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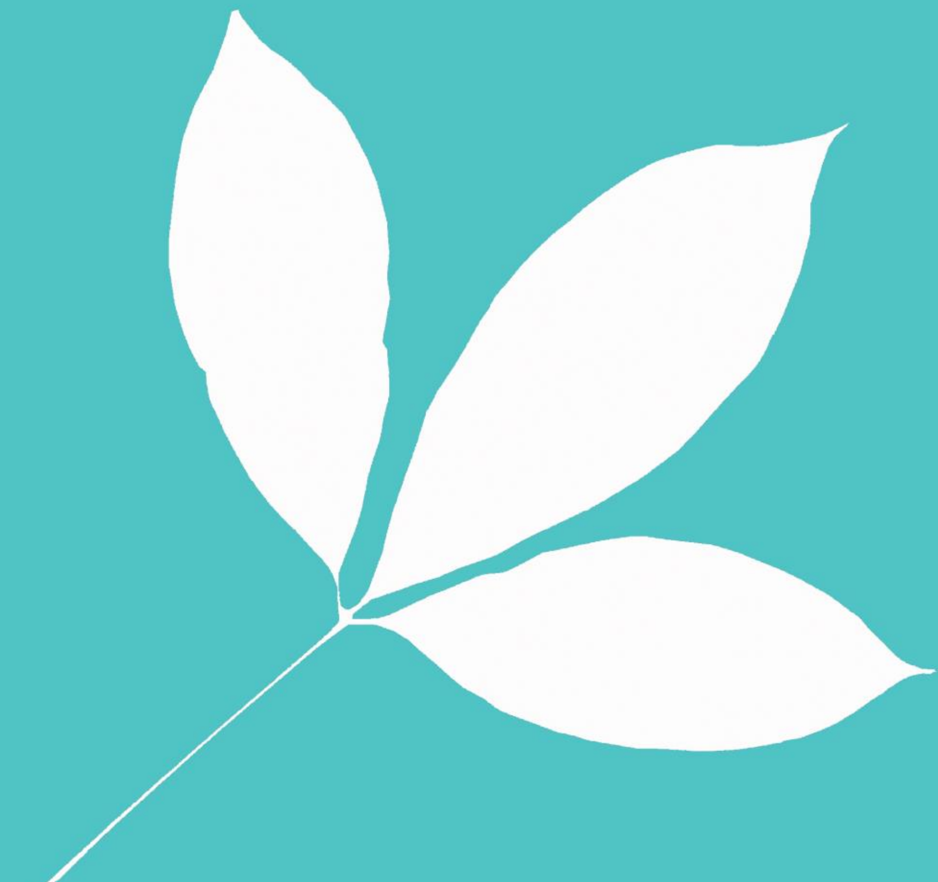

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CONTENTS

Property improvements of natural rubber and low density polyethylene blends through dynamic vulcanization

W D M Sampath*, D G Edirisinghe* and S M Egodage**
* *Rubber Research Institute of Sri Lanka, Telewala Road, Ratmalana, Sri Lanka*
** *Department of Chemical & Process Engineering, University of Moratuwa, Sri Lanka*

Abstract

Polymer blends are prepared by melt mixing, solution or latex blending. Thermoplastic vulcanizates (TPVs) are generally produced by melt mixing. TPVs can be co-injected or co-extruded to produce complex articles built from very soft/hard components. Dynamic vulcanization would give rise to a uniform and fine distribution of rubber and thermoplastic phases. The aim of this study was to identify a suitable dynamic vulcanization system to produce natural rubber (NR)/low-density polyethylene (LDPE) blends with improved chemical, ageing and morphological properties. Three 50:50 NR/LDPE blends were prepared by varying the vulcanizing agent as sulphur, dicumyl peroxide (DCP) and 50:50 sulphur: DCP. A control was prepared without any vulcanizing agent. All these blends were prepared by melt mixing. The blends were characterized using FTIR spectroscopy and SEM analysis of tensile fracture surfaces. Water absorption, swelling and ageing behaviour of the blends were investigated.

Water absorption percentage of blends prepared with DCP and the mixed curing system was zero. Analysis of gel content indicated a higher crosslink density for the blend prepared with the mixed curing system compared to the other blends. Also, the former blend showed the highest retention of strength properties and elongation at break compared to the other two blends prepared with vulcanizing agents. SEM analysis showed a fine and smooth textured morphology for this blend. Results in overall indicated that the 50:50 NR/LDPE blend prepared with the mixed curing system would be suitable to manufacture heat and solvent resistant products.

Key words: ageing properties, dynamic vulcanization, morphology, NR/LDPE blends, swelling properties

Introduction

Polymer blends may be categorized in general into two broad classes: immiscible and miscible blends. Immiscible blends are those which exist in two distinct phases, but they are practically still very useful materials, *e.g.,* toughened plastics. Miscible blends are those, which exist in a single homogeneous phase and may exhibit synergistic properties, different from the pure components (Utracki, 1990). Thermoplastic elastomers (TPEs) may be divided into the following classes according to their chemistry and morphology such as (soft) triblock copolymers, (hard) multi block copolymers and blends of rubbers with thermoplastics and application of dynamic vulcanization. The thermoplastic vulcanizates (TPVs) based on PP/EPDM blends by dynamic vulcanization exhibit superior tensile properties including higher tensile strength and greater elongation and also exhibit better processability (Koning *et al.,* 1998). In dynamic cross-linking, the rubber and thermoplastic are pre-mixed with the curative and other additives are then added. The rubber crosslinks *in-situ* to give typically a form of semi-interpenetrating polymer network, which is subsequently capable of being molded or extruded (Wu, 1987).

The morphology of a material is organized on a supra-molecular scale, *i.e.*, the curing agent, size and orientation of crystallites, domains, the structure of groups of molecules of the components and of their boundaries, and the degree of crystallinity. The morphology of polymer blends is understood as the mostly qualitative description of the spatial arrangement of the blend-component phases (Reijden-stolk, 1989). However, in the case of dynamic vulcanization an inverse phase morphology characterized by a matrix phase representing the minor content. Therefore, no shear deformation as the main mixing mechanism, but mainly elongation deformations are required as a precondition for an effective dispersion process (Coran and Patel*,* 1996).

Several crosslinking agents have been employed to crosslink the EPDM phase in PP/EPDM blends: co-agent assisted peroxides; activated phenol-formaldehyde resins, commonly known as resol-resins; platinum catalyzed hydro siloxane; vinyltrialkoxy silane/moisture; catalyzed quinine dioxime and bisthiols; *etc.* (Naskar, 2004). Special crosslinking agent like benzene-1,3-bis (sulfonyl) azide for the preparation of TPVs was investigated by Lopez Manchado and Kenny, 2001. They concluded that the sulfonyl-azide group can act as an effective crosslinking agent for the elastomeric phase and also as a coupling agent between the elastomeric and thermoplastic phases. The cure rate, the final crosslink density, the thermal stability of the crosslinks formed, the safety, health and environmental characteristics of the chemicals used and the cost price are relevant parameters for the final choice of the crosslinking system (Naskar *et al.,* 2004).

Sulfur vulcanization of EPDM rubber and other elastomers is normally performed in the presence of activator and accelerators. It is proven, that the mechanism of sulfur vulcanization of EPDM is more or less similar to the mechanism, which is normally accepted for polydiene elastomers (Chapman and Porter, 1998). Sulfur/accelerator combinations have in principle been demonstrated to be applicable to dynamic vulcanization of PP/EPDM blends by Coran and Patel, 1980. They observed improvement of tensile properties of PP/EPDM blends. However, sulfur crosslinking systems are not applied in commercial PP/EPDM TPVs, since PP has a relatively high melting point and the crosslinks lack in thermal and UV-stability. Moreover, the production and processing of these TPVs suffer from severe problems (Koning *et al.,* 1998).

Peroxide cure can be used to vulcanize both unsaturated and saturated elastomers. Vulcanization of the latter is not possible with sulphur. Nevertheless, there are some polymers which cannot be crosslinked by peroxide vulcanization. The efficiency of the total crosslinking reaction depends mainly on the type of peroxide and polymer radicals formed during the process. The relationship between peroxide structure and crosslinking efficiency has been described by Endstra, (1985). However, when peroxide is added to a PP/EPDM blend, two competing processes may take place simultaneously: EPDM-crosslinking and PP-degradation. It is generally accepted that degradation proceeds through β-chain scission by abstraction of tertiary hydrogen atoms from the main chain of the olefin polymer (Coran *et al.,* 1982).

In peroxide vulcanization, the crosslinks formed consist of carbon-carbon bonds between the polymer chains, while in sulphur vulcanisation the crosslinks between the chains consist of sulphur bridges: carbon-sulphur and sulphur-sulphur bonds (Dluzneski, 2001). The carbon-carbon bond is more rigid and stable (bond energy is 351 kJ/mol) than the carbon-sulphur (285 kJ/mol) and sulphur-sulphur (267 kJ/mol) bonds. This difference in network structure gives the two vulcanisation systems their different characteristic properties, since the lower the bond energy the easier the breakage of bonds under influence of mechanical or thermal energy (Dluzneski, 2001). This will be reflected, for instance, in ageing properties: peroxide vulcanisates have much better heat ageing characteristics than sulphur cured elastomers, due to the fact that the carbon-carbon bonds are more stable than the sulphur bridges. Brodsky, (1994) reported combined sulphur/accelerator and peroxide cure for a blend of EPDM/BR/NR. He used a variety of mixed peroxide-sulphur curing systems and tested to study the mechanical properties of the vulcanizates. In regard to the curing behaviour, the results show that the mixed curing system leads to compounds with low scorch safety and low cure rate when compared to the usual sulphur/accelerator system. On the other hand, the mixed vulcanizates show a reduced reversion, proportional to the amount of peroxide used. In regard to physical properties, it was observed that vulcanizates produced with the mixed curing system have improved tensile strength and similar tear resistance. Ageing resistance, compression set and fatigue life of vulcanizates prepared with the mixed curing system was also found to be improved when compared to those of the sulphur/accelerator cured rubber. Vulcanization of EPDM with sulphur

and peroxide in two curing steps was studied by van der Burg (Van, 1998). The vulcanisates were found to have lower crosslink density and intermediate tear strength and compression set.
The cross-linking of the NR and LDPE in our work was carried out using sulphur and peroxide individually and with 50:50 sulphur : peroxide (mixed) systems. Therefore, aim of this study was to identify a suitable dynamic vulcanization system to produce NR/LDPE blends. Because of the high incompatibility between the two components, compatibilization is necessary to improve the mechanical performance for commercial applications.

Materials and Methodology

Commercially available low density polyethylene (LDPE) and ribbed smoke sheet rubber (RSS 2) were used in the preparation of the blend compounds. LDPE having melt flow index, density and crystalline melting temperature of 2.4 g/10 min., 0.923 g/cm^3 and115 ^{0}C, respectively was supplied by Deluxe Plastics Ltd., Sri Lanka. RSS 2 having a plasticity retention index (PRI) of 64 was obtained from the Rubber Research Institute of Sri Lanka. Other compounding ingredients, sulphur and dicumyl peroxide (DCP) as the vulcanizing gents and BKF (2, 2'-methylene bis (4-methyl-6-tert butyl phenol)) as the antioxidant are industrial grade chemicals and were purchased from Glorchem Enterprises, Sri Lanka.

Blend preparation

Four 50:50 NR/LDPE blends were prepared with and without vulcanizing agents and the formulations are given in Table 1. The blends were prepared by melt mixing using a Brabender plasticorder operated at a temperature of 130 ^{0}C, and at a rotor speed of 60 rpm. Total mixing time was kept constant at 10 minutes. The mixing cycle used in the preparation of NR/LDPE blend compounds is also given in Table 1. NR/LDPE blend compounds were compressed in an electrically heated hydraulic press at 150 ^{o}C under 3.5 bar pressure for 15 minutes to produce 2 mm thick sheets. Test specimens were cut from these sheets according to the standard.

Table 1. *Formulation and mixing cycle of 50:50 NR/LDPE blends*

Ingredient	Parts by weight				
	Control	Sulphur system	DCP system	Mixed system	Total mixing time (min.)
LDPE	50	50	50	50	0
NR	50	50	50	50	4
Antioxidant	1	1	1	1	5
TBBS	0	1	0	0.5	6
Sulphur	0	1	0	0.5	8
DCP	0	0	1	0.5	10

Chemical properties of 50:50 NR/LDPE blends

Swelling behavior

ISO 1817:2011 describes methods of evaluating the resistance of vulcanized and thermoplastic rubbers to the action of liquids by measurement of properties of the rubbers before and after immersion in test liquids. Two test pieces (30mm x 10mm x 2mm) from each of the treatments were tested. The test pieces were immersed in p-xylene for 72 hours in closed lid bottles and the swollen weight was obtained. Calculated the degree of swelling according to the formulation given below.

$Q (\%) = (m - m_0/m_0) \times 100$

Where, m_0 and m are the masses of the sample before and after swelling in p-xylene, respectively.

Gel content

Three test pieces approximately 1cm × 3cm × 0.2cm in size from each of the samples were tested. The test pieces were measured to the nearest 0.1 mg and covered with a mesh, then placed in boiled p-xylene solution for 16 hours according to ASTM D2240. Thereafter, the samples were taken out and the final weight was measured. Then the test pieces were oven aged at 70 ^{0}C and the weight after each hour was measured until the readings became constant.

