Effect of Water Deficient Stress on Early Vegetative Growth Stage of Black Pepper (*Piper nigram* L.)

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ABSTRACT

Water deficient stress is one of the key abiotic stresses affecting plant growth including black pepper (*Piper nigram* L.) growth. With the projected climate change, it is important to screen climate resilient black pepper cultivars to sustain the productivity. Accordingly, this study was conducted to identify the plant response to water deficient stress at the early vegetative growth stage of two pepper cultivars namely; Panniyur-1 and local in a green house. Pepper plants that are ready for field planting were subjected to five water deficit regimes *viz.* soil field capacity (FC; control), 80, 60, 40 and 20% of the FC. Growth parameters (shoot length, root length, root volume, number of leaves, leaf area), total chlorophyll content, shoot and root dry matter content were evaluated at 25 day intervals under the five moisture levels. The results indicated that the moisture stress reduced the plant growth, and moisture deficient stress induced growth retardation was clearly visible at 75 days. From the tested cultivars of pepper, Panniyur-1 performed better compared to local under all the moisture levels in terms of higher leaf area, higher shoot dry matter and higher root dry matter except 20% FC level in which the local cultivar showed higher values. The effect of water stress differently affected the root growth compared to shoot growth. Even though the root growth was poor under 20% FC moisture, it was fluctuated in varying degrees under 80, 60 and 40% FC moisture levels compared to control. With regards to the leaf number, water stress did not significantly affect the number of leaves in the local cultivar whilst it reduced in Panniyur-1 under 20% FC moisture. These findings can be considered when screening pepper cultivars for drought tolerance in the future.

KEYWORDS: Cultivar, Early growth stage, Moisture stress, *Piper nigram* L., Vegetative growth

INTRODUCTION

Black pepper (*Piper nigram* L.) is a perennial climber belonging to the family Piperaceae and it is the most widely used spice in the world which is popular as the "king of spice". Sri Lanka is the world's 6th largest black pepper producer contributing 5.7% to the world's pepper production in 2014 (Anon, 2014). In Sri Lanka, black pepper is mainly cultivated in low and mid country, wet and intermediate agro climatic zones (Anon, 2009).

Black pepper is mainly grown as a rain-fed crop in Sri Lanka. Total rainfall and its distribution play an important role in pepper growth and productivity. The rainfall requirement of the crop varies from 2000 to 3000 mm per annum. However, with the global climatic changes, rainfall patterns have become unpredictable and the frequency of drought has increased (Niranjan *et al.*, 2013). Sri Lanka is more vulnerable to impacts of climate change such as changes in temperature, amount of precipitation and pattern, increase in extreme climate events and rise in sea level (Sugirtharan and Venuthasan, 2012).

These factors highlight the need of climate resilient cultivars of pepper which are better adapted to the drought condition. These selected cultivars could be used as parent material for developing new cultivars that perform better under the changing climate in Sri Lanka (De Costa, 2010).

Currently, introduced pepper cultivars (Panniyur-1 from India and Kuchin from Malaysia) and superior quality local selections are popular among Sri Lankan farmers (Anon, 2009). However, there are limited researches that have been conducted related to drought tolerance and plant response to moisture stress in different black pepper cultivars. Such studies would provide new directions to varietal improvement in black pepper under changing climate.

Therefore, this research was conducted to identify the plant response to water deficient stress in the early vegetative growth of two pepper cultivars, Panniyure-1 and a local selection. This study primarily focused to assess the early vegetative growth of these two cultivars under different water levels.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out from December 2015 to May 2016, at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) which is located Low Country Intermediate Zone (IL_{1a}).

Planting Materials and Layout

Two cultivars, namely; Panniyur-1 and, a local selection were selected for the study. Healthy, homogeneous nursery plants from both cultivars that were ready to field planting were obtained from Plant Production and Tissue Culture Research Centre, Department of Export Agriculture, Walpita.

Nursery plants that were transported to the University were kept nearly two weeks before transplanting for acclimatization. Then they were transplanted in black polythene bags (10"×15") that were filled with recommended soil mixture used in field planting of pepper (Anon, 2009) two weeks before transplanting. Each vine was tied to a peeled gliricidia (*Gliricidia sepium*) pole which was established in the corner of the polybag. Re-potted polybags were arranged in Completely Randomized Design (CRD) in the greenhouse.

