When taking into consideration the climate (rainfall, temperature, humidity and sunshine hours) and the soil features over the two-year experimental period for the three different sites (Table 1), differ-

Table 4 - Total phenol content (TPC), radical scavenging activity (IC50) and antioxidant activity (AEAC) in fruits from dry, intermediate and wet cultivation zones

Cultivation zones studied	Fruit numbers per plant	Fruit weight (g)	TSS °Brix
Dry	3 ± 0.25 a	74.7 ± 3.8 a	8.4 ± 0.1 a
Intermediate	8 ± 0.28 b	299.8 ± 41.5	7.9 ± 0.1 b
Wet	7 ± 0.30 b	271 ± 19.7 b	7.7 ± 0.1 b

Mean ± SE is shown, n = 10. There was a significant difference in TPC ANOVA (F df (2, 21) = 0.2139, (p<0.0001), IC50 values ANOVA (F df (2, 21) = 3.760, (p<0.0001) and AEAC content ANOVA (F df (2, 21) = 17.58, (p<0.0001). For given cultivation zones, means not sharing the same letters are significantly different using Tukey's test.

ences in plant growth rates and the physiological performance is clearly due to the differences in water and nutrient availability. Water availability, together with most required essential nutrients such as N, K, Ca, and Mg was sufficient in the intermediate and wet zones compared to the dry zone.

Highest yielding plants that also maintained higher photosynthesis, transpiration and water use efficiency occurred at the intermediate zone. In comparison to dry and wet zones, this cultivation zone had moderate amount of rainfall and good soil quality overall. Highest plant growth rate (Table 2), high gas exchange (Fig. 2) and high fruit yields (Table 3) in the intermediate zone showed that water availability and nutrients were not an issue for this zone. Even though the annual total rainfall in this zone was less than the dry zone, the dry months (May to October) here had considerably higher rainfall compared to the dry zone and lower rainfall compared to wet zone (Table 1). During the dry months, the zone's total precipitation was 553.5 mm which falls in the range required to grow high yielding noni plants. Nelson and Elevitch (2006), also stated that for high yields, noni cultivation site should receive moderate rainfall preferably 500-1500 mm annually that is distributed evenly throughout the year. Higher growth in the intermediate zone was complemented by the high mineral nutrient content of the soil. Major essential mineral nutrients required for plant growth such as N, K, Ca, Mg including P levels were considerably higher than their ideal range in the soil at this site (Table 1). Water availability was highest in the wet cultivation zone together with adequate soil nutrients (Table 1). This site had the highest mean rainfall within the two-year period (Table 1). The soil

N and K content were comparable to the intermediate zone. Ca, and Mg were considerably higher in this zone (Table 1). P content, however, was lowest in the wet zone. With generally good soil properties plus plentiful of water, the growth and physiological performance of noni in the wet zone were expected to be much higher. Sunshine hours of 4.9 hours (Table 1) in the wet zone was lowest compared to the other zones as the area had cloudy and rainy days more often. Similarly, transpiration rates and  $WUE_i$  were also comparable between the two zones. One crucial factor that may have influenced the physiological performance of noni plants in this zone by limiting photosynthetic activity is the much lower soil P content (Table 1). Short-term inadequate supply of P limits the photosynthesis rates due to restriction of photophosphorylation during the light reactions (Rychter and Rao, 2005). However, the effect of low P content on the physiology of noni is not that strongly evident. Phosphorous deficiency symptoms were not seen on the leaves as well. Noni may have some adaptive mechanism for surviving in P limited soils. Mo *et al.* (2019), showed that some tropical lowland forest plants are able to acclimatize to low P levels by changing the foliar P allocation to fulfil the P demand for photosynthesis. Acclimatization of noni plants to low phosphorous levels requires further investigation. The dry cultivation zone had significantly lower water and nutrient availability (Table 1). The combined effects of water deficit and nutrient deficiency stress lowered the growth of noni in the dry zone. Low photosynthesis and transpiration rates, together with low  $WUE_i$  of plants (Fig. 2) in this agro-climatic zone can also be attributed to both water and nutrient deficiency. The climatic conditions on days of measurement, especially during the dry season were optimal (i.e. fine sunny days) for high transpiration rates at the dry site, but the rates were significantly low. This was mainly due to partial stomatal closure as indicated by the low stomatal conductance (Fig. 2D). Low stomatal conductance indicates a low degree of stomatal opening, resulting in low rates of incoming  $CO_2$  and outgoing water vapour. The stomatal conductance is usually higher in K deficient plants (as was the case here) under drought stress since K deficiency impairs stomatal function by signalling for ethylene production which in turn inhibits the action of abscisic (ABA) on stomata delaying the closure (Wang *et al.*, 2013). However, low K levels in the dry zone did not appear to influence the stomatal closure. During drought stress in a drought-resistant