FTIR spectra of gel samples

FTIR is a powerful technique to qualitatively identify organic materials and to determine molecular structure. The test pieces which were obtained from the gel content test were used to obtain the FTIR spectra. Nicolet 380 FTIR spectrometer was used for the testing.

Water absorption

Water absorption data was obtained by immersing 72 hours in water at 27 °C. Upon removal, specimens were dried and weighed immediately. The readings were taken after each hour until the weight was constant. The increase in weight was reported as a percentage.

Ageing properties of 50:50 NR/LDPE blends

Tensile and tear properties were evaluated after ageing. Ageing was carried out in an air circulating oven at 70 ^{0}C for 72 hours according to BS ISO 37:2010 (E) and BS ISO 34-1:2010 (E).

Morphology of 50:50 NR/LDPE blends

Surface morphology of tensile fracture surfaces of NR/LDPE blends was examined by a ZEISS EVO LS 15 Scanning Electron Microscope (SEM). The specimens for SEM observation were prepared by lay down the as–spun NR/LDPE fine droplet on a copper stub. Each sample was coated with a thin layer of gold prior to observation under SEM.

Results and Discussion

Chemical properties of 50:50 NR/LDPE blends

The swelling behaviour of a thermoplastic material defines the chemical or liquid absorption capacity of it or compound. If the compound shows a higher percentage of swelling it can absorb a high amount of chemical/liquid without dissolving in the same. The mobility of the penetrant through the polymer chains depends upon the free volume in the matrix. According to free volume theory (Harogoppad and Aminabhavi, 1991), the rate of diffusion depends upon the ease with which polymer chain segments exchange their positions with penetrant molecules.

Figure 1 shows that percentage of swelling is highest in the 50:50 NR/LDPE blend prepared with sulphur as the curing agent. C-C bonds in a peroxide vulcanizate is more rigid and stable compared to C-S in a sulphur vulcanizate (Dluzneski, 2001). Also, C-C crosslinks are shorter than sulphur crosslinks and hence penetration of solvent molecules through a peroxide vulcanized network would be difficult than through a sulphur vulcanized network and this results in a higher swelling value for the latter. A higher swelling value reflects a lower gel content. The Control shows a value less than 100 for the percentage of swelling and this indicates that the blend dissolves into p-xylene because non-crosslinked materials do not exhibit good resistance towards organic solvents.

According to the above figure, blends prepared with peroxide and the mixed curing system have good solvent resistance compared to the blend prepared with sulphur and the Control.

The gel content test is used for defining the solvent resistance of a thermoplastic, plastic or rubber compound. Crosslink density of a material is proportionate to the gel content. When the gel content is high, percentage swelling will be reduced. Figure 2 shows that the gel content of the blend prepared with peroxide is markedly higher than that of the blend prepared with sulphur as the curing agent. The blend prepared with the mixed curing system has the highest gel content and indicates the highest crosslink density due to good chemical interaction with NR and LDPE.

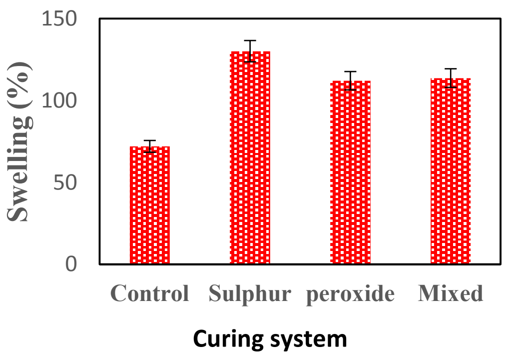

Fig. 1. Swelling behavior of 50:50 NR/LDPE blends prepared with different curing systems

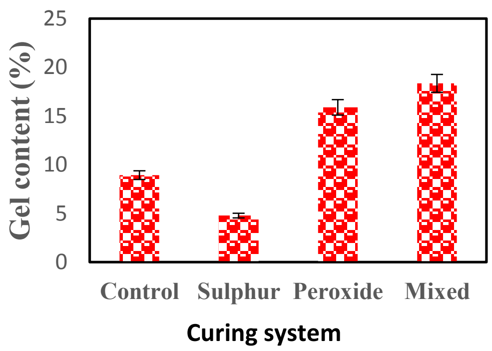

Fig. 2. Gel content of 50:50 NR/LDPE blends prepared with different curing systems

Figure 3, shows the FTIR spectra of NR/LDPE blends prepared with DCP and the mixed curing system. Both these NR/LDPE blends have a strong plastic stretching absorption around 1376 cm-1. This peak confirms the presence of gel and it is evidence for the amount of crosslinks present in the LDPE phase of the blend. In addition, the peak at 840 cm-1 corresponding to natural rubber appears to be weak in both the blends.

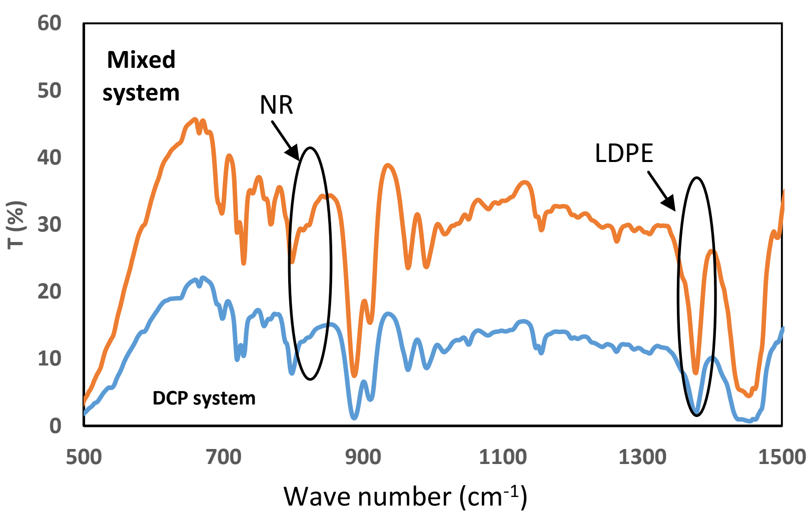

Fig. 3. FTIR spectra of 50:50 NR/LDPE blends prepared with DCP and the mixed curing system

Water absorption of 50:50 NR/LDPE blends

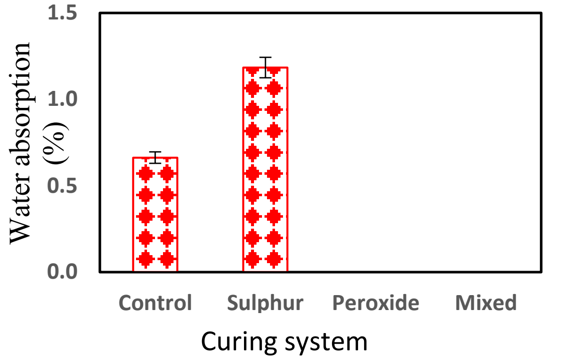

Fig. 4. Water absorption of 50:50 NR/LDPE blends prepared with different curing systems

According to Figure 4, blends prepared with DCP and the mixed curing system show a zero water absorption indicating higher water resistance. Under dynamic vulcanization, 50/50 NR/LDPE phase changed from co-continuous to plastic phase and plastics have good water resistance compared to rubber (Wang *et al.,* 2005).

Ageing properties of 50:50 NR/LDPE blends

The curves in Figure 5, which was obtained from tensile testing of aged test pieces shows almost a similar pattern of variation. However, the blend prepared with the mixed curing system shows a semi crystalline behavior compared to the other three composites. The highest strain value elucidates from the former blend and hence it indicates good heat resistance compared to the others.

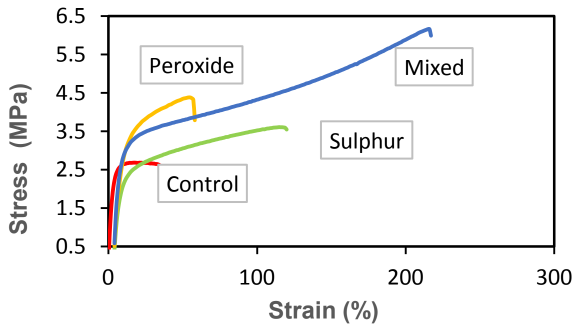

Fig. 5. Stress – strain curves of 50:50 NR/LDPE blends, after ageing

Table 2. *Percentage retention of properties of 50:50 NR/LDPE blends, after ageing*

System	Retention of tensile strength (%)	Retention of elongation at break (%)	Retention of tear strength (%)
Control	65	22	82
Sulphur	44	24	87
Peroxide	35	12	85
Mixed	54	40	90

All the blends show low retention of tensile strength and elongation at break, but high retention of tear strength. Therefore, these blends are not at the required level in regard to resistance to thermal degradation. According to Table 2, peroxide loaded blend shows very low ageing properties and indicates the initiation of radical reaction between C-C under the ageing condition. Carig and white (2006) has reported the changes in the polymer properties under the different environmental and processing conditions are termed ageing. Polymers have been known to demonstrate two kinds of ageing: chemical and physical. Chemical ageing is thermal degradation, photo oxidation, etc. and the changes are connected with degradation and lead to molecular chain scission and/or crosslinking. The result is cracking and chemical disintegration of polymers. Therefore, percentage retention of properties after ageing is generally less than 100.

Morphology of 50:50 NR/LDPE blends

Figure 6, (c) and (d) show smoother and fine fracture surface compared to (a) and (b) and indicate good interfacial adhesion between NR and LDPE. Figure 6, (a) and (b) show coarse phase morphology. Figure (a) shows a clear separation of NR and LDPE phases, probably due to poor interfacial adhesion between the phases due to the absence of any crosslinks. This phase separation indicates incompatibility between NR and LDPE, which results in poor mechanical properties. The morphological analysis shows that interfacial adhesion between NR and LDPE phases is at the highest level when a mixture of sulphur and peroxide is added as the curing system. Further, the 50:50 NR/LDPE blend prepared with this mixed curing system shows a higher gel content, which means there is good interaction between the NR and LDPE phases. This blend is the least heterogeneous according to its phase morphology shown in Figure 6 (d). Results in overall indicate that the 50:50 NR/LDPE blend prepared with the mixed curing system would be suitable to manufacture heat and solvent resistant products.

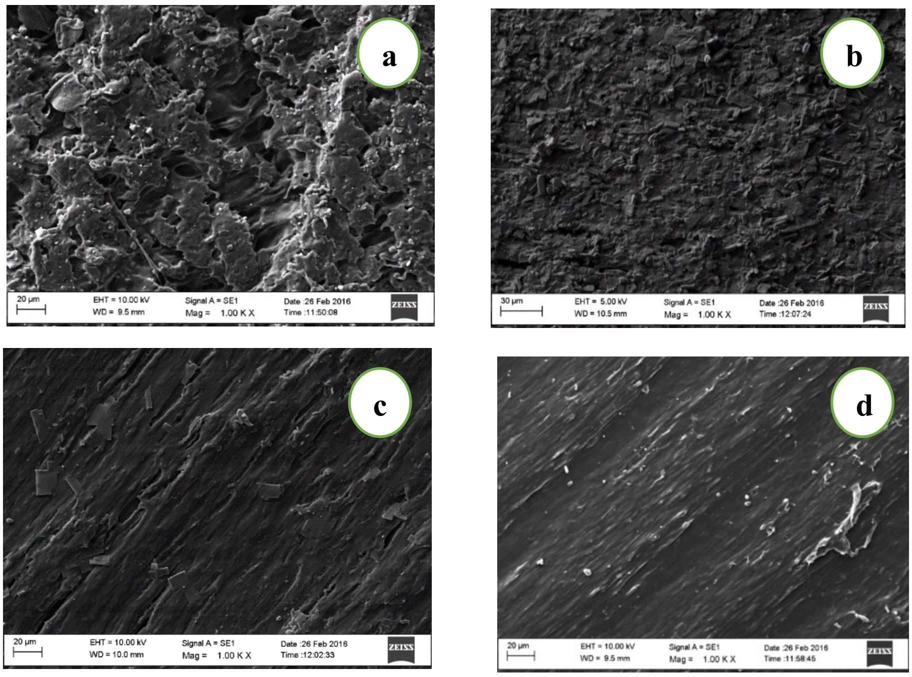

Fig. 6. Scanning electron micrographs of tensile fracture surfaces of 50:50 NR/LDPE blends at x1000 magnification **(a)** control **(b)** vulcanized with sulphur **(c)** vulcanized with DCP **(d)** vulcanized with the mixed curing system

Acknowledgements

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Address for correspondence: Mr W D M Sampath, Research Officer, Rubber Technology & Development Dept., Rubber Research Institute of Sri Lanka, Telewala Road, Ratmalana, Sri Lanka.
e-mail: wikcramage@yahoo.com

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Early growth of rubber in the Dry Zone of Sri Lanka: an investigation in Vavuniya District

S M M Iqbal*, V H L Rodrigo*, E S Munasinghe*, B M D C Balasooriya*, K V V S Kudaligama*, P M M Jayathilake* and R P S Randunu*

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

Abstract
In view of supporting the rubber product manufacturing sector in Sri Lanka and providing stable income for the peasant community, rubber cultivation is in the process of expanding to the drier regions of the country. Under this programme, rubber was introduced to the Northern region (Dry Zone) in 2010 with a tentative set of agronomic protocols. The present study showed that rubber cultivated in this region under smallholder conditions together with irrigated short term crops, has shown reasonable growth with an average annual girth expansion rate of 8.6cm. A growth curve for the immature phase of rubber was established for the first time for the dry zone of Sri Lanka. Despite some adverse effects of dry weather on photosynthesis and associated physiological parameters, no evidence of permanent damages to photosynthetic apparatus was recorded. Further actions to be taken in the expansion process of rubber cultivation in this region are also discussed.

