DOI: http://doi.org/10.4038/jrrisl.v97i0.1879

Journal of the Rubber Research Institute of Sri Lanka (2017) **97**, 21-34

Role of salicylic acid in irrigation scheduling for rubber nurseries in the Intermediate zone of Sri Lanka

S A Nakandala*, K D N Weerasinghe, P Seneviratne*, S M M Iqbal*** and P D Pathirana***

* *Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka*

** *Department of Agricultural Engineering, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka*

*** *6/10 Rajamahavihara Road, Pitakotte, Sri Lanka*

Abstract

Salicylic acid (SA)is an important signaling molecule known to alleviate the negative effects of drought stress in plants. The present study was conducted in a protected house at Moneragala substation of Rubber Research Institute of Sri Lanka to examine the possibility of using salicylic acid to minimize the net irrigation water requirement (NIR) of rubber nurseries in the Intermediate Zone of Sri Lanka which has the sub-optimal environmental condition for rubber. A split-plot design with the treatment combination of different soil moisture depletion levels of irrigationnamely10%, 30%, 50%, 70%, and 90%) and salicylic acid (0.5 mM) application frequencies (once in two weeks, once a month, once in three months and no application (control)). The effect of treatment combination was assessed on morphological and physiological growth attributes of seedling rubber plants. NIR was estimated based on maximum allowable depletion level, crop evapotranspiration (ET_c) and crop factor in irrigation scheduling.

Results revealed that the growth of nursery plants with irrigation at a 10% moisture depletion level was greater those. Moderate to severe water stress conditions were developed with the moisture levels at 50 to 90% moisture depletions respectively. The plants treated with 0.5 mM SA at monthly intervals have shown a higher growth when compared with bi-weekly or once in three months SA applications. Therefore, plants treated with 0.5 mM SA as monthly application at 50 % moisture depletion level exhibited higher tolerance to water stress compared to non-treated plants. Estimated NIR has shown that SA applications have had significant effect on lowering ET_c of rubber nursery plants and reduced the NIR of the rubber nursery by 25% of the total irrigation requirement.

***Key words**:* abiotic stress, drought tolerance, rubber and salicylic acid

Introduction

Water is one of the most essential substances for the existence of plant life. Water helps to dissolve nutrients in the soil solution which enables plant uptake. It is a reactant in many metabolic reactions in plant cells namely; photosynthesis, respiration and other enzymatic activities. Another important function of water is the thermoregulation, through the process called transpiration. In this process, a large amount of heat energy is released from the plant maintaining its temperature at a tolerable range (De Costa, 2001). Plant-water relationship is considered an important aspect in crop production under all types of climatic conditions. Therefore, a supplement of water from outside known as irrigation performs an important role in plant growth and development. A sufficient amount of rainfall supports agricultural crop production throughout the year. In many areas in wet zone of the country, receive an adequate amount of rainfall for two growing seasons per year. However, irrigation is necessary to supply moisture for plants whenever the rainfall and soil water storage are insufficient for crop growth (Kramer, 1983).

The ideal annual rainfall for growing rubber is 1650 to 3000 m which should be evenly spread throughout the year (Yogaratnam, 2001). Therefore, rubber grows well in the Wet Zone. However, there is a potential to grow rubber under sub-optimal conditions in the Intermediate Zone and some parts of northern regions of Sri Lanka. When the annual variation of rainfall in the Intermediate Zone is considered, nearly 60% of the rainfall experiences during the North-East monsoon in a range of 1200 – 2500 mm. Rainfall received during South-West monsoon is less than 1000 mm and a lesser amount is received subsequent months from May to September. Therefore, the probability of occurrence of dry months in the Intermediate Zone from May to August every year is well defined. The effect of the drought depends on the duration and the severity of the drought (Wijesuriya *et al.*, 2010). The influence of long drought with severe growth reduction and longer immaturity periods of rubber plants as a results of long period have been reported by many researchers (Samarappuli *et al.*,1996, Vijayakumar *et al.*, 1998 and Iqbal *et al.*, 2010).