Treatments

In growth experiments, plants were subjected to five water deficit regimes with five replicates. The soil field capacity (FC) served as the control (T_1), 80% (T_2), 60% (T_3), 40% (T_4), and 20% (T_5) FC water regimes served as different water deficit conditions (Boutraa *et al.*, 2010). All pots were weighed on four day intervals. The loss in pot weight represented transpiration and evaporation. Cumulative water loss was added to each pot to compensate the transpiration and evaporation. Accumulated water loss was calculated as the differences in pot weights between successive weights (Akhkha *et al.*, 2011).

Measurement and Data Analysis

Un-destructive sampling was done to measure plant height (collar region of the plant up to the terminal bud) and leaf number (every unfolded leaf was counted) throughout the experiment period. Destructive sampling was done at 25, 50 and 75 days after the commencement of the experiment to take the length (grid paper) and volume of roots (Sumanasena, 2003), oven-dried biomass (80 °C for 48 h) of roots and shoots, leaf area (leaf area meter, LI- 3100C) and chlorophyll content (UV visible spectrophotometer, UV160A) (Parthasarathy *et al.*, 2008) of leaves. Data were analysed using analysis of variance (ANOVA) using Minitab software version 16.

RESULTS AND DISCUSSION

Variation of Growth Parameters

Shoot Length

The shoot length significantly varied within treatments, time and the cultivar ($P<0.05$). From the two cultivars tested, Panniyur-1 showed higher shoot length compared to the local all the time; and shoot length in both cultivars significantly increased with time. There was no significant difference in the shoot length of local cultivar within the treatments at 25 and 50 days after commencement of the treatments. However, a significant difference was observed at 75 days after commencement of the treatments in the local cultivar in which, plants treated with FC (T_1) and 80% FC (T_2) showed higher shoot length (90 cm and 88 cm respectively) compared to that of other moisture levels. Significantly lowest plant height in the local cultivar (64 cm) was recorded in plants treated with 20% FC when compared to all other treatments except T_4 (40% FC; Figure 1a).

Panniyur-1 showed significant difference in the shoot length within the treatments at 50 days from the commencement of the treatments with the highest shoot length (86 cm) in T_1 and the lowest (44 cm) in T_5. At 75 days after the treatments, both T_1 and T_2 showed higher shoot length (112 cm and 104 cm respectively) compared to other treatments and the lowest (56 cm) was observed in T_5 (Figure 1b).

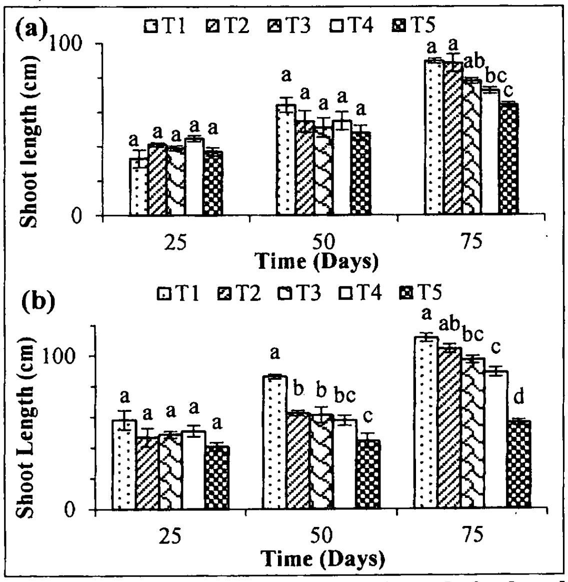

Figure 1. Variation of shoot length in local (a) and Panniyur-1 (b) within the treatments with time. *Means with the same letter within each time are not significantly different at 0.05 confidence levels. T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC*

Root Length and Volume

During the first 25 days, the highest overall root length was reported in T_2 (80% FC) followed by T_1 (FC), T_3 (60% FC), T_4 (40% FC) and the lowest root length was observed in T_5 (20% FC). The overall root length of the plants treated with T_2, T_1 and T_4 were higher compared to those of T_3 and T_5 at 50 days from the commencement of treatment; however those were not significantly different from each other.

The lowest overall root length was recorded in the plants treated with 20% FC (T_5) at both 50 days and 75 days from the commencement of the treatments (Figure 2a). Further, Panniyur-1 showed higher overall root length than local cultivar in all the treatments except T_3 (Table 1).