Key words: dry climate, growth curve, *Hevea,* photosynthesis

Introduction

Urbanization and industrialization prevent the expansion of rubber cultivation further in its traditional rubber growing wet regions (WZ). Consequently, the Government of Sri Lanka (GoSL) has decided to expand rubber cultivation to drier non-traditional regions of the country. For this purpose, East and North regions of the country have been focused not only because their land availability per capita is reasonably higher but also because of livelihood consideration of the people, who were entangled in 30 year war. In this endeavor, rubber was initially grown in the Intermediate Zone (IZ) of Eastern province and it was found to be successful in terms of both agronomic (Iqbal *et al*., 2010) and social aspects (Rodrigo *et al*., 2009). With that back ground, GoSL suggested to expand the rubber cultivation to the Dry zone (DZ) of East and North regions. However DZ being drier than IZ, agro-management practices in DZ would be more problematical than those in either WZ or IZ. As experienced in India, such condition may prolong the immature period and result in decline in yields (Datta *et al*., 2010; Vijayakumar *et al*., 1998). Water availability for irrigation is very limited in this region and the lands where water available are devoted for more remunerative short-term cash crops (Rodrigo *et al*., 2009). It is expected to grow rubber under rain fed conditions; however, some farmers are interested in growing rubber with some level of irrigation. Therefore, a set of tentative recommendations was issued to cultivate rubber in DZ targeting initially the Vavuniya District (Rodrigo *et al*., 2011a). Accordingly, rubber cultivation began in this region in 2010 with few farmers engaged in cultivating annual and semi-perennial crops. By end of 2015, about 31ha of rubber have been cultivated with 20 farmers in Vavuniya district (under the area of Vavuniya South Divisional Secretariat).

Further expansion of rubber in this region or any other area coming under DZ is to be decided upon the success of the already planted rubber. Although rubber trees planted in Vavuniya have not been harvested yet, the growth pattern and associated physiological performance of rubber should provide sufficient information on the success of rubber cultivation in this region. Therefore, the present study was aimed to quantify the growth of immature rubber in this region and to investigate the impact of the prevailing climatic conditions on its physiological parameters.

Materials and Methods

Site details;

The study began in 2010 with the initial establishment of rubber in the Northern region. Details of rubber fields established in Vavuniya District (Vavuniya South and North) from 2010 onwards are shown in Table 1. All of these rubber holdings were established jointly by the Rubber Research Institute of Sri Lanka (RRISL) and Rubber Development Department (RDD) as a smallholder development programme. It was observed that successful sites were confined to the Divisional Secretariat region of Vavuniya South. To all sites, rubber, genotype RRIC 121, was introduced as an intercropping system in which annuals (*e.g.* Capsicum and Tomatoes) and semi-perennials (*e.g.* Papaya and Banana) were grown along with rubber (Fig. 1). Being a crop with no quick return, rubber was the subsidiary crop in the management of these intercropping systems in these regions. Since seasonal and semi-perennials were irrigated with drip or low height sprinkler systems, rubber plants also received reasonable amount of water for a period about 2-3 years after which cultivation of annuals and semi-perennials were discontinued due to the shade of the rubber canopy. Nevertheless, water received by rubber plants during dry spells was just sufficient for survival. Thick (15 cm) mulch was applied around the rubber plants mostly with crop debris to conserve the soil water. Fertilizer to rubber plants was applied with

N:P:K:Mg ratio of R/SA 7:9:9:4 thrice a year with doses of 450g, 900g, 1350g, and 1800g per plant per year from 1st to 5th year of growth, respectively (Advisory Circular 2012). Other agro-management practices were conducted as per the tentative recommendations issue to cultivate rubber in DZ targeting initially the Vavuniya District (Rodrigo *et al.*, 2011a). The rainfall data in Vavuniya for the study period were collected from the Meteorological Department of GoSL.

Table 1. *Details of rubber fields successfully established in the Northern Province (Vavuniya district) of Sri Lanka*

Year	Extent (ha)	No. of holdings
2010	2.6	07
2011	17.8	15
2012	2.1	15
2013	31.6	113
2014	6.1	01

Fig. 1. Rubber (Clone RRIC 121) planted with annuals (Capsicum and Tomatoes) and semi-perennial (Banana) in Vavuniya South

Growth and physiological measurements;

Growth of rubber in terms of tree circumference (girth) at 120cm height was measured continuously at approximately one year intervals in two sites planted in December 2010. In addition, five sites selected from subsequently planted years (three from 2011 and one from 2012 and 2013 plantings) were also used for the same growth measurement. In each site, 100 trees were selected at the centre of the plot for girth measurements.

Physiological assessments comprised building up the light response curve for photosynthesis (LRC) with associated parameters, *i.e.* apparent quantum yield (ϕ_{app}) and light saturated rate of photosynthesis (A_{max}), measurements on stomata conductance and chlorophyll *a* fluorescence. A portable infra-red gas analyser (IRGA) with open system (LI-6400, Li-Cor Inc., Lincoln, NE, USA.) was used for building up LRC whilst porometer (Delta T AP4, UK) was used for the measurements of stomatal conductance under field conditions. Assessments were done in 2014 in two sites planted in 2010 and 2011 (*i.e.* at the stages of three and four years after planting) with two sets of measurements representing wet and dry seasons (N.B. irrigation has been stopped at these stages). In each season, LRCs were established with photosynthetic measurements taken at 11 light levels (given with built in 6400-02B Red/Blue light source in IRGA) in the morning (0800-1000 h) and afternoon (1400-1600 h) hours of the day. In the case of stomatal conductance, diurnal measurements were conducted at three time points representing morning (0800-1000 h), midday (1100-1300h) and afternoon (1400-1600 h) hours of the day. Both measurements were confined to the leaves in the recently matured leaf whorl (*i.e.* leaves considered to be most active). At each time point, LRC was built up with single set of measurements with a single leaf whilst measurements on stomatal conductance were confined to twelve leaves at a time from four plants in the most recently matured leaf whorl.

Leaf chlorophyll *a* fluorescence emission was measured in terms of the ratio of variable (F_v) to maximal (F_m) fluorescence with the fluorometer OptiSc (OSP 5, UK). Measurements were taken at three time points representing morning (0800-1000 h), midday (1100-1300h) and afternoon (1400-1600 h). At a given time point, nine leaves from three plants selected from the most recently matured leaf whorls (*i.e.* leaves considered to be most active) were used for the measurements after 20 minutes of dark adaptation. Although the assessments on photosynthesis and stomatal conductance represented both wet and dry periods, fluorescence measurements were confined to the dry period in 2016 due to the unavailability of the instrument.

Data analyses;

Plants growth is not generally linear; hence, a logistic function representing the typical 'S' type curve (Equation 1) was fitted to time bound repeatedly measured girth values of rubber using Genstat Statistical Package (GenStat Release 11.1UK).

Equation 1:

$$Girth = a + \frac{c}{1+\exp(-b(x-m)}$$

Logistic function fitted to the girth expansion of rubber plants where a, b, c and m are constants and x stands for months after planting. Of the growth

function, parameters' a' plus 'c' represent the upper asymptote whilst 'b' is a slope parameter. The time point of inflexion of the growth curve is given by 'm'.
Light-response curve for CO_2 assimilation were derived by fitting the data to a non-rectangular hyperbola (Equation 2) (Prioul and Chartier, 1977) using a computer software package (Photosyn Assistant, Ver. 1.1.2.) and the parameters, light saturated rate of photosynthesis (A_{max}) and the apparent quantum yield of rubber (ϕ_{app}) were derived.

Equation 2:

$$A = \frac{\emptyset appQ + Amax - \sqrt{(\emptyset appQ + Amax)^2 - 4\emptyset appQkAmax}}{2k}$$

Where, Q is the incident photon flux density, ϕ_{app}, k and A_{max} are the initial slope (apparent quantum yield), convexity and upper asymptote of the light-response curve (light saturated rate of photosynthesis). The value for convexity was kept as a constant (0.65).
Presentation of data is rather descriptive showing the mean values for respective periods along with Standard Error.

Results

Total amount of rainfall received per year within the study period was in the range of 1319 to 2106 mm (Fig. 2). Depicting the dry climatic conditions of the region, only 2-3 months per year received rainfall over 200 mm and 1-2 months with over 300 mm. In most of years, over 3 months dry periods (less than 50 mm per month) were recorded and there was an instance of having nine months dry period (*i.e.* in 2014) with only one month receiving over 50 mm but less than 100 mm rainfall. In general, annual rainfall distribution is rather unimodal and dry conditions prevailed in the months of May, June, July, August and September.
Irrespective of the site differences, the logistic function fitted for plants girth has been able to demonstrate the typical 'S' curve of plant growth with two dominant phases, i.e. initial increasing rate and then declining rate of plant growth (Fig. 3). The point of inflexion given by the parameter 'm' shows that phase change occurred at 38 MAP. If plant growth is assumed to be linear over time then girth expansion shows 8.6 cm increase per year.
LRCs built up for wet and dry periods and then morning and afternoon hours of the day showed significant differences (Fig. 4). In particular, light saturated rate of photosynthesis (A_{max}) varied largely with greater values during wet than in dry periods. Also, A_{max} was higher in morning hours than in afternoon in both wet and dry periods. Further, the effect of dry weather on A_{max} was more severe than that of wet period. However, with the afternoon value in the dry period being higher

than the morning value in the dry period. Nevertheless, A_{max} was higher in the four year (planted in 2010) than in three year (planted in 2011) old plants (Table 2). Mean apparent quantum yield (ϕ_{app}) was least affected with values around 0.04, irrespective of the season and the time of the day. However, it also showed higher values in the four than in the three year old plants.

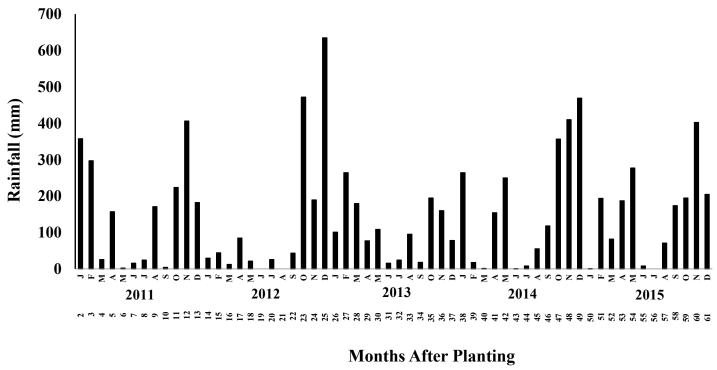

Fig. 2. Monthly distribution of rainfall in the Dry zone of Northern Province, Vavuniya South from January 2011 to December 2015

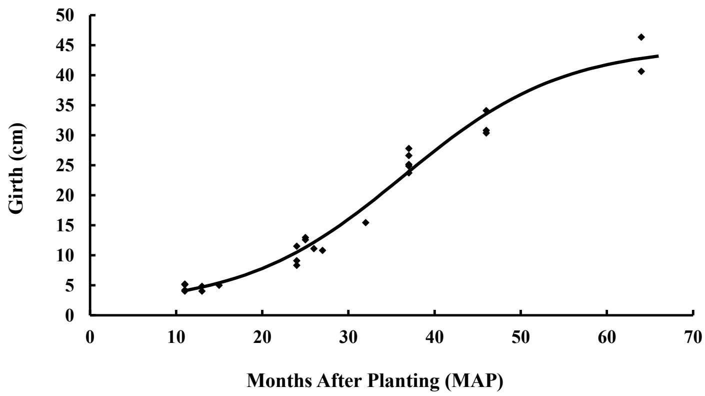

Fig. 3. Girth expansion of rubber trees in the Dry zone of Northern Province, Vavuniya South. The best fitted line obtained using the logistic function (Equation 1); Girth = 1.47+43.71/[1+exp(-0.108*(MAP-36.36))] R^2– 97%

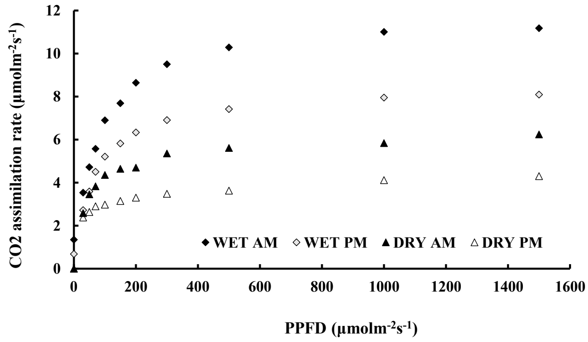

Fig. 4. Diurnal variation in photosynthetic CO_2 assimilation of rubber with respect to different light levels under wet and dry conditions (pooled values of rubber planted at the ages of three and four). Light was measured as the photosynthetically-active photon flux density (PPFD)

Table 2. *The mean apparent quantum yield (ϕ_{app}) and maximum light saturated rate of photosynthetic CO_2 assimilation (A_{max}) determined from the light response curves shown in Figure 3.*

Year of planting (Age)	Mean apparent quantum yield (ϕ_{app})		Mean A_{max} (μmol CO_2 m^{-2} s^{-1})	
	Wet period		**Wet period**	
	AM	**PM**	**AM**	**PM**
2010 (4 years)	0.058 (±0.016)	0.064 (±0.003)	12.27 (±0.197)	8.55 (±0.312)
2011 (3 years)	0.037 (±0.012)	0.045 (±0.015)	10.00 (±0.410)	5.36 (±0.445)
	Dry period		**Dry period**	
	AM	**PM**	**AM**	**PM**
2010 (4 years)	0.047 (±0.006)	0.055 (±0.028)	7.27 (±0.264)	3.87 (±0.329)
2011 (3 years)	0.036 (±0.015)	0.053 (±0.019)	5.04 (±0.209)	3.41 (±0.498)

According to the diurnal variation in chlorophyll *a* fluorescence emission, F_v/F_m ratio was greater in the morning than in afternoon, and also despite the high level of variation, the midday day values showed the lowest F_v/F_m (Fig. 5). However in general, all values recorded for F_v/F_m were above 0.6.