Prolonged drought impairs many physiological and metabolic processes in plants, which may lead to reduced plant growth and development than any other abiotic stresses (Anosheh *et al*., 2012). Many recent research studies have reported that salicylic acid is an important signal molecule known to modulate plant responses to abiotic stresses such as drought, temperature stress, etc. Salicylic acid has an ability to confer stress tolerance in plants (Senaratna *et al*. 2000; Anosheh *et al.*, 2012 and Habibi, 2012). Results of recent research carried out in rubber nurseries revealed that the exogenous application of salicylic acid alleviates water stress in young plants while improving their growth attributes (Nakandala *et al.*, 2016). Since the government has decided to expand rubber cultivation into non-traditional areas, sustainable planting materials production has become very important. Irrigation is, therefore, a necessity in those drier areas to ensure high-quality and healthy plants. Since water has been a scarce resource and also to improve the water use efficiency in plants, it is very important to have proper water management practices with the selection of an appropriate irrigation method for rubber nurseries.

Hence, this present study aim to examine the possibility of using salicylic acid in irrigation scheduling to minimize the net water requirement for rubber nurseries in the Intermediate Zone of Sri Lanka as a novel approach of irrigation technology.

Materials and Methods

The experiment was designed under protected conditions at Substation of Rubber Research Institute of Sri Lanka which is located in Moneragala District (060 50/ 06//N Latitudes and 810 18/ 55//E Longitudes). It belongs to the agro-ecological region of the Intermediate zone, IL1c (Wijesuriya *et al*., 2010).

A young budding nursery having 1000 one-month-old seedling plants was established in the protected house at Moneragala substation. Plants were arranged in a split-plot design with 20 treatment combinations which replicated three times. The treatments were five soil moisture depletion levels (10%, 30%, 50%, 70%, and 90%) of irrigation and four salicylic application frequencies (*i.e.;* once in two weeks, once a month, once in three months and no application) arranged in main and sub-plots respectively. Salicylic acid at a concentration of 0.5mM was applied as soil drench application for each plant before the irrigation was done. Preliminary testing on texture, bulk density, field capacity (FC), permanent wilting point (PWP), moisture depletion level and net irrigation requirement were done as pretest to determine the characteristics of soil, used in preparation of poly seedling nursery.

Plant stem diameter at 1 cm from the base of the seedling was measured by using a digital venire caliper. Leaf chlorophyll content was measured by using a chlorophyll meter (SPAD 502DL Plus). The meter reads chlorophyll content by an index value [SPAD (soil-plant Analyses Development) value] that is proportional to the amount of chlorophyll content of leaves. Dry matter content of the stem, leaves, and roots were assessed at the bud grafting stage. Samples were oven-dried at 85 ^{0}C for 48 hours until reaching a constant weight. Dry weights of each five plants were recorded and shoot: root ratio were calculated accordingly.

The available soil moisture content in 10 cm depth of poly bags was measured daily using a theta probe (ΔT Model DL6) with an external moisture meter. Statistical analysis was done by the analysis of variances followed by a mean separation procedure and Duncan's Multiple Range Test (DMRT), at a probability level of 0.05. SAS statistical software package – version 9.0 (SAS Inc., USA) was used to analyze data.

Estimation of net irrigation requirement of rubber nursery

Daily rainfall, maximum and minimum temperatures, pan evaporation and wind velocity were recorded at the Meteorological station at Moneragala. Net irrigation requirement (NIR) of the rubber nursery was estimated by using following equations which determine available soil moisture content, crop evapo-transpiration (ET_c), reference evapo-transpiration (ET_0) and dependable rainfall of irrigation scheduling as described in FAO Irrigation and Drainage paper 56 (Allen *et al.*, 1998).

- ***Available soil moisture content (D)***

D = (FC-PWP).BD.h/100 (Eq. 1)

Where,
D = Avalable soil moisture content (cm)
FC = soil moisture % at field capacity
PWP = soil moisture % at permanent wilting point
BD = Bulk density of soil (gcm^{-3})
h = Root zone depth (cm)

- ***Crop evapo-transpiration (ET_c)***

ET_c = ETo x Kc (Eq.2)

Where,
ET_c - crop evapotranspiration (mm/day)
K_c - crop coefficient
ET_o - reference evapotranspiration (mm/day)

- ***Reference evapotranspiration (ETo)***

ET_0 has been calculated by using average pan evaporation data. Pan co-efficient (Kp) for the Class A pan was used to determine ETo as described in FAO Irrigation and Drainage paper 56 (Allen *et al.*, 1998).