plant, ABA level initially increases leading to stomatal closure, but as the drought stress is continued, ABA levels decrease markedly, and stomatal closure becomes water potential-driven (Brodribb and McAdam, 2013). This strategy allows the plants to respond to any rainfall quickly and open the stomata much faster for gas exchange than a non-drought tolerant plant. Drought tolerant plants are also able to maintain gas exchange to gain carbon for a longer period of time during drought (Tardieu and Davies, 1993). For drought tolerant plants, complete stomatal closure is avoided or delayed during drought stress, this helps to maintain the carbon balance (Brodribb and McAdam, 2013). This appeared to be the case for noni plants growing in the dry zone as the stomatal conductance measurements of noni leaves over the two-year period did not show complete closing of stomata in the dry zone. Noni has also been claimed to be a drought-tolerant plant (Nelson, 2003; Singh and Rai, 2007).

Being drought-tolerant,  $WUE_i$  in noni plants from the dry area was also expected to be comparable to wet and intermediate zone plants. However, the  $WUE_i$  was significantly lower in the dry zone (Fig. 2C) indicating that carbon gain per water loss was low, which resulted due to the low photosynthesis rates and subsequently smaller plants. Nutrient deficiency stress being an add-on to water stress in the dry zone limited photosynthesis activity leading to low  $WUE_i$ , hence overall productivity of the plant was low. A decrease in photosynthesis was also due to low amounts of  $CO_2$  entering the leaves as a result of low stomatal conductance. In addition, an increase in leaf temperature may have enhanced the suppression of photosynthesis in the dry zone. According to Haldimann *et al.* (2008), reduced stomatal conductance and reduced transpiration rate raise leaf temperature by several degrees resulting in suppression of photosynthesis due to reversible inactivation of Rubisco. There was no considerable difference between the mean air temperature and the maximum air temperature between the three zones (Table 1). However, an increase in leaf temperature in the dry zone may have resulted from significantly longer sunshine hours (Table 1). Marias *et al.* (2017), showed that an increase in leaf temperature due to reduced stomatal conductance could be very dramatic in the full sun compared to partial sun.

High fruit yield in the intermediate zone (Table 3) also showed that climate and soil features of the intermediate zone were ideal for growing noni under

rain fed conditions. Effects of water stress on fruit production and yield is highly evident as seen from the lowest yield in the dry zone. Similar results under drought conditions have been reported for apples (Yao *et al.*, 2001), peach (Rahmati *et al.*, 2018), citrus (Huang *et al.*, 2000), oranges (Perez-Perez *et al.*, 2009) and tomatoes (Sivakumar and Srividhya, 2016). According to Rahmati *et al.* (2018), fruit sizes during drought stress decrease because of reduced water flow to the fruits due to fruit stomatal closure, development of thick cuticles or due to reduction in micro-crack occurrences. Nutrient deficiency can also be a factor contributing to fruit size as fruit size is also depended on the number of cells at anthesis (Bohner and Bangerth, 1988). Ca and K, which have crucial roles in cell division and stomatal conductance respectively are lower in the dry zone leading to smaller fruit size. Despite being smaller in size and producing a low number of fruits per plant, fruit sweetness (in terms of TSS) was not low as expected. Interestingly, it was significantly higher compared to the intermediate and the wet zones (Table 3). Increase in fruit sweetness under moderate water stress has been reported for tomatoes (Veit-Kohler *et al.*, 1999; Bertin *et al.*, 2000; Nahar and Ullah, 2018), peach (Kobashi *et al.*, 2000), plums (Maatallah *et al.*, 2014) and nectarines (Thakur and Singh, 2012). Sugar levels notably increase under drought stress to affect osmotic potentials and high sugar levels in cell vacuoles helps to produce high turgor pressure (Ma *et al.*, 2017).