Diurnal variation in stomatal conductance (g_0) was more prominent in the wet than in the dry period, with the lowest values in the midday and then in the afternoon (Fig. 6). Stomatal conductance recorded in the morning of the wet period was the highest with the values exceeding 1 cms^{-1}. Although some level of diurnal variation of g_0 was shown during dry period, the values recorded for g_0 in the morning were less than the values for midday in the wet period. Also, diurnal variation of g_0 in the dry period was in the range of 0.2 cms^{-1}.

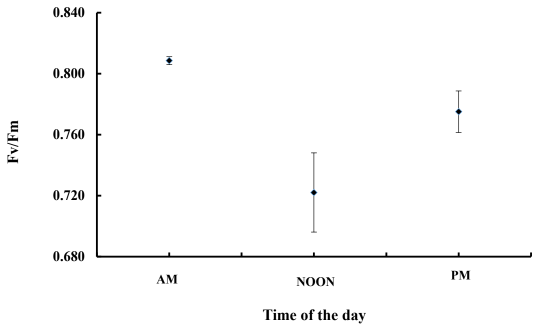

Fig. 5. Leaf chlorophyll *a* fluorescence emission *Fv/Fm* ratio of rubber in dry period (pooled values of year 2010 and 2011). Error bars represent ± standard errors for nine values

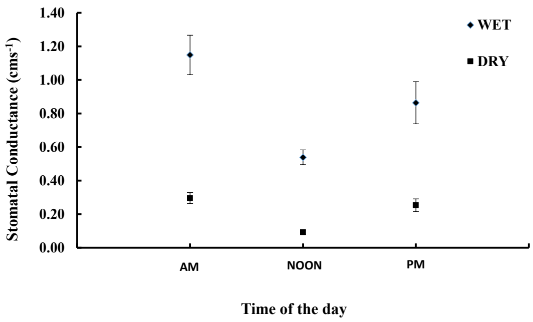

Fig. 6. Stomatal conductance (g_0) of rubber planted in 2010 (at the age of four years). Each g_0 value represents the mean of 12 measurements recorded during the morning (0900-1100), midday (1200-1400) and evening (1500-1700) in wet and dry periods in year 2014. Error bars indicate standard error of means

Discussion

Rubber cultivation in suboptimal conditions is important to meet the in-country demand for raw rubber from the rubber product manufacturing sector. The present level of value addition to raw rubber produced within the country has already exceeded 70% and at present, raw rubber imports in Sri Lanka are more than exports (MPI, 2015). Hence, in order to provide uninterrupted supply of raw rubber, rubber cultivation has to be expanded from traditional to other potential areas. With low level of opportunity cost for labour, rubber cultivation in North and East regions would create a win-win situation providing additional rubber to the manufacturing sector of the country and also a stable income to the resource poor farmers in the region.

Growth of rubber as shown by the girth expansion has been satisfactory with an average of 8.6 cm per year which is comparable with general circumstance in the traditional Wet Zone. This is mainly due to the irrigation provided at early stages of growth. If this could be continued, rubber trees in this region would come to the tappable stage (*i.e.* 50 cm girth) within seven years. Although this would be slightly higher than the expected number years in immaturity (*i.e.* 5-6 years), the growth rate achieved under suboptimal conditions in Sri Lanka is rather remarkable as nine years of immaturity has been recorded for rubber in suboptimal regions in India, *i.e.* Konkan region of Western India (Chandrashekar *et al.,* 1998) and Bastar region in Chattisgarh state located in central India (Krishan *et al*., 2007; Krishan 2013; 2015). However, the situation might have differed with no irrigation provided in early stages. As indicated by the parameter 'm' in the logistic function fitted to girth values, the transition in growth rate from increasing to decreasing took place at about 38 MAP. Evidence in traditional WZ shows that this occurs at about 42-48 MAP (Rodrigo *et al*., 2005). Intercrops grown with rubber faded off after 3rd year of growth due to the canopy cover given by rubber and then, irrigation to rubber fields was also terminated. This would be the reason for such earlier transition in growth rates. If water supply were continued, increased growth rates would have continued with trees coming to harvestable stage by 5-6 years as experienced in the WZ. Perhaps, plant growth and then yield of rubber would be higher in DZ under irrigated conditions since plants would receive greater level of total sunlight than in WZ for a given period time.

Seasonal variation in rainfall would have caused seasonal fluctuation in girth expansion as evidenced elsewhere (Chandrashekar *et al.*, 1998) However, no such changes could be detected since the frequency of girth measurements (*i.e.* once a year) is not intensive enough for such analysis. However, such study should be useful in quantifying the drought effects on tree growth and yield, hence proposed for future research.

Effect of drought on plant growth is illustrated by the decrease in the light saturated rate of photosynthesis (A_{max}**).** The sensitivity of A_{max} even to short-term water stress is shown by the decrease in A_{max} in the afternoon from the values recorded in the morning. As evidenced by the values recorded for apparent quantum yield (ϕ_{app}) in photosynthesis and the ratio of variable to maximum fluorescence $F_v/F_{m,}$ (*i.e.* values above 0.035 and 0.6, respectively), no permanent damage to photosynthetic mechanism or photoinhibition has occurred due to the dry weather. However, reduced water status in the plants would have resulted in stomatal closure (Chandrashekar *et al*., 1990) as shown by low levels of stomatal conductance during dry period and then, down regulation in photosynthesis would have reduced A_{max} and overall in photosynthesis rate. Though not up to the same levels in dry periods, the same phenomenon would occur even in the afternoon hours of the wet periods with time bound reduction in water status of plants (*i.e.* due to heavy radiation loads received during midday period). Such diurnal effects on photosynthesis and other physiological parameters have been recorded in several previous studies on rubber (Senevirathna *et al*., 2003 and Iqbal and Rodrigo, 2006) and other crops (Mohotti and Lawlor, 2002; Ramalho *et al.,* 1997 and Dang *et al*., 1991).

The response of plants to dry weather would vary with the genotype. For instance in India, Chandrashekar *et al.* (1990) has recorded such genotype variation in stomatal conductance under drought conditions. Therefore, testing of rubber clones in DZ is to be given as a priority basis (N.B. this is in progress at present) with that clones performing better than the presently used genotype could be identified for this region.

Acceptable level of growth rates recorded for rubber in DZ confirms the suitability of the agronomic protocol developed (*i.e.* the tentative recommendation issued) for rubber cultivation in this region (Rodrigo *et al*., 2011a). Therefore, it could continuously be adopted in the expansion process of rubber in DZ. However, the interest shown for rubber cultivation by the Tamil community in North is not encouraging. Although a few social studies on rubber cultivation have been conducted in this region (Rodrigo *et al.,* 2011b), the real cause is still unknown. Therefore, systematic social studies are required to identify causal factors for the same before investing on rubber in Tamil dominated areas in the Northern Province.

In conclusion, the present study showed that the growth of rubber in Northern region (DZ) of Sri Lanka under smallholder conditions (having irrigated intercrops in first three years) has been able to achieve reasonable growth with an average annual girth expansion rate of 8.6 cm. Despite some effects of dry weather on photosynthesis and associated physiological parameters, no evidence for permanent damages to photosynthetic apparatus was recorded.

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Address for correspondence: Dr S M M Iqbal, Principal Research Officer, Adaptive Research Unit, Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka.
e-mail: *smmiq57@gmail.com*

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Effectiveness of low frequency harvesting systems in rubber smallholder sector of Eastern province (IL2) of Sri Lanka

R G N Lakshman*, K V V S Kudaligama*, V H L Rodrigo*, A Nugawela, A P Attanayake*, M K P Perera* and P D T L Madushani***

* *Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka*
** *Department of Plantation Management, Faculty of Agriculture & Plantation Management, Wayamba University of Sri Lanka, Makandura, Gonawila*

Abstract
With no experience on Low Frequency harvesting (LFH) systems in the drier climates, tapping half of the tree circumference once in four days (S/2 d4) instead of traditionally doing once in two days (S/2 d2) was tested in rubber smallholdings established in Eastern Province of Sri Lanka. Yields given by S/2 d4 was slightly higher than that of S/2 d2 and also, found financially more profitable, particularly under hired labour conditions. Quality of raw rubber produced was also not affected by LFH. Seasonal distribution of rubber yields showed that S/2 d4 system outperforms in rainy period whilst yields of S/2 d2 was higher in dry months. Also, demand for labour was higher in the rainy period due to the establishment of seasonal crops. Therefore, it is suggested to apply S/2 d4 only during wet periods (September to March in the following year) and S/2 d2 in dry months (April to August). Reasons for the above responses are also discussed.

Keywords: Bark consumption, cost of production, latex harvesting, low frequency harvesting

Introduction

Rubber industry play a vital role in Sri Lankan economy and it is generating income by raw rubber and rubber product. The amount of foreign exchange by exporting raw rubber and end products in year 2014 was about USD million 940 (Anon, 2014). Rubber plantations in Sri Lanka lie in the Wet zone (WZ) of the country and no sufficient lands for further cultivation of rubber in traditional growing areas, based on the research findings of the rubber Research Institute of Sri Lanka, the Ministry of Plantation Industries had decided to expand rubber in to the Intermediate and Dry zone areas in Eastern and Northern provinces. In addition to the rubber production, Government of Sri Lanka (GoSL) seeks multiple benefits in the expansion process, *i.e.* improved environment with forest cover and enhanced rural livelihood. The Ministry of Plantation

Industry targeted to cultivate 10,000ha in Ampara District and 1,400ha had been cultivated by the end of 2013 (Anon, 2014).

Traditionally, rubber tree is tapped with S/2 d2 system (tapping a half of the circumference of the trunk at the frequency of once in two days). Hence, a harvester could tap only two blocks of trees (tapping blocks) making harvesting a labour intensive activity in rubber cultivation. Though local and international attempts have been tried to mechanize tapping in order to reduce the cost on tapping and to remove the skill factor, a commercially practical device has not yet been developed. Hence, reduction of harvesting frequency to improve the efficiency in the usage of skilled harvesters has become an internationally accepted management system in rubber cultivation. Low frequency harvesting (LFH) allows each harvester to be allocated a higher number of tapping blocks resulting in reduced harvester requirement when compared with the traditional S/2 d2 tapping system. In order to compensate the yield loss due to less number of tapping days per tree, ethephon is used as the yield stimulant to increase the yield per tree per tapping. This results in enhanced daily intake per harvester and therefore, their wages can be increased. Furthermore, overall increase in harvesters' productivity results in reduction of cost of production (COP). Less bark consumption in LFH results in increased economic life of the tree providing an additional benefit to the growers.

Experiments done in India (Karunaichamy *et al.,* 2001; Vijayakumar *et al.,* 2002), Malaysia (Ahmad *et al*., 1991; Kewi and Sivakumaran, 1994) and China (Xuehua *et al.,* 2004) were in favour of LFH systems and with that, tapping a tree once in four, five and six days was recommended. Response of LFH systems depend on the climatic conditions (Vijayakumar *et al.,* 2002). First recommendation on LFH *i.e.* half spiral tapping once in three days with the application of 2.5% ethephon, 4-5 round per year was issued to Sri Lankan growers in 1994 and this system would bring down the harvester requirement by 1/3 (Nugawela, 2001; Nugawela *et al.,* 2000). Recently, Rubber Research Institute of Sri Lanka has recommended S/2 d4 system with application of 12 rounds of 3.3% ethephon at monthly intervals (Rodrigo *et al.,* 2011). This system reduced the intensity of harvesting by 50% and increase the harvester productivity by 100% (Kudaligama *et al.,* 2015). Though this system has been successful in Wet zone of the country, its effectiveness under the suboptimal climatic conditions in Intermediate zone has not been studied systematically. Therefore, this study was aimed to investigate the effectiveness of S/2 d4 system under dry climatic condition in Eastern province and benefits to the rural rubber farmers in the area.

Materials and Methods

Three mature rubber smallholdings replanted with RRIC 121 genotype in Padiyathalawa of Ampara district which lies in IL 2 Agro-ecological zone was selected for testing the effectiveness of S/2 d3 (tapping half spiral of the tree once in three days frequency) and S/2 d4 (tapping half spiral of the tree once in four days frequency) systems under prevailing dry climatic conditions in the Intermediate zone. Trees were planted in 2003 and tapping was started in 2011. Approximate number of trees in each field were between 200- 250 and about 60 - 75 trees was selected to harvested with S/2 d3 and S/2 d4 systems with the application of 2.5% ethephon quarterly (4 rounds per year) and 3.3% ethephon monthly (10 rounds per year), respectively as the yield stimulant to compensate the yield loss due to lowering the tapping frequency. June – July period stimulation was suspended due to prevailing dry condition in the area.