ET_0 = Epan x Kp (Eq.3)

Where,
ETo - reference evapotranspiration (mm/day)
Kp - pan coefficient (0.8)
Epan - pan evaporation (mm/day)

- ***Monthly dependable rainfall (DF)***

Monthly dependable rainfall was calculated by Weibull's method for a period of 15 years at a 75% expectancy level. The computation was carried out as described below:
The annual rainfall data set was arranged in the descending order of rainfall magnitudes. Data were ranked as, m = 1, 2, 3 up to the last record. Cumulative Probability (*P*) was determined by using the following equation (Eq. 4)

$$P = \frac{M}{N+1} \text{ x } 100 \qquad \text{(Eq. 4)}$$

Where,
M = rank number
N = total number of rainfall records

- ***Net irrigation requirement (NIR) of rubber nurseries***

NIR = ET_C – DF (Eq. 5)

Where,
NIR - Net irrigation requirement for a given month (mm/month)
DF - Effective rainfall for a given month (mm/month)
ET_c - Crop Evapotranspiration (mm/month)

Results

Soil moisture depletion pattern

Figure 1 illustrates the soil moisture depletion pattern for each depletion level at 10 cm depth of soil in the nursery plants one month before the salicylic acid application. Daily moisture measurements in the soils for a month were taken by using a Theta probe. The data were expressed as percentage of moisture availability in soil on volume basis and build up a moisture curve under each depletion level.

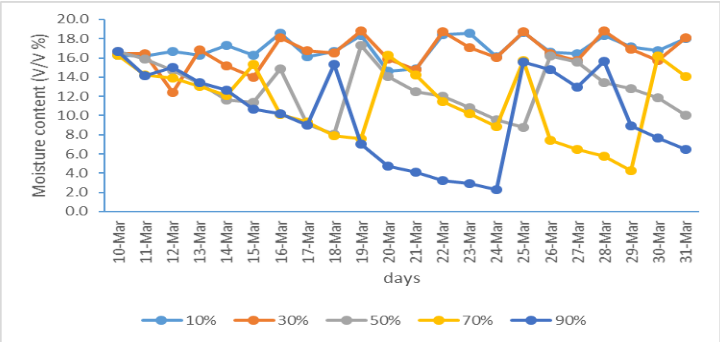

Fig. 1. Moisture curves at different soil moisture depletion levels

According to the result, soil moisture reached 17% when the soil was irrigated up to FC and it reached 4%, which was identified as the wilting point of seedling plants at higher depletion levels 70 to 90% (Fig. 1). Depletion levels also represent the intervals of irrigation scheduling. For example, at 30% depletion level, eight irrigations were carried out in a month with two-day intervals. Similarly, five- and four-times of irrigations were carried at 50 and 70% depletion levels with four to five days irrigation intervals, respectively (Fig. 1).

Growth responses of plants

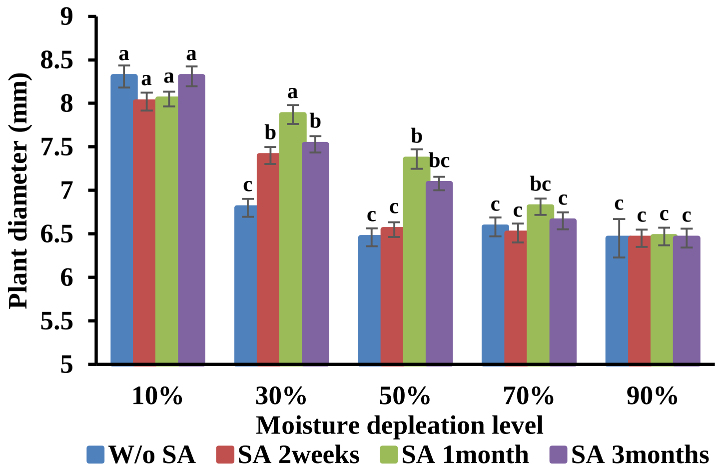

Fig. 2. The effect of frequency of application of salicylic acid (SA) under at different moisture depletion levels on plant stem diameter