There was a significant difference in the root volume among treatments. The maximum root volume was observed in T_1 (2.1 cm^3) and that was not significantly different from T_2 (2 cm^3) whilst the lowest root volume was observed in T_5 (1.3 cm^3) and T_3 (1.3 cm^3) (Figure 2b). From the two cultivars tested, Panniyur-1 showed higher root volume than local in all five treatments (Table 1).

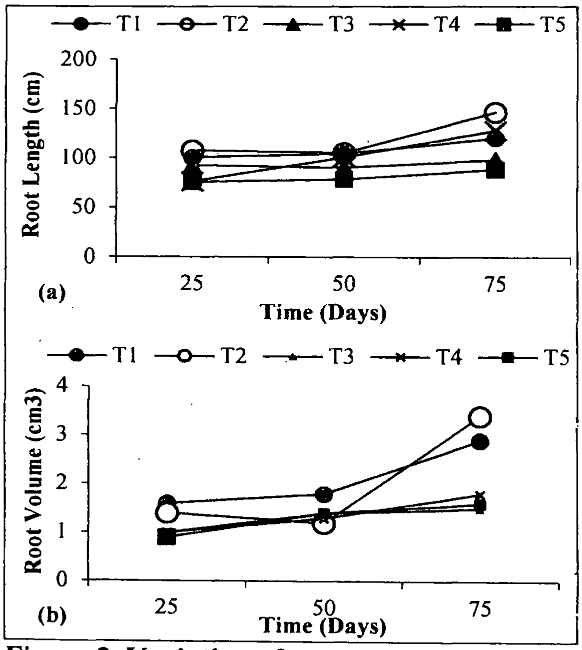

Figure 2. Variation of average root length (a) and average root volume (b) among five treatments with time. *T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC*

Table 1. Average root length and average root volume in two cultivars under different treatments

Treatment	Average root length (cm)	
	Local	Panniyur-1
T_1	104.6±3.94 ^{bc}	112.3±4.45 ^{b}
T_2	98.5±6.35 ^{bcd}	141.3±5.33^{a}
T_3	106.5±3.44 ^{bc}	81.2±2.11 ^{de}
T_4	97.5±3.43 ^{bcd}	106.3±9.0 0^{bc}
T_5	69.2±2.85 ^{c}	92.3±4.17 ^{cd}
	Average root volume (cm^3)	
	Local	Panniyur-1
T_1	1.7±0.17 ^{b}	2.5±0.19 ^{a}
T_2	1.4±0.11 ^{b}	2.6±0.47 ^{a}
T_3	1.1±0.10 ^{b}	1.5±0.09 ^{b}
T_4	1.2±0.03 ^{b}	1.6±0.16 ^{b}
T_5	1.2±0.10 ^{b}	1.4±0.09 ^{b}

Means with the same letter within the cultivars are not significantly different at 0.05 confident levels. T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC

Number of Leaves and Leaf Area

The number of leaves in the local variety did not significantly vary within the treatments at all times ($p>0.05$). However, Panniyur-1 showed differences in leaf numbers within the treatments at 25 and 75 days, where plants treated with 20% of FC produced the lowest number of leaves (8 and 12 respectively; Table 2).

Even though the number of leaves in the local cultivar did not show a differences within the treatments, a significant difference ($P<0.05$) could be observed in treatments with respect to the leaf area in both local and Panniur-1 cultivars. This variation was clearly observed during 75 days from the commencement of treatments, where in local cultivar, the highest leaf area was recorded in the plants treated with FC moisture (T_1) and 80% FC (T_2) (530.8 cm^2 and 476.4 cm^2 respectively) compared to all other treatments (Figure 3a) and in Panniyur-1, the highest leaf area was recorded in T_1 followed by T_2, T_3, T_4 and T_5 (Figure 3b). Plants treated with 20% FC moisture level showed the lowest leaf area in both cultivars (Panniyur-1: 342.8 cm^2 and Local: 369.2 cm^2; Figure 3a and b). When varietal difference in leaf area is considered, Panniyur-1 recorded higher leaf area compared to local in all the treatments except T_5 (20% FC).