Rest of the trees in each field were harvested with traditional once in two days (S/2 d2) harvesting system without stimulation for comparison. Experiment began in 2013. In each tapping day latex volume and dry weight of rubber was recorded. In every six months girth of trees, bark thickness, bark consumption and incidence of tapping panel dryness were also assessed. Ribbed smoked sheets (RSS) processed from the latex collected under S/2 d2, S/2 d3 and S/2 d4 harvesting systems were assessed for raw rubber properties *i.e.* initial plasticity (Po), plasticity retention index (PRI), Mooney viscosity (V_R), colour, nitrogen and ash content using the standard test methods stipulated by the International Standard Organization (Anon, 1984).

Local weather condition was monitored with a simple metrological station established at Komana Maha Vidyalaya in the area. It comprised a rain gauge, maximum and minimum thermometers, wet and dry bulb thermometers and a pan evaporator.

From daily records on latex yields and the actual number of tapping days, mean values for yield per tree per year (YPT), yield per hectare per annum (YPH) and daily intake per harvester (IPH) were derived for each tapping system. Similarly, growth data and at tree level were processed into mean values in each treatment of each plot. Three harvesting systems tested were statistically analysed using the statistical package the SAS system 9.2.

Financial viability of S/2 d4 harvesting system was assessed at commercial level with the knowledge on both cost and benefit components for a given period of one year. Tapping cost was based on the current daily wage of workers (LKR 800/=) in the area and ethephon application cost (chemical cost, LKR 5,425/= per 5 litre plus the labour cost of LKR 400/= per tapping block per application). Labour for weed control, cost of chemicals and maintenance of latex collecting utensils were the other costs taken for calculating the cost of production.

Prevailing prices for ribbed smoked sheets (RSS) and scrap rubber *i.e.* LKR 200/= and LKR 80/=, respectively were taken for the financial analysis.

Results

Rainy season to Padiyathalawa area started in mid October and lasted till February in the following year. Highest rainfall was received during December – January period and few showers were also available till April. Drought period started from end of April and came to peak during June. Annual rainfall and number of rainy days were 900.1mm and 51, respectively. However, 70% of the rainfall was received during November - January period and average relative humidity (%RH) during these wet months were above 90% and average falls to about 78% during dry months (Fig. 1a and 1b). During initial three months of the year pan-evaporation was about 3.00mm/day and this was increased to about 5 mm/day during April - July period and thereafter gradually decreased with the receiving rainfall (Fig. 1c). Average minimum and maximum temperature were 24.13˚C and 34.90˚C, respectively in the area. During January - May, observed minimum and maximum temperatures were below the average and thereafter these increased giving the highest during June – September period. With the beginning of monsoon towards the end of year both values gradually decreased (Fig. 1d).

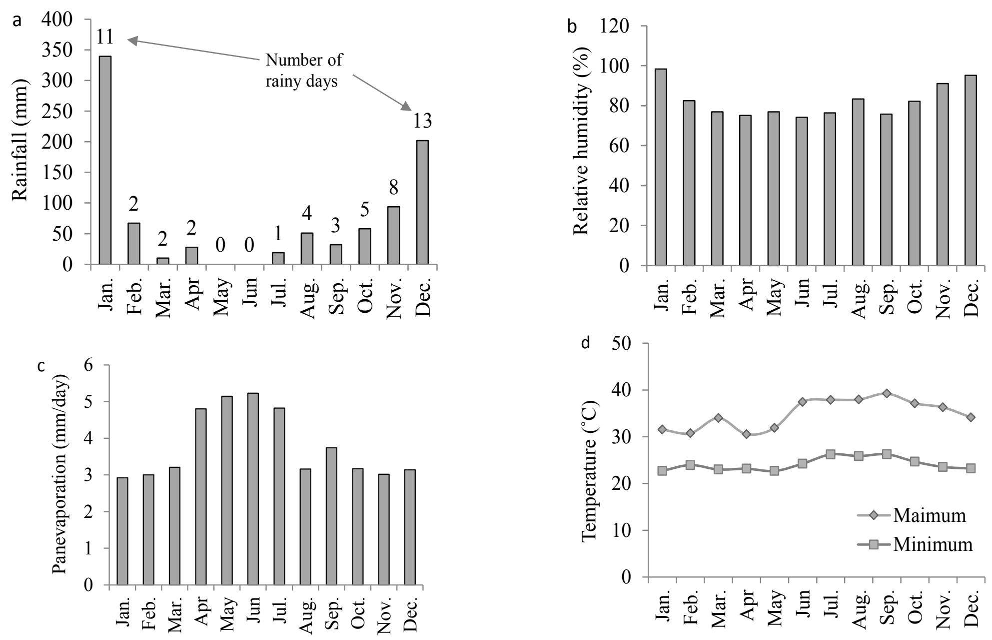

Fig. 1. Environmental conditions in Padiyathalawa area

Table 1. *Average yield parameters of S/2 d2 and S/2 d4 harvesting systems (Yield per hectare has been derived at the standard planting density of 400 tree/ha)*

Tapping system	Actual tapping days (per tree)	Dry rubber content (%)	Yield per tree per tapping (g)	Yield per tree per year (kg)	Yield per hectare per year (kg)
S/2 d2	128	38.17±0.45	21.05±1.03	2.69	1078
S/2 d4	69	37.99±0.45	44.75±2.86	3.09	1235

In all three fields monitored, dry rubber content of latex (%DRC) of S/2 d4 system had not shown any significant difference from that of S/2 d2 system and values did not fall below 35% in both harvesting systems throughout the period (Table 1).

Though not statistically significant, average annual yield received by S/2 d4 was higher than that of S/2 d2 system. Similarly, average yield received from a tree tapped under S/2 d4 system was greater than that of S/2 d2 system (Table 1).

When compared to the expected level, bark consumption per tapping was slightly higher in S/2 d4 system. However, annual bark consumption was 22.30cm and 12.20cm in S/2 d2 and S/2 d4 systems, respectively. Therefore, expected years for harvesting in base panels in S/2 d4 system showed 77% increase over that of S/2 d2.

Trees affected with tapping panel dryness (TPD) were below 1% in both harvesting systems and the incidence of TPD was not significantly different among S/2 d2 and S/2 d4 harvesting systems. Average girth and bark thickness of trees in the fields monitored were 52.77cm and 5.92cm, respectively. Though not significant, annual rate of increase of girth and bark thickness of trees harvested with S/2 d4 system were lower than that of S/2 d2 system.

Initial plasticity (Po), plasticity retention index (PRI), Mooney viscosity (V_R), ash%, nitrogen% and Lovibond colour did not show any significant variation between two harvesting systems (Table 2).

Table 2. *Variation in raw rubber properties of ribbed smoked sheets (RSS) produced under S/2 d2 and S/2 d4 systems*

Harvesting systems	Po (Wallace units)	PRI	V_R (ML 1+4@ 100°C)	Nitrogen (%w/w)	Ash (%w/w)
S/2 d2	35.35±0.96	86.26±1.96	62.26±1.59	0.28±0.01	0.26±0.012
S/2 d4	34.81±1.03	84.65±1.63	59.87±1.60	0.29±0.01	0.26±0.013

During wet months average yield and income was significantly higher when trees were tapped with S/2 d4 system by farmers. However, the difference in yield and income in S/2 d2 and S/2 d4 systems were not significant during dry months. At any time harvesting trees with S/2 d2 system by hired labour gave the least financial benefit. In some months financial benefit is higher in S/2 d4 system even with use of hired labour than use of own labour in S/2 d2 harvesting (Fig. 2 & 3).

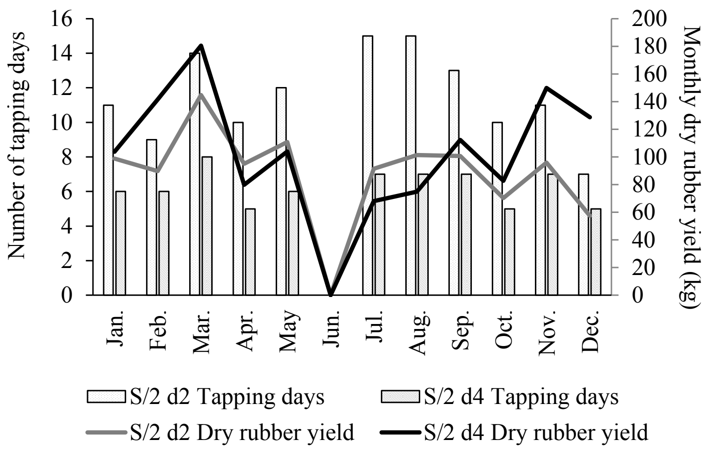

Fig. 2. Seasonal variation in tapping days and expected dry rubber yield to be received from a one hectare of rubber land

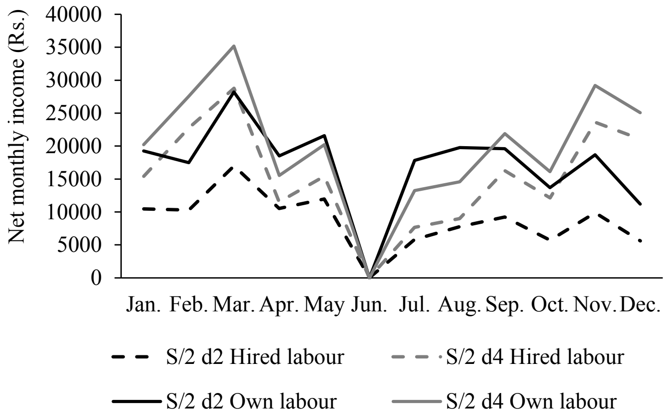

Fig. 3. Net monthly income of one hectare of rubber land managed with hired labour and own labour condition

Other than the tapping, costs involved for cost of production (COP) were cost for weed control, chemicals and maintenance of tapping and latex collecting utensils in the field. Under hired and own labour condition COP other than tapping cost was LKR 5.50 and LKR 1.50, respectively per production of one kg of dry rubber. Profit from 1 ha of rubber land tapped with hired labour using S/2 d2 system was LKR 40,102/= per year and with the adoption S/2 d4 system net profit per ha of rubber land further increased by LKR 65,738/=. If the trees were tapped with own labour, the profitability from 1 ha of rubber land tapped with S/2 d2 and S/2 d4 system were LKR 189,964/= and LKR 193,627/=, per year respectively.

Discussion

The success of expanding process of rubber cultivation to drier regions depends on the latex yield given and how effectively it can be harvested in the socio-economic environment in the peasant community. The present study has shown not only the applicability of a newly developed low frequency harvesting system (*i.e.* S/2 d4) but also how best the usually practiced S/2 d2 fits into such environment. With no detailed studies carried out on yield pattern of rubber in this climatic conditions before, the present study is unique in providing necessary information to apply the suitable harvesting systems to meet the climatic and social conditions in the Intermediate Zone (IZ) of Sri Lanka. Rainfall pattern in the experimental area is rather unimodal with very distinct dry period; hence it features drier conditions than other IZ areas (having bimodal rainfall) where rubber is grown (*e.g.* Monaragala). Therefore, findings of this study will also be applicable to Dry Zone areas where newly planted rubber fields are yet to be harvested.

The yield given by both systems of harvesting exceeded 1000 kg/ha/year. Although this level might be below the general expectation (*i.e.* 1500 kg/ha/year), it depicts national average yield which is principally based on the yields in traditional wet areas of the country. Rubber trees are still in the early stage of harvesting and yields should increase further with the growth of trees. Also, number of tapping days recorded in both harvesting systems was much below the value to be and that is also a major contributory factor for low yields. Nevertheless, total yield given by S/2 d4 low frequency harvesting (LFH) system was slightly superior to that of usually practised S/2 d2, hence its worthiness would be out of question. This is further confirmed by the financial analysis. Latex harvesting is the most costly operation in rubber fields and therefore with comparatively less labour requirement in S/2 d4, the gap in profitability between two tapping systems increases under the condition of hired labour.

The reason for comparatively poor performance of S/2 d4 in dry months would be that more water is to be taken out as latex on each tapping day of this system and this is affected by the poor water status in the plant (N.B. *ca.* 90% of latex is water). Nevertheless, latex quality in terms of quality parameters tested in raw rubber produced (RSS) was not affected by LFH. Another advantage of LFH shown in the study was the potential of keeping the rubber tree longer with reduced bark consumption. In drier climates, early establishment of rubber plants is a challenge and therefore, once established, tapping is to be undertaken carefully to keep trees longer. Unlike wet region, incidence of root diseases does not influence to reduce the tree density to a greater extent in drier climates. Therefore, this advantage can be explored with S/2 d4 LFH system to meet the need of extended tree lifespan in drier climates.