The values are means and SE of n = 10 seedlings after 3 months of SA application for four months old seedling. Values followed by the same letter are not significantly different at (P≤0.05)

Treatments effect on plant stem diameter is depicted in Figure 2. Plants that were irrigated frequently at 10% depletion level indicate a higher growth ($p \leq 0.05$) irrespective of SA application. Plants that were irrigated at 30% depletion level and treated with SA at monthly intervals also showed a higher plant diameter ($p \leq 0.05$) compared with other SA application intervals and similar trend was also found at 50% depletion level. However, plants that were exposed to moisture stress at 70% to 90% depletion levels showed a notable decrease in stem diameter irrespective of the application of salicylic acid.

Leaf chlorophyll content

As shown in Figure 3, chlorophyll content is higher in the well-watered plants irrespective of SA applications. Leaf chlorophyll content reduced gradually with the increased of soil moisture depletion showing the highest reduction at 90% soil moisture depletion level. Moreover, a mild chlorosis was observed on the leaves when irrigated at 50 % depletion level and treated with salicylic acid at a monthly interval while acute chlorosis was observed in plants exposed to moisture stress at 70% and 90% depletion levels.

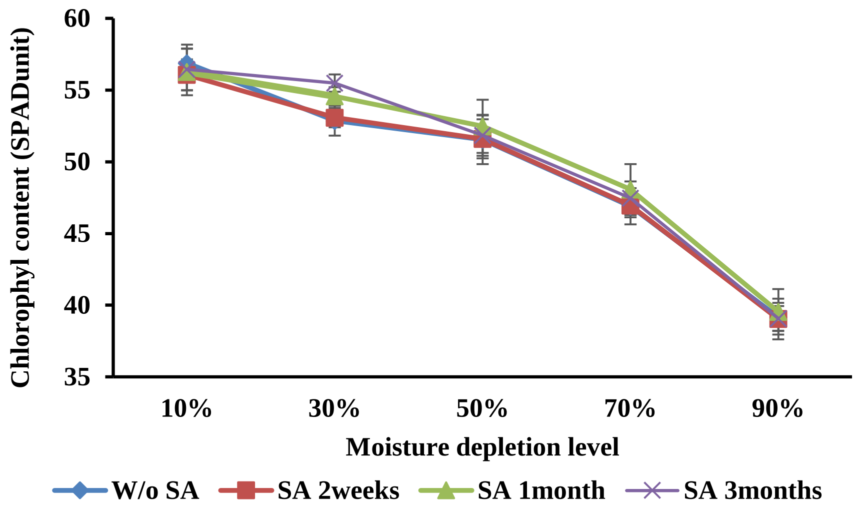

Fig. 3. The effect of frequency of application of salicylic acid (SA) at different moisture depletion levels on chlorophyll content of rubber seedlings

Dry matter content and shoot: Root ratio

There is a significant ($p \leq 0.05$) increase in total dry matter of plants when were irrigated at a 10% moisture depletion level irrespective of SA application frequency (Table 1). Regular irrigation at a 10% depletion level ensured 90% of soil water availability to plants which did not affect plant growth. Similarly, plants irrigated at 30% depletion level and SA application at two to four weeks intervals, also recorded a significant ($p \leq 0.05$) increment of dry matter content. Further, 30% depletion level and monthly application of SA showed a positive effect on shoot: root ratio (Table 1). In response to the 50% depletion level plants treated with SA at monthly intervals showed a significant ($p \leq 0.05$) increase in total dry mater content compared to the other application intervals (Table 1). Moreover, at 70% and 90% depletion levels, plants showed a significant ($p \leq 0.05$) reduction in dry matter accumulation irrespective of the frequency of SA application.

The overall results on plant growth indicated that, a 50 % depletion level of soil moisture, could be considered as the minimum level of soil moisture depletion that can be maintained with one-month interval of application of salicylic acid for saving of irrigation water during prominent dry periods prevailed in the Intermediate Zone of Sri Lanka.