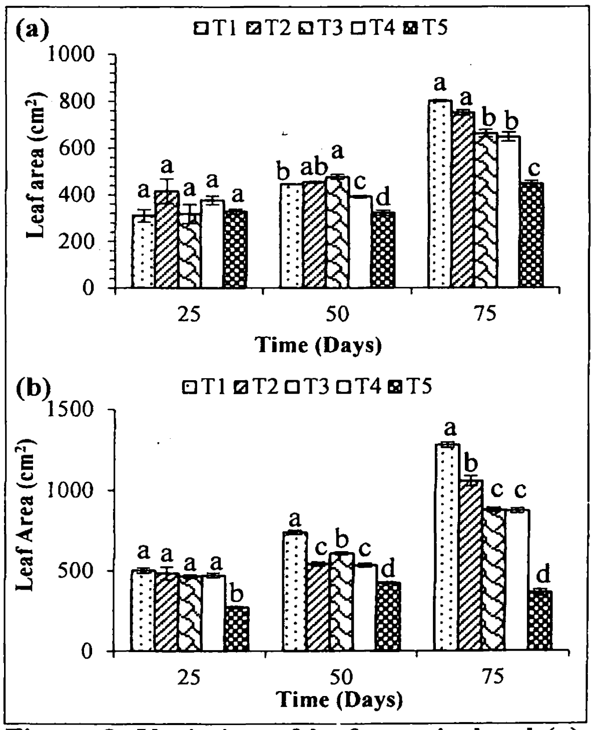

Figure 3. Variation of leaf area in local (a) and Panniyur-1 (b) among the treatments. *Means with the same letter within each time are not significantly different at 0.05 confidence levels. T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC*

Table 2. Variation of number of leaves in local and Panniyur-1 within the treatments

Trt	25 Days		50 Days		75 Days	
	L	P	L	P	L	P
T_1	08± 1a	12± 1a	11± 1a	15± 1a	16± 1a	18± 1a
T_2	10± 2a	09± 2ab	12± 1a	11± 2a	16± 2a	19± 1a
T_3	09± 1a	09± 1ab	11± 2a	14± 3a	15± 2a	16± 1a
T_4	10± 2a	11± 1ab	11± 1a	12± 1a	14± 1a	16± 2a
T_5	09± 1a	08± 2b	11± 1a	11± 2a	13± 1a	12± 1a

Means with the same letter within the variety are not significantly different at 0.05 confidence levels. L- Local, P- Panniyur-1, T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC

Variation of Plant Dry Matter

Shoot Dry Weight

Shoot dry weight was significantly affected by moisture deficient stress in both cultivars ($p<0.05$). The highest shoot dry weight was observed in Panniyur-1 under FC moisture (T_1) compared to local. When the plants were subjected to water deficient regimes, shoot dry weight was significantly reduced in both cultivars and the lowest shoot dry weight was shown by panniyr-1 (3.9 g/plant) and local (4.9 g/plant) treated with 20% FC moisture (Figure 4). Shoot dry matter content in plants treated with 80% (T_2) and 60% (T_3) FC moisture were not significantly different whereas, plants treated with 40% FC moisture (T_4) recorded significantly lower shoot dry weight than T_2.

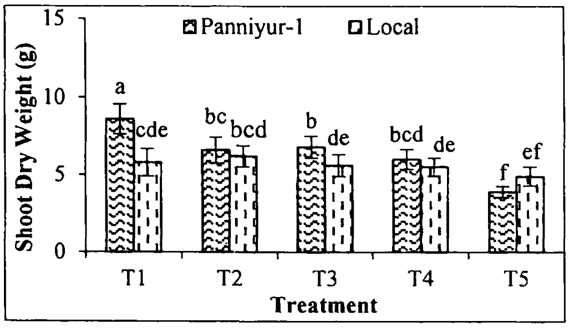

Figure 4. Variation of shoot dry weight in two cultivars under different water stress regimes. *Capital letters indicate the significance among the treatments and simple letters indicate the significance within the cultivars. Means with the same letter are not significantly different at 0.05 confidence level. T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC*

Root Dry Weight

Similar to shoot dry weight, the highest dry weight of roots (1.1 g/plant) was observed in Panniyur-1 that was treated with FC moisture (T_1) compared to that of local cultivar (0.8 g/plant). Root dry weight in all other treatments was not significantly different to each other. With regards to the cultivar effect on root dry matter under different moisture stress regimes, Panniyr-1 showed higher root dry weight in all the treatments except T_5 (20% FC) than the local (Figure 5).