Despite excellent physiological performance and yield, fruit antioxidant properties were lowest in the intermediate zone (Table 4), which clearly indicated that plants in this zone were not significantly affected by any abiotic stress conditions. Significantly higher TPC and ACEA in dry and wet zones (Table 4) indicated considerable abiotic stress. Low antioxidant properties of fruits in the intermediate zone on the other hand clearly indicated that the plants at the intermediate zone were not significantly affected by any abiotic stress conditions. Antioxidant compounds in plants increase in response to abiotic stress. Phenolic compounds are powerful antioxidants that protect plants from oxidative stress by scavenging harmful reactive oxygen species (ROS) under different abiotic and biotic stresses (Balasundram *et al.*, 2006; Lattanzio *et al.*, 2006; Li *et al.*, 2012; Kulbat, 2016; Naiko *et al.*, 2019; Samec *et al.*, 2021;). Highest phenol content in the dry zone is undoubtedly due to antioxidant defense activation. An increase in pheno-



lic compounds under drought stress has been also reported for fruits such as mulberry (Khamjad *et al.*, 2021), grapevine (Irani *et al.*, 2021), pomegranate (Farji *et al.*, 2020) and strawberries (Unal and Okatan, 2023). The increase in antioxidant properties may also be in response to high oxidative stress caused by adverse environmental conditions created by nutrient deficiency, high temperature and high UV light (high sunshine hours) (Das and Roychoudhury, 2014). Comparable and high antioxidant properties in the wet and dry zone (Table 4) indicated that plants were under considerable environmental stress. Abiotic stress in the wet zone can be attributed to either excessive precipitation or low phosphorous availability, as discussed earlier. Alfaro *et al.* (2013) reported an increase in polyphenol content and antioxidant activity in murtilla fruits with an increase in rainfall. Extreme precipitation in the area may have also resulted in soil compaction lowering the oxygen levels in the soil leading to activation of the antioxidant defense system. ROS generation also occurs during oxidative stress due to hypoxia (lack of oxygen) which can result from soil compaction (Blokina *et al.*, 2003; Ali and Alqurainy, 2006). Vergara *et al.* (2012), also found activation of the antioxidant defense system in grapevines in response to hypoxia. P deficiency stress, as discussed earlier, maybe the second reason for noni plant stress and high antioxidant production in the wet zone. Increase in antioxidants in response to phosphorous deficiency has been reported by Tewari *et al.*, (2007); Zhang *et al.*, (2014) and Joel *et al.* (2017). Mineral deficiency stress can also be added to the list of stresses producing high antioxidants in fruits from the dry zone. Tewari *et al.* (2007), showed that antioxidant activity increased in mulberry plants under N, P and K deficiencies.

In conclusion, this study showed that the physiological performance, fruit yield and antioxidant properties of cultivated noni were significantly influenced by water availability and soil nutrient content. The physiological performance including fruit yield was optimum in the intermediate zone which was characterized by a moderate rainfall and fairly good soil properties while poorest physiological performance was in the dry zone. Fruit yield was highest in the intermediate zone with lowest antioxidant properties while it was lowest with highest antioxidant properties in the dry zone. Overall the physiological performance plus fruit yield and antioxidant properties were greatest in the wet zone where plants appear to have a moderate level of abiotic stress. This study

also implies that under cultivation, moderate abiotic stress can enhance the antioxidant properties of noni.

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