Table 1. *Dry matter content and shoot: Root ratio*

Soil moisture depletion level	Frequency of salicylic acid application	Total dry weight (mg)	Shoot: root ratio
10%	Control (without SA application)	22.15±1.00[a]	4.17±0.56[a]
	Once in 2 weeks	21.15±1.26[a]	3.93±0.23[a]
	Once a month	20.91±0.94[a]	3.89±0.56[a]
	Once in 3 months	21.22±0.99[a]	4.10±0.18[a]
30%	Control (without SA application)	17.21±1.05[b]	2.66±0.15[b]
	Once in 2 weeks	19.92±0.98[a]	2.80±0.20[b]
	Once a month	17.21±1.25[b]	3.08±0.17[a]
	Once in 3 months	14.47±2.01[c]	2.41±0.59[c]
50%	Control (without SA application)	13.22±1.09[b]	3.70±0.27[a]
	Once in 2 weeks	14.79±2.40[b]	3.33±0.31[b]
	Once a month	16.00±1.87[a]	2.86±0.24[c]
	Once in 3 months	14.77±2.03[b]	3.03±0.30[b]
70%	Control (without SA application)	10.42±1.31[b]	3.33±0.09[a]
	Once in 2 weeks	11.04±0.94[a]	3.23±0.12[a]
	Once a month	10.45±1.00[b]	2.70±0.05[b]
	Once in 3 months	10.30±1.12[a]	3.03±0.08[b]

Soil moisture depletion level	Frequency of salicylic acid application	Total dry weight (mg)	Shoot: root ratio
90%	Control (without SA application)	8.48±1.07[b]	4.00±0.10[a]
	Once in 2 weeks	11.48±1.11[a]	3.70±0.09[a]
	Once a month	10.37±0.95[a]	3.85±0.97[a]
	Once in 3 months	10.53±0.98[a]	3.85±0.97[a]

The values are means and SE of n = 5 seedlings. Values followed by the same letter are not significantly different at ($P \leq 0.05$)

Estimation of net irrigation requirement for rubber nurseries

The above results highlighted that the monthly application of 0.5 mM salicylic acid as a soil drench at 50% depletion level enhances growth of rubber seedling plants when considered the stem diameter which is more importantly reveal at initiating bud grafting programme. With that, the crop water requirement of rubber nursery plants with SA application was estimated to scheduling the net irrigation water requirement (NIR) for rubber nurseries in the Intermediate Zone. For an effective irrigation scheduling in rubber nurseries, NIR was calculated by using derived equations as described in the methodology section.

Reference evapotranspiration (ET_0)

Reference evapotranspiration was estimated based on climatological data *i.e.;* pan evaporation, temperature, humidity, wind, *etc*. in the nursery site. Climatological data were obtained from the meteorological station located near the experimental site at a distance of about 100 m and estimation was done by using Eq.3 as described by Allen *et al*, (1998). ET_0 was calculated with a selection of pan coefficient (Kp) of 0.8 according to FAO Irrigation and Drainage paper 56 (Allen *et al.*, 1998) (Table 2).

Table 2. *Estimation of reference evapotranspiration (ET_0) on pan evaporation data*

Month	Pan evaporation (mm/day)	Pan Co-efficient (Kp)	ET_0 (mm/day)
January	2.94	0.8	2.35
February	3.76	0.8	3.01
March	4.26	0.8	3.41
April	3.38	0.8	2.70
May	4.13	0.8	3.30
June	4.51	0.8	3.61
July	4.37	0.8	3.50
August	4.55	0.8	3.64
September	4.36	0.8	3.49
October	3.31	0.8	2.65
November	2.49	0.8	1.99
December	2.74	0.8	2.19

Crop evapotranspiration (ET_c) rate with and without salicylic acid application

The crop evapotranspiration (ET_c) differs distinctly from the reference evapotranspiration (ET_o) as the ground cover, canopy properties and aerodynamic resistance of the crop are different from grass. The effects of characteristics that distinguish field crops from grass are integrated into the crop coefficient (K_c) (Allen *et al.*, 1998). In the crop coefficient approach, crop evapotranspiration is calculated by multiplying ET_o by K_c (Eq. 2). In the nursery condition of rubber nurseries at the Intermediate zone, estimated K_c values for rubber seedling plants were considered as 0.9 and 1.1 respective to with and without application of salicylic acid as a soil drench (Nakandala *et al.*, 2012). Monthly crop evapo-transpiration without and with the salicylic acid application was calculated accordingly and recorded in Table 3.