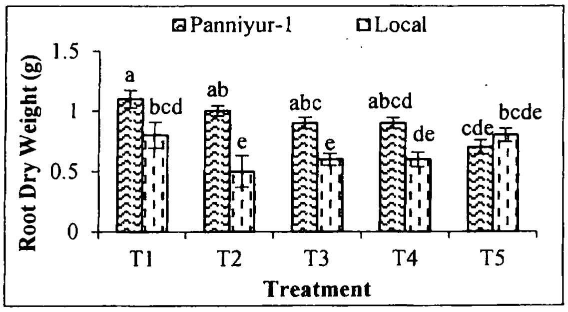

Figure 5. Variation of root dry weight gained by two cultivars under different water stress regimes. *Capital letters indicate the significance among the treatments and simple letters indicate the significance within the cultivars. Means with the same letter are not significantly different at 0.05 confidence levels. T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC*

Chlorophyll Content

With regard to the chlorophyll content, the highest value was observed in local cultivar treated with FC moisture (T_1; 1.5 mg/g of FW) compared to T_5 and that was higher compared to Panniyur-1 (1.3 mg/g of FW). Both cultivars showed lower chlorophyll content, when the plants were treated with 20% FC (Panniur-1: 0.9 mg/g of FW and Local: 1.0 mg/g of FW; Figure 6).

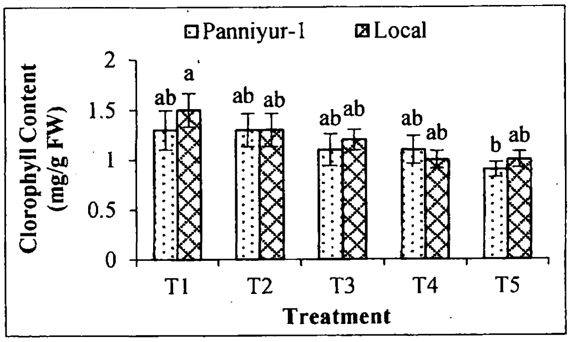

Figure 6. Variation of total chlorophyll content in two cultivars under different water stress regimes. *Capital letters indicate the significance among the treatments and simple letters indicate the significance within the cultivars. Means with the same letter are not significantly different at 0.05 confidence levels. FW- Fresh weight. T_1- Field capacity (FC), T_2- 80% FC, T_3- 60% FC, T_4- 40% FC, T_5- 20% FC*

There are many literature where the effect of moisture deficient stress on plant growth is discussed (Boutraa *et al.*, 2010). However, effect of water stress may depend on the severity of the stress, duration of the stress and the cultivar of the particular plant species

(Boutraa *et al.*, 2010). The study too revealed that the water deficient regimes tested in the study were differently affected for the growth and dry matter content in two black pepper cultivars. With respect to the effect of water deficient stress on shoot length, root length and root volume resulted reduction in those parameters under water deficient conditions compared to the control in both tested cultivars. Literature revealed that drought stress has significant effect on morphological and other growth characters of coffee in their study (Chemura *et al.*, 2014).

Even though the number of leaves was not significant among the treatments, leaf area was gradually reduced when plants were exposed to the water deficient stress. This can be attributed with reduction of cell elongation under water stress that leads to decrease in cell size (Schuppler *et al.*, 1998). In line with this, chlorophyll content also reduced gradually in the plants experienced with moisture stress. Drought stress imposed at the vegetative stage, significantly decreased chlorophyll a, b and total chlorophyll content both at the vegetative and flowering stages in plants (Mafakheri *et al.*, 2010).

CONCLUSIONS

Plant growth parameters and dry matter contents were gradually decreased in two cultivars when the degree of drought stresses increased. From the two cultivars tested, Panniyur-1 performed better in all the moisture levels except 20% FC moisture in which local performed better than Panniyur-1 in terms of higher leaf area, higher shoot dry matter and higher root dry matter. The effect of drought clearly visualized by the plants at 75 days after the commencement of treatments compared to 25 and 50 days. Therefore, this experiment has to be extended further to evaluate both morphological and biochemical changes under different water deficient regimes.

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