Farmers in traditional wet region do not usually change the harvesting frequency adopted due to the convenience in hiring workers for tapping. However, this may not be an issue in the social conditions in drier regions where farmers use own or shared labour in on-farm operations. They need to manage themselves rubber tapping as well as other farm operations during peak labour demanding periods associated with the establishment of other crops (seasonal crops). Therefore, farmers may forgo tapping on some days and this effect is more prominent in high frequency systems as shown in the present study (N.B. Reduction in tapping days from the expected level was higher in S/2 d2 system which requires more labour inputs than in S2 d4 LFH). On other hand, the yield performance of S/2 d4 was better during wet periods whilst S/2 d2 performed better in dry periods. During wet periods, farmers need to be engaged in other farm operations required for seasonal crops. Therefore, the best would be to have a combination of harvesting systems in which all issues are addressed. Considering the temporal (seasonal) advantages of different harvesting systems, S/2 d4 can be practiced only during wet periods (September to March in the following year) whilst engaging in farm operations for other crops and then S/2 d2 is to be practiced during dry months (April to August). In this way, higher monthly and then annual yields could be obtained than what were recorded in the present study. Also, number of ethephon stimulations are limited to the months of S/2 d4 and thereby, the cost involved can be minimized.

Conclusion

Newly developed S/2 d4 low frequency system could be successfully be adapted in combination with S/2 d2 for harvesting rubber in drier areas. Considering the seasonal advantage of different harvesting systems, S/2 d4 can be practiced during wet period (September – March in the following year) limiting the use of S/2 d2 to dry months (April – August).

Acknowledgement

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Address for correspondence: Dr (Mrs) K V V S Kudaligama, Senior Research Officer, Biochemistry & Physiology Dept., Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka.
e-mail: *vskudaligama@yahoo.com*

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Treatment with nitric oxide and seed coat removal improve germination and growth of rubber (*Hevea brasiliensis*)

N M C Nayanakantha*, K D Madhushani, L A R Amarathunga*, G A S Wijesekera*, P D Pathirana*, W Karunatilaka*, D L N de Zoysa*, M N de Alwis*, R Handapangoda* and P Seneviratne***

* *Plant Science Department, Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka*
** *Department of Crop Science, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka*

Abstract
Studies were carried out on the effect of seed coat removal and nitric oxide (NO) donor sodium nitroprusside (SNP) treatment on germination and growth of seedling and budded plants of rubber (Hevea brasiliensis). Interaction effects were observed between seed coat treatment and SNP treatments for germination time and percentage germination. Decoated seeds germinated earlier and improved synchronicity than intact seeds (with seed coat) and achieved 50% germination within nine days. SNP treatment at all four concentrations (20, 50, 100 and 200 µM) hastened the germination and increased the percentage germination especially when decoated seeds were used. Shoot and root attributes of seedlings (stock plants) derived from the seeds treated with SNP, especially at 50-100 µM, were significantly improved as compared to those from control and mock treated seeds. Growth of the budded plants were better when stock plants derived from SNP treated seeds were used. Therefore, NO treatment coupled with decoating could effectively be utilized to improve the germination and growth of seedling and budded plants of rubber. To our knowledge, this is the first report on redox priming of rubber seeds with SNP.

Key words: germination, growth, nitric oxide, rubber, sodium nitroprusside

INTRODUCTION

The quality of planting material and an optimum stand of vigorous plants are of utmost importance to achieve the potential yield of rubber (*Hevea brasiliensis*) clones (Nugawela, 2010). In order to produce high quality rubber plants, both the rootstock and the budwood should be of high quality (Seneviratne and Wijesekara, 2011). Rubber seeds are used to generate root stock plants to be budgrafted with desirable clones. Rubber seeds are recalcitrant and have high moisture content at maturity (Chin, 1995). Recalcitrant seeds germinate rapidly when sown fresh, but are sensitive to desiccation and freezing and hence difficult to store (McDonald, 2004). Fresh rubber seeds generally take 7-10 days to start germination in a sand bed. Vigorous seeds should be selected to raise stock plants by collecting early germinates. About 50% of the early germinates should be harvested every other day, only for three rounds, although entire population germinates (Anon, 2013). Vigorous seedlings become buddable in 3-4 months under proper agro- management practices (Anon, 2013).

However, for government rubber nurseries, seeds are purchased from different suppliers who collect and store seeds for a few days to weeks to supply them in bulk quantities. These seeds generally take 2-3 weeks to start germination and 3-4 weeks to achieve 50% germination. Although it is recommended to transplant the seedlings into polybags at the radicle emergence stage under normal condition (Anon, 2013), transplanting is generally delayed by the nursery men until the leaf emergence stage due to various reasons. Due to asynchronous germination of rubber seeds, a number of vigorous seedlings that immerge earlier grow faster when the rest germinate. During transplanting, somewhat mature seedlings are compelled to be discarded and hence the vigorous seedlings are lost. Further, it frequently forces the nurseries to extend the budgrafting period for up to six months as they wait for late-germinating seedlings also to achieve buddable size. Therefore, it is essential to find ways to speed up and improve synchronous germination and increase percentage germination of rubber seeds.

The coat is thick and hard in rubber seeds and therefore can act as a physical barrier for germination. Seed coat removal is a mechanical scarification method to improve the germination and ensure uniform seedling emergence (Kimura and Islam, 2012). Previous studies indicated that removal of seed coat of certain woody perennial species producing orthodox seeds *viz.,* pine (Saeed and Thanos, 2006), *Rauvolfia serpentine* (Paul *et al.,* 2008), *Quercus serrata* Thunb. and *Quercus semecarpifolia* Sm. (Pandey and Tamta, 2013), *Grevillia* spp (Morris *et al.,* 2000) and citrus (Girardi *et al.,* 2007) would enhance germination and uniformity, increase percentage germination and enhance seedling performance. However, only a few reports are available on studies of seed coat removal in recalcitrant woody perennials such as Mango (Muralidhara *et al.,* 2016) and Avocado (Bergh, 1988). Therefore, one can expect that decoating (complete removal of seed coat) would improve germination of rubber seeds.

Soaking of seeds in water (hydropriming), inorganic salt solution (halopriming) or hormone solution (homopriming) has been reported as common priming techniques for enhanced germination dynamics in many crop plants such as wheat, rice (Dorna *et al.,* 2014) and lentil (Ghassemi-Golezani, 2008). It is well known that plant hormones can impact seed dormancy and improve germination in crop species. For example, abscisic acid (ABA) generally inhibits germination (Gubler *et al.,* 2005), whereas gibberellic acid (GA) promotes germination (da Silva *et al.,* 2004).

More recently, it has become apparent that molecules, such as nitric oxide (NO) can significantly impact on plant growth and development from seed germination up to senescence (Mur *et al.,* 2012). Redox priming with NO donor sodium nitroprusside (SNP) has been shown to enhance germination and/or break dormancy in orthodox seeds such as Barley (Bethke *et al.,* 2004), lettuce (Beligni and Lamattina, 2000), rice (Habib *et al.,* 2010), wheat (Hua *et al.,* 2003), switchgrass (Sarath *et al.,* 2006) including *Arabidopsis thaliana* (Bethke *et al.,* 2006a). Nevertheless reports on NO implication in recalcitrant seeds, including rubber, is scarce (Bai *et al.,* 2011).

NO is required for root organogenesis (Pagnussat *et al.,* 2002), lateral root development (Correa-Aragunde *et al.,* 2004) and improve growth and development of many crop plants especially under abiotic stress conditions (Mur *et al.,* 2012, Kong *et al.,* 2014). Nayanakantha *et al.* (2014) demonstrated that treatment with NO donor SNP improved growth and root characteristics of rubber seedlings in a dose dependent manner. Therefore, this study was undertaken to ascertain the effectiveness of seed coat removal and NO treatments on germination dynamics and growth performance of seedling and budded plants of rubber. We report here that, exogenous NO donor SNP and decoating treatments hastened germination, improved synchronicity and percentage germination and growth of rubber plants.

Materials and methods

The study was conducted in a nursery at the Dartnofield Estate of the Rubber Research Institute of Sri Lanka (RRISL). Fresh rubber seeds from the clone BPM 24 were collected at the early seed fall from Millewa estate, Padukka and brought to RRISL. Half of the seed lot was manually decoated and other half left intact. Both intact and decoated seeds were treated as follows: no soaking (control), soaked in water for 6 hours or in 20, 50, 100 and 200 µM SNP for 6 hours.

Seeds were sown in a germination bed filled with sand in to a randomized complete block design (RCBD) with four blocks. There were 4 replications of 50 seed each in each treatment in each block, with a total of 2,400 seeds. Germination beds were watered once daily. Cumulative seed germination percentage was recorded at 7, 9, 11, 13, 15, 17, 19 and 21 days after sowing. Seeds were considered as germinated when the radicle had protruded about 2 mm, regardless of seed coat.

Each germinated seedlings were transplanted in black polythene bags, gauge 300 and having lay-flat dimensions of 15 cm diameter and 37 cm height, filled with soil. Soils from the Agalawatta series were collected from the surface layer (0 – 15 cm depth), sieved through a 1 cm mesh to remove large lateritic gravel before filling the bags. Polybags were arranged in a nursery according to a RCBD with eight blocks, each treatment containing five plants so that the total number of plants for each treatment was forty. Two weeks after transplanting, application of chemical fertilizers, *i.e.* young budding fertilizer mixture in liquid form, into seedlings was started and carried out at two-week intervals. All other management practices were same as recommended by the Rubber Research Institute of Sri Lanka (Anon, 2013).

Measurement of growth parameters of seedlings

Growth attributes of rubber seedlings were assessed after three months from transplanting. Ten plants from each treatment were removed and root system was washed gently under running water over a 0.5 mm sieve and the adhering soil and dust particles were carefully removed. Morphological attributes *viz.,* diameter of stem, plant height and number of leaves were recorded. Leaf area was determined by leaf area meter (Model L1-3100, LI-COR, USA). Chlorophyll content was measured using SPAD 502 Plus chlorophyll meter. Dry weights (DW) of shoots and roots were obtained by oven-drying the samples at 70 ^{o}C for 48 hours.

Measurement of growth parameters of budded plants

Seedlings were budgrafted with PB 260 clone after three months from transplanting. Percentage budgrafting success was recorded after one month. When the first leaf whorl was matured, growth attributes *viz.,* number of leaves, leaf area, chlorophyll content of leaf and dry weight of scion shoots were recorded.

Data analysis

Germination data was subjected to arc-sin transformation before being submitted to analysis of variance. However, actual percentages are presented. Growth data before and after budgrafting were analyzed according to two-factor factorial experiment (with/ without seed coat and five levels of SNP). Significance of the observed treatment differences was tested by analysis of variance using proc GLM procedure of the SAS software package (version 9.1) and significant means were separated using Duncan's Multiple Range Test (DMRT) at the 5% probability level.

Results

Seed germination

Results of the present study revealed that there were interaction effects between seed coat treatment and soaking treatments for germination time and percentage germination. A significantly higher germination percentage was recorded from decoated seeds than from intact seeds for up to thirteen days from sowing when the factor 1 (soaking treatment) was fixed (Fig. 1). The first visible germination occurred as early as four days after sowing when several seeds with radical perturbation were observed from decoated seeds treated with SNP. However, by seventh day after sowing, a significantly higher germination percentage was recorded from seeds treated with SNP, especially at 50 µM-100 µM, when the factor 2 (seed coat treatment) was fixed (Fig. 2).

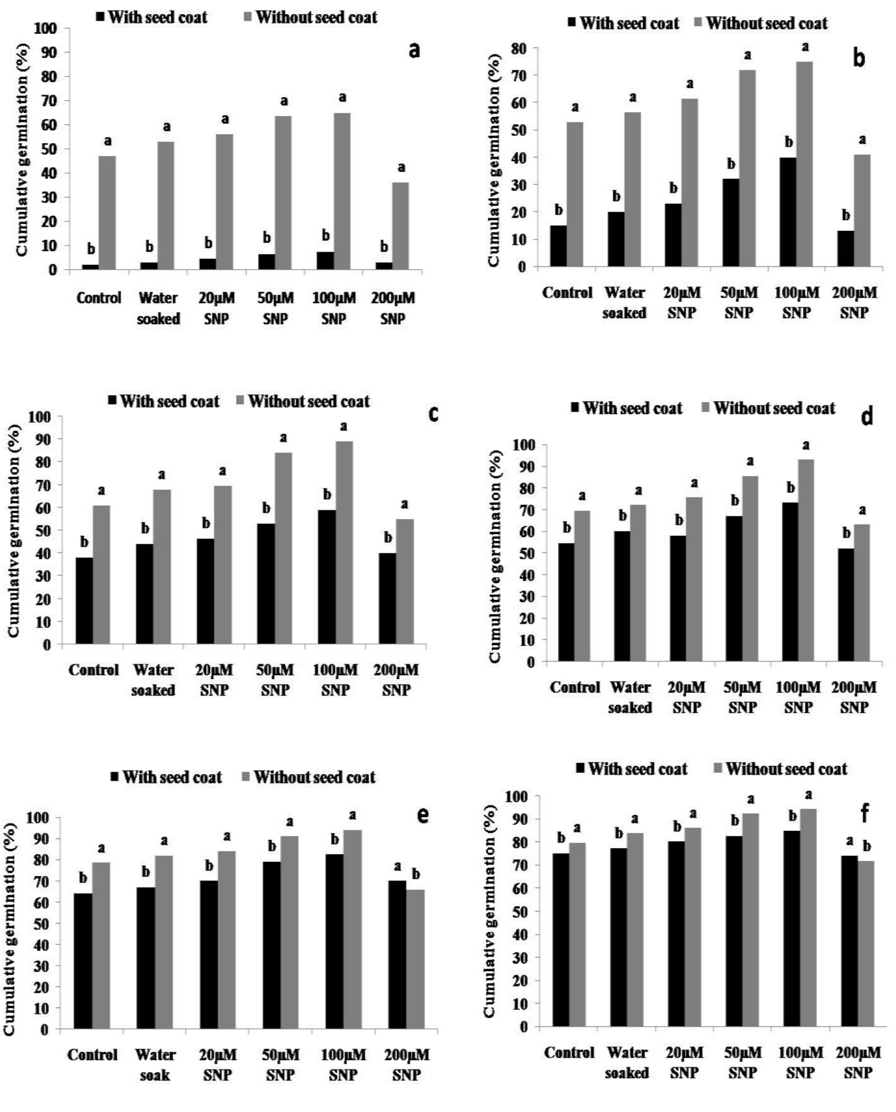

Fig. 1. Effect of seed coat on germination percentage of rubber seeds when the factor 1 (soaking treatments with SNP) was fixed. **a)** after 7 days, **b)** after 9 days, **c)** after 11 days, **d)** after 13 days, **e)** after 15 days and **f)** after 21 days from sowing. Means with same letter between two adjacent bars are not significantly different at $p \leq 0.05$

Decoated seeds devoid of soaking treatments took only 9 days to complete 50% germination whilst intact control seeds required 13 days (Fig. 1).

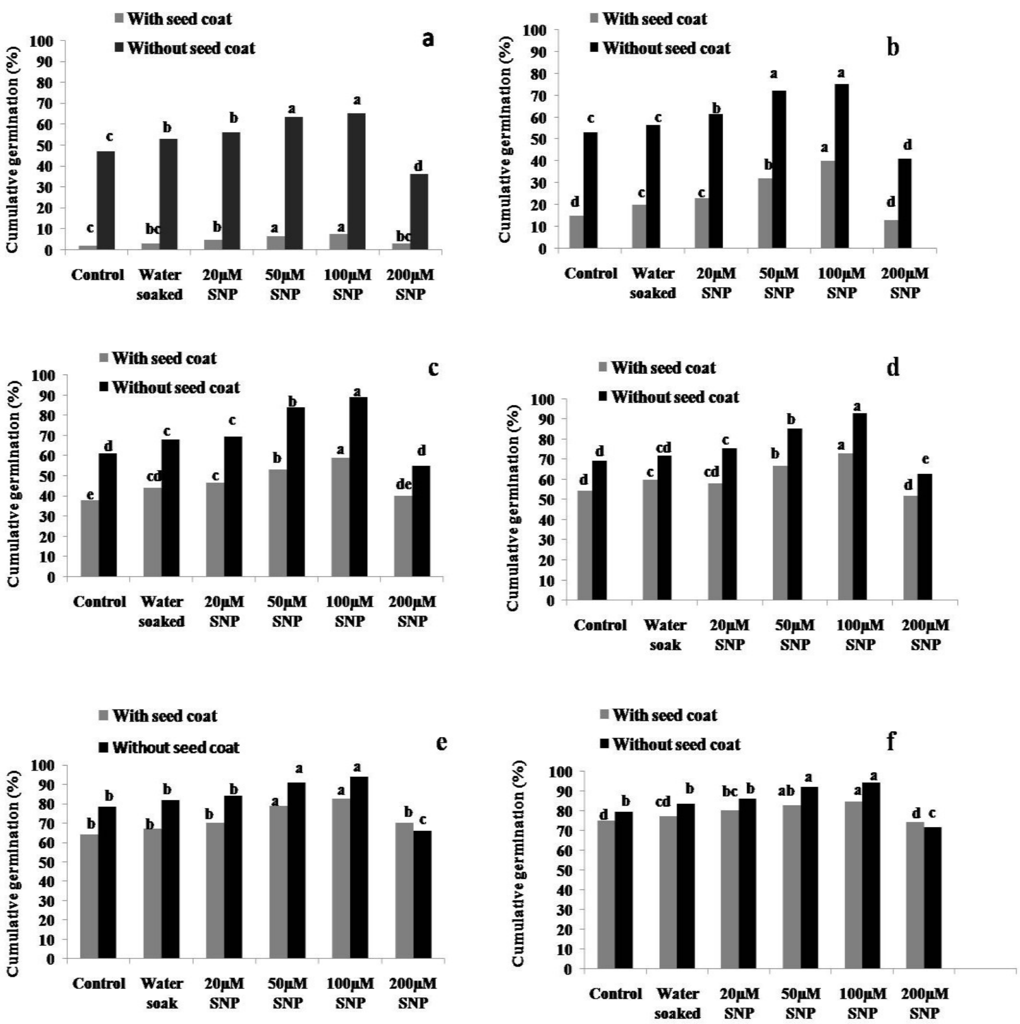

Fig. 2. Effect of NO donor SNP on germination percentage of rubber seeds when the factor 2 (seed coat treatment) was fixed. **a)** after 7 days, **b)** after 9 days, **c)** after 11 days, **d)** after 13 days, **e)** after 15 days and **f)** after 21 days from sowing. Means with same letter among bars for different SNP concentrations are not significantly different at $p \leq 0.05$.

SNP treatment greatly hastened the germination and increased percentage germination in both decoated and intact seeds. By the 13th day after sowing, 93% of decoated seeds and 73% of intact seeds had germinated upon SNP treatment at 100 µM as compared to those devoid of soaking treatments, 69.5% for decoated seeds and 54.5% for intact seeds (Fig. 2). Nevertheless, by the 21st day after sowing, 75% of the intact seeds (control) had germinated while 79.5% of the decoated seeds devoid of soaking treatments had germinated (Fig. 1).

Seedling attributes

Interactive effects were observed only for dry weight of shoots and chlorophyll content between seed coat treatment and SNP treatments after three months from transplanting into polybags (Fig. 3, 4, 5 & 6). Seedlings derived from SNP treated seeds, in dose dependent manner, showed improved shoot and root characteristics as compared to those derived from control and mock treated (water soaked) seeds after three months from transplanting into polybags.

Seedlings derived from decoated seeds devoid of soaking treatments and SNP treatment at 20 µM showed a higher chlorophyll content than that from their respective intact seeds (Fig. 6). Nevertheless, seedlings derived from intact seeds with mock treatment and treated with SNP at 50-200 µM showed a higher chlorophyll content than that from their respective decoated seeds (Fig. 5). Nevertheless, 100 µM SNP was most effective for the increment in chlorophyll content in seedlings derived from both decoated and intact seeds. Interestingly SNP treatment at all four concentrations (20 µM, 50 µM, 100 µM and 200 µM) showed a significantly higher shoot dry weight as compared to control and mock treatment when intact seeds were used (Fig. 3). However, when decoated seeds were used, 200 µM SNP was not effective (Fig. 6). Nevertheless, soaking of seeds in a 100 µM SNP solution was most effective for the increment in shoot dry weight in seedlings derived from both decoated and intact seeds.

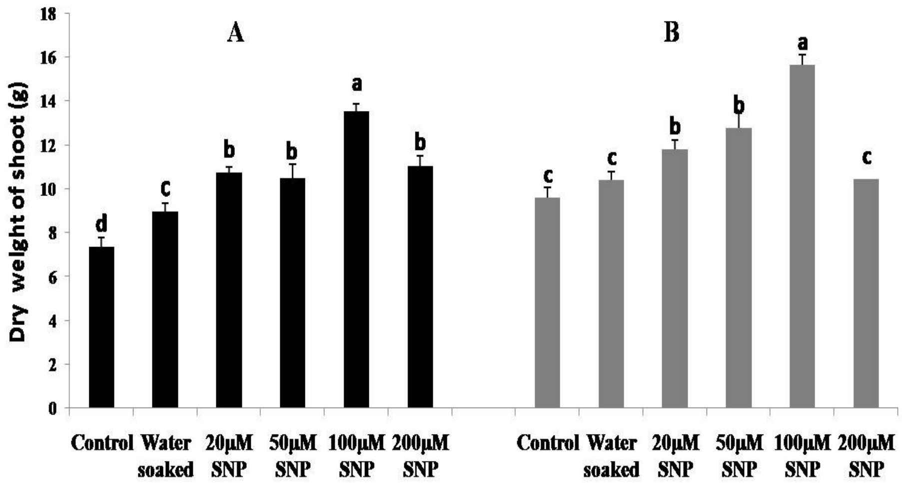

Fig. 3. Effect of NO donor SNP on shoot dry weight of rubber seedlings after three months from transplanting, when the factor 2 (seed coat treatment) was fixed. A) intact seed, B) decoated seed. Letters indicate significant difference at $p \leq 0.05$ according to DMRT.

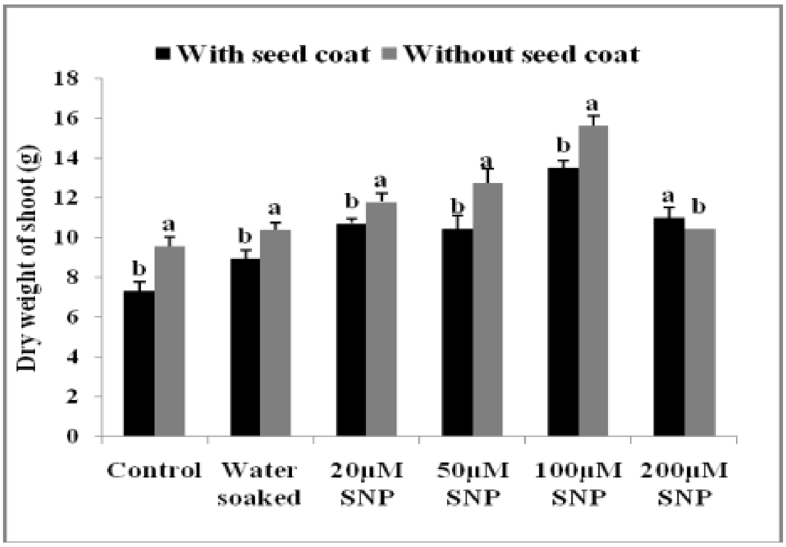

Fig. 4. Effect of seed coat treatment on shoot dry weight of rubber seedlings after three months from transplanting, when the factor 1 (soaking treatment with SNP) was fixed. A) intact seed, B) decoated seed. Letters indicate significant difference at $p \leq 0.05$ according to DMRT

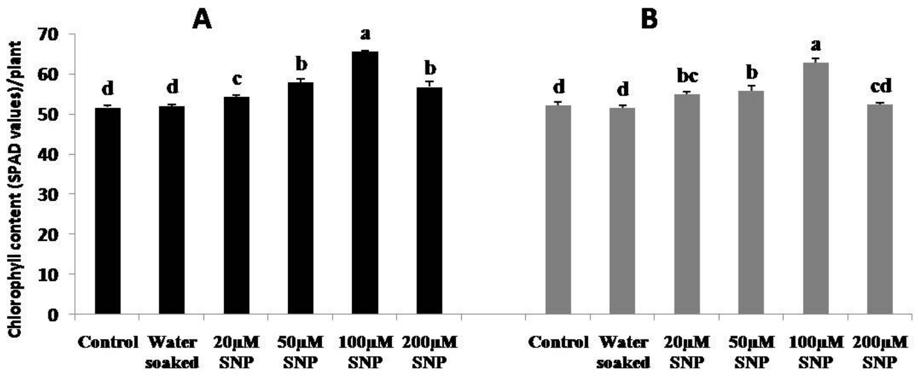

Fig. 5. Effect of NO donor SNP on chlorophyll content of rubber seedlings after three months from transplanting, when the factor 2 (seed coat treatment) was fixed. A) intact seed, B) decoated seed. Letters indicate significant difference at $p \leq 0.05$ according to DMRT.

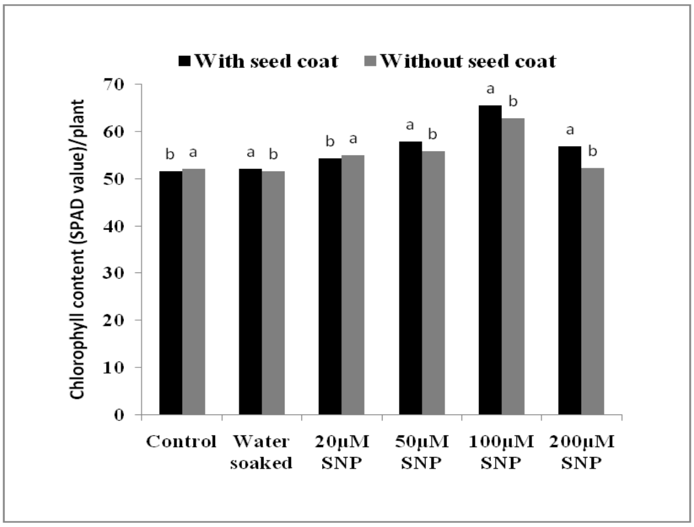

Fig. 6. Effect of seed coat removal on chlorophyll content of rubber seedlings after three months from transplanting, when the factor 1 (soaking treatment with SNP) was fixed. A) intact seed, B) decoated seed. Letters indicate significant difference at $p \leq 0.05$ according to DMRT

Shoot characteristics *viz.,* stem height, stem diameter and leaf area significantly increased with SNP treatment, especially at 100 µM, as compared to control and mock treatment (Table 1). Nevertheless, there was no significant difference in stem height, stem diameter, number of leaves and leaf area between seedlings derived from decoated seeds and intact seeds (Table 2). Tallest plants were produced when seedlings were derived from seeds treated with SNP at 200 µM (Table 1).

Interaction effects were not observed for any of the root attributes between seed coat treatment and soaking treatments with SNP (Table 3 & 4). All the concentrations of SNP (20-200µM) resulted in increase in root attributes of rubber seedlings *viz.,* dry weights of early secondary roots (ESR) + secondary roots (SR), tap root and total roots as compared to control and mock treatment (Table 3).

Table 1. *Effect of seed treatment with NO donor SNP on shoot characteristics of rubber seedlings after three months from transplanting into polybags*

Treatment	**Stem height (cm/plant)**	**Stem diameter (mm/plant)**	**No. of leaves (per plant)**	**Leaf area (cm^2/plant)**
Control	65.36 ± 1.18^{d}	8.07 ± 0.07^{cd}	9.8 ± 0.44^{a}	888.55 ± 20.75^{c}
water soaked	66.86 ± 1.13^{cd}	7.88 ± 0.14^{d}	9.8 ± 0.36^{a}	921.71 ± 16.67^{bc}
20 µM SNP	68.86 ± 1.11^{c}	8.61 ± 0.16^{b}	9.8 ± 0.36^{a}	977.66 ± 19.21^{b}
50 µM SNP	69.72 ± 0.83^{c}	8.75 ± 0.12^{b}	10.1 ± 0.46^{a}	963.89 ± 19.63^{bc}
100 µM SNP	73.01 ± 1.54^{b}	10.63 ± 0.31^{a}	10.0 ± 0.21^{a}	1260.68 ± 14.88^{a}
200 µM SNP	84.31 ± 1.82^{a}	8.44 ± 0.19^{bc}	9.6 ± 0.43^{a}	980.09 ± 33.37^{b}

Values followed by the same letter in a column are not significantly different at $p \leq 0.05$.

Table 2. *Effect of seed coat removal on shoot characteristics of rubber seedlings after three months from transplanting into polybags*

Treatment	**Stem height (cm/plant)**	**Stem diameter (mm/plant)**	**No. of leaves (per plant)**	**Leaf area (cm^2 /plant)**
Intact seeds	68.99 ± 1.24^{a}	8.50 ± 0.15^{a}	10.1 ± 0.16^{a}	1018.85 ± 24.22^{a}
Decoated seeds	73.73 ± 1.35^{a}	8.96 ± 0.21^{a}	9.6 ± 0.25^{a}	978.68 ± 30.91^{a}

Values followed by the same letter in a column are not significantly different at $p \leq 0.05$.

Table 3. *Effect of seed treatment with NO donor SNP on root characteristics of rubber seedlings after three months from transplanting into polybags*

Treatment	**DW of ESR+ SR (g/plant)**	**DW of taproot (g/plant)**	**DW of total roots (g/plant)**
Control	1.18±0.03[c]	2.50±0.07[d]	3.69±0.08[d]
Water soaked	1.26±0.05[c]	2.64±0.07[d]	3.90±0.08[d]
Soaked in 20µM SNP	1.48±.05[b]	2.86±0.07[c]	4.35±.08[c]
Soaked in 50µM SNP	1.54±0.03[b]	3.10±0.07[b]	4.65±0.08[b]
Soaked in 100µM SNP	2.02±0.08[b]	4.29±0.08[a]	6.31±0.12[a]
Soaked in 200µM SNP	1.44±0.08[b]	3.15±0.09[b]	4.60±0.10[bc]

Values followed by the same letter in a column are not significantly different at $p \leq 0.05$ according to DMRT.

Table 4. *Effect of seed coat treatment on root characteristics of rubber seedlings after three months from transplanting into polybags*

Treatment	**DW of ESR+ SR (g/plant)**	**DW of taproot (g/plant)**	**DW of total roots (g/plant)**
Intact seeds	1.45±0.05[a]	3.03±0.11[a]	4.49±0.16[a]
Decoated seeds	1.52±0.05[a]	3.15±0.11[a]	4.68±0.16[a]

Values followed by the same letter in a column are not significantly different at $p \leq 0.05$ according to DMRT.

Budded plant attributes

There was no effect of SNP or seed coat treatment on budgrafting success. The budgrafting success was 80-85% in all cases, including the control. Interactive effects were not observed for any of the parameters *viz.,* diameter of the scion shoot, length of the scion shoot, leaf area and chlorophyll content of the leaves between seed coat treatment and SNP treatment (Table 5 & 6). Nevertheless, growth characteristics of the scion shoots of stock plants derived from seeds with SNP treatment, especially at 20µM, were relatively better though statistically not significant.

Table 5. *Effect of seed treatment with NO donor SNP on scion shoot characteristics of budded rubber plants*

Treatment	Shoot diameter (mm/plant)	Shoot length (cm/plant)	Leaf area (cm^2 /plant)	Chlorophyll content (SPAD value)
Control	5.13 ± 0.18^{a}	21.08 ± 0.73^{a}	204.48 ± 19.59^{a}	40.73 ± 2.03^{b}
Water soaked	5.69 ± 0.28^{a}	26.75 ± 1.84^{a}	157.37 ± 23.72^{a}	44.55 ± 1.68^{ab}
Soaked in 20µM SNP	5.84 ± 0.31^{a}	27.36 ± 2.74^{a}	229.68 ± 48.67^{a}	45.23 ± 1.61^{ab}
Soaked in 50µM SNP	5.74 ± 0.25^{a}	24.73 ± 2.53^{a}	164.11 ± 28.65^{a}	40.81 ± 1.59^{b}
Soaked in 100µM SNP	5.44 ± 0.25^{a}	21.47 ± 1.61^{a}	209.33 ± 42.15^{a}	42.61 ± 1.65^{ab}
Soaked in 200µM SNP	5.62 ± 0.35^{a}	22.23 ± 1.68^{a}	183.13 ± 32.93^{a}	46.27 ± 1.05^{a}

Values followed by the same letter in a column are not significantly different at $p \leq 0.05$ according to DMRT.

Table 6. *Effect of seed coat treatment on scion shoot characteristics of budded rubber plants*

Treatment	Shoot diameter (mm)	Shoot length (cm)	Leaf area (cm^2)	Chlorophyll content (SPAD value)
Intact seeds	5.54 ± 0.16^{a}	24.77 ± 1.31^{a}	172.43 ± 17.80^{a}	42.74 ± 0.91^{a}
Decoated seeds	5.62 ± 0.16^{a}	23.14 ± 1.05^{a}	210.27 ± 20.46^{a}	43.99 ± 1.07^{a}

Values followed by the same letter in a column are not significantly different at $p \leq 0.05$ according to DMRT.

DISCUSSION

Seed germination is temporally defined as the sequence of molecular and physiological events initiated upon imbibition in non-dormant seeds leading to the radicle protrusion through the seed external envelopes (testa and endosperm) that marks the end of germination (Bewley, 1997). Germination ability is regulated by a combination of environmental and endogenous signals with both synergistic and antagonistic effects. The ABA/gibberellins (GAs) balance coordinates the last step of germination with a decrease in ABA leading to a progressive release of its inhibitory effect on endosperm rupture while increase in bioactive GAs levels and enhancing the growth potential of the embryo and inducing hydrolytic enzymes that weaken the barrier tissues (Arc *et al.,* 2013).

Nitric oxide (NO) is an uncharged, gaseous and lipophilic free radical that can readily diffuse across biological membranes. Thus, NO can interact with numerous distinct molecules in plant cells and then acts as a signaling element (Beligni and Lamattina, 2000).

NO is a potent dormancy releasing agent in many species, including *Arabidopsis*, and has been suggested to behave as an endogenous regulator of this physiological blockage (Arc *et al.,* 2013).

Rubber seeds have not been tested for their response to NO but only for seed coat removal. Data presented in this study, for the first time, demonstrated that exogenous NO donor SNP elicited a significant enhancement in germination as compared to the control in both decoated and intact seeds. NO has been shown to act as a germination promoter and enhance the germination responsive genes to improve the germination in wheat (Hua *et al.,* 2003). Exogenous SNP has been reported to improve germination of rice seeds under salt stressed condition (Habib *et al.,* 2010). Seed pre-incubation (priming) with SNP (redox priming) was also shown to increase salt stress tolerance in wheat (Duan *et al.,* 2007). NO also enhances the seed vigor index under abiotic stress by enhancing germination promoting proteins (Duan *et al.,* 2007). NO has been shown to break seed dormancy in *Arabidopsis* and barley (*Hordeum vulgare* L.) (Bethke *et al*., 2006b) suggesting that NO is an endogenous regulator of seed germination in these two species.

ABA inhibition of seed germination in many species is well established (Arc *et al.,* 2013). ABA and NO are antagonistic for seed germination (Bethke *et al.* 2004). NO donor SNP enhanced the positive effect of norfluorazon, an ABA synthesis inhibitor, on dormancy release of *Arabidopsis* C24 seeds (Bethke *et al.,* 2006a). Moreover, SNP was shown to reduce seed sensitivity to exogenous ABA (Bethke *et al.,* 2006b). Liu *et al.* (2009) demonstrate that rapidly accumulated NO induces an equally rapid ABA decrease that is required for the breaking of seed dormancy and germination in *Arabidopsis*. While ABA determines seed dormancy and inhibits seed from germination, GAs are necessary for seed germination (Liu *et al.,* 2009). Through its antagonistic effects with ABA, GAs are able to release seeds from dormancy. NO was proposed to act downstream of H_2O_2 in enhancing GA biosynthesis (Liu *et al.,* 2009). Considering the previous studies, results of the present study postulate that exogenous NO donor SNP can antagonize ABA activity but promotes GAs activity in rubber seeds and thereby hastened germination and increase percentage germination in a dose dependent manner.

The present study revealed that decoating (complete removal of seed coat) resulted in significant promotion of rate and final germination of rubber seeds regardless of SNP treatment. Mock treated seeds (decoated seeds soaked in water) also showed an enhanced germination as compared to decoated seeds alone. Since the coat is thick and hard in rubber seeds, decoating apparently relieved any mechanical restraint and/or barriers to gas exchange and water imbibition and thereby hastened a synchronous germination. According to Filho (2005), seed coat regulates the seed imbibition speed, controls gas exchange, causes dormancy, prevent seed deterioration and acting as a barrier to microorganisms entry.

Rawat *et al.* (1998) demonstrated that the seed coat was very much hard and thick in *Q. serrata* and therefore inhibited the rate of seed germination. Seed coat removal increased the emergence rate of several citrus rootstocks by 30-100% (Radhamani *et al.,* 1991). The presence of the seed coat in citrus also works as a mechanical barrier to root development during germination because of the reduction in oxygen and carbon dioxide diffusion and seed imbibition (Girardi *et al.,* 2007). Seed coat removal allowed plant emergence about one week earlier than coated seeds, leading to 25% higher scion and root dry weight for both rootstocks (Rangpur lime and Swingle citrumelo) (Girardi *et al.,* 2007).

There is a correlation between seed coat thickness and germination rate. The thicker the seed coat, the greater the relative increase in plant emergence after seed coat removal. Seed coat external texture also influences plant germination. Therefore, smooth seed coats usually correspond to fast-germinating species (Girardi *et al.,* 2007). Although external seed coat removal is a labor-intensive activity, this procedure may increase seedling emergence and improve plant quality (Sempionato *et al.,* 1997). Since the seed coat of rubber is thick, it is amenable to mechanical removal and hence automation of seed coat removal needs to be investigated as a practical solution for large scale nurseries.

Growth of the rubber seedlings derived from SNP treated seeds, especially at 100 µM, was improved as compared to those derived from control and mock treated seeds when both decoated and intact seeds were used after three months from transplanting into polybags. Interaction effects were observed between seed soaking treatments with SNP at least for shoot dry weight and chlorophyll contents of leaves. Leaf area increased with SNP pretreatment in a dose dependent manner. Studies conducted during the induction of diverse plant responses have demonstrated that NO may also affect biosynthesis, catabolism/ conjugation, transport, perception, and/or transduction of almost all the phytohormones*, i.e.* auxins, gibberellins, cytokinins, abscisic acid, ethylene, salicylic acid, jasmonates, and brassinosteroids (Mur *et al.,* 2012). NO is known to enhance de-etiolation and promote greening in young seedlings (Beligni and Lamattina, 2000). Chohan *et al.* (2012) demonstrated that in chick pea seedlings, leaf areas increased with exogenous SNP as a NO donor. Further, SNP treatment enhanced the accumulation of total dry matter content and partitioning towards different plant parts in chick pea. This could be due to the cumulative effect of SNP through the regulation of cell division, xylem differentiation and exo-and endo-β-D-glucanase in the cell wall (Zhang *et al.,* 2003).

Root dry weights also increased significantly at 50-100 µM SNP. Synergistic effects of auxin and NO have been observed during the regulation of a series of plant responses, including the interplay between these molecules during adventitious root formation (Pagnussat *et al.,* 2002) and lateral root development (Correa-Aragunde *et al.,* 2004). Improved germination dynamics and growth characteristics of rubber seedlings could be attributed to the beneficial effect of SNP as a NO donor in modifying phytohormone signalling pathways and thereby increasing nutrient uptake and effective allocation of photoassimilates. Further research is needed to determine the NO accumulation in germinating rubber seeds. The practicality of decoating treatment under commercial scale nursery conditions is also required to be explored. Abiotic stress tolerance capacity of rubber plants derived from NO treated seeds should also be investigated.

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Address for correspondence: Dr N M C Nayanakantha, Head, Plant Science Dept., Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka.
e-mail: *nayanakanthachamil7@gmail.com*

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Exogenous application of Salicylic Acid alleviates drought stress of rubber nursery plants in the Intermediate Zone of