Table 3. *Monthly crop evapotranspiration (ET_c) of rubber plants with and without salicylic acid application*

Month	ET_0 (mm/day)	ET_c without salicylic acid application (mm/day)	ET_c with salicylic acid application (mm/day)	ET_c without salicylic acid application (mm/month)	ET_c with salicylic acid application (mm/month)
January	2.35	2.59	2.12	80.20	65.62
February	3.01	3.31	2.71	92.65	75.80
March	3.41	3.75	3.07	116.21	95.08
April	2.70	2.97	2.43	89.23	73.01
May	3.30	3.63	2.97	112.67	92.18
June	3.61	3.97	3.25	119.06	97.42
July	3.50	3.85	3.15	119.21	97.54
August	3.64	4.00	3.28	124.12	101.56
September	3.49	3.84	3.14	115.10	94.18
October	2.65	2.91	2.38	90.30	73.88
November	1.99	2.19	1.79	65.74	53.78
December	2.19	2.41	1.97	74.75	61.16
Total				**1,199.25**	**981.20**

Monthly dependable rainfall

Mean monthly dependable rainfall was computed by using the probability method (Eq. 4) for 15 years at a 75% expectancy level. Estimated values for monthly dependable rainfall at Moneragala is depicted in Figure 4.

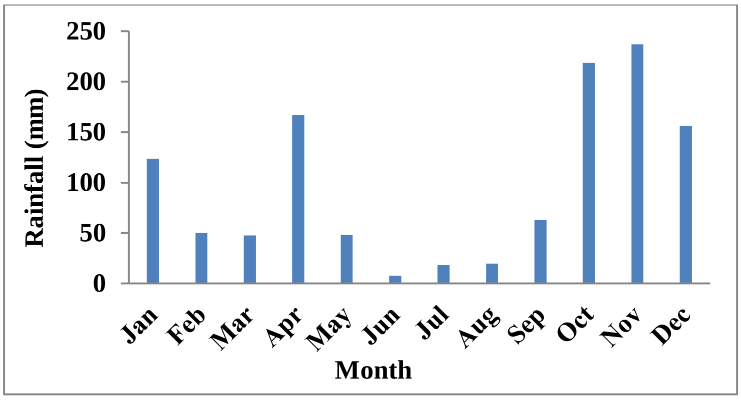

Fig. 4. *Monthly dependable rainfall*

As evident in Figure 4, monthly dependable rainfall varied from the maximum (237 mm) in November to the lowest (8 mm) in June. Only five months were seemed to have dependable rainfall exceeding the 100mm level (April, October to January). Remaining seven months are relatively dry and hence, plants should be irrigated for the better growth performances.

Net irrigation requirement of rubber nursery with salicylic acid application

Net irrigation requirement is the depth of irrigation water required to accomplish the crop water requirement or crop evapotranspiration (ET_c). As shown in Figure 5, irrigation is needed to fill the gap between precipitation and crop evapotranspiration during the dry months from February to September except for April. The results indicate that the net irrigation requirement was comparatively low in plants treated with a salicylic acid.

The net irrigation requirement (NIR) with and without salicylic acid application have been given in Table 4. It shows that NIRs for rubber nursery plants with and without salicylic acid application are 398.9 mm and 544.2 mm respectively. With that, the annual saving of irrigation water for the rubber nursery is about 145 mm per plant which is about 25%.

Table 4. *Net irrigation requirement (NIR) for the Rubber Nursery*

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
Rainfall (mm)	123.60	50.00	47.7	167.20	48.20	7.70	18.20	19.80	63.20	218.70	237.00	156.4	1157.70
ET_c without salicylic acid application (mm)	80.20	92.65	116.21	89.23	112.67	119.06	119.21	124.12	115.1	90.3	65.74	74.75	1199.25
ET_c with salicylic acid application (mm)	65.62	75.80	95.08	73.01	92.18	97.42	97.54	101.56	94.18	73.88	53.78	61.16	981.20
Net irrigation requirement without salicylic acid application (mm)	0	42.65	68.51	0	64.47	111.36	101.01	104.32	51.9	0	0	0	544.22
Net irrigation requirement without salicylic acid application (mm)	0	25.80	47.38	0	43.98	89.72	79.34	81.76	30.98	0	0	0	398.96

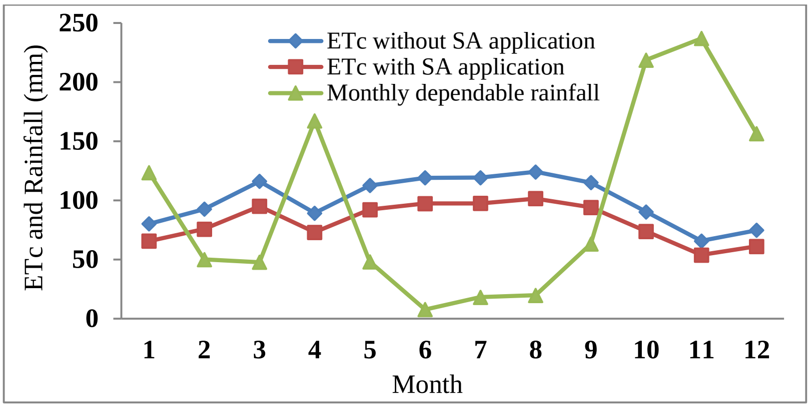

Fig. 5. *Dependable rainfall and crop ET*

Discussion

The results of the study indicates that the growth of rubber seedlings with irrigation at a 10% depletion level was greater than those to plants under water stress conditions. Moderate to severe water stress conditions were developed with the moisture levels at 50 to 90% depletions respectively. It is also evident that increasing the depletion level in the soil retarded the growth of seedling plants irrespective of the salicylic acid (SA) application (Fig. 2). Samarappuli (1992) also reported that the immature rubber plants which were grown at higher moisture depletion levels exhibit varying degrees of physiological and morphological impairments resulting in severe growth reduction and longer immaturity periods of rubber. However, results on the present study revealed that plants that were treated with 0.5 mM of salicylic acid at 50% moisture depletion level showed significantly ($p \leq 0.05$) higher growth performance than non-treated plants (Fig. 2).

Further, SA concentration at 0.5 mM applied at monthly intervals at 50% moisture depletion level enabled the rubber seedlings to maintain higher chlorophyll content compared to other treatments (Fig. 3). Leaf chlorophyll is a key component of the photosynthetic system that governs plant growth. Further, it found that the decrease in chlorophyll content was greater at higher soil moisture depletion levels *i.e.*; 70 and 90% irrespective of SA application. Similar observations were recorded by Singh and Usha (2003) for wheat seedlings.

It was found that plants treated with 0.5 mM of SA increased plant dry matter content by lessening the adverse effect of water stress at higher depletion levels. Gomez *et al.* (1993) also reported an improvement in plant biomass and yield of wheat genotypes under water stress with the application of salicylic acid.

Thus, these results highlighted that the moisture stress at higher soil moisture depletion levels decreases the plant growth severely and monthly application of 0.5 mM salicylic acid as a soil drench at 50% moisture depletion level enhances the plant growth performance by adjusting physiological processes of plants which are negatively affected by drought stress.

Meteorological calculations in Tables 3 and 4 have shown that SA application have had a significant effect on lowering ET_c of rubber nursery plants. These results indicate that the net irrigation water requirement of the rubber nursery was low with salicylic acid application. Further, it reveals that the saving of the net irrigation water requirement of the rubber nursery is about 25% of the total irrigation water requirements of the nursery.

Conclusion

In conclusion, irrigation with a monthly application of 0.5mM salicylic acid at 50% soil moisture depletion level of rubber nursery plants minimizes negative effects of drought stress and SA can be recommended to minimize the net irrigation water requirement of rubber nurseries in the Intermediate Zone of Sri Lanka.

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Address for correspondence: Dr (Mrs) S A Nakandala, Research Officer, Plant Science Dept., Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka.
e-mail*: sanakandala@gmail.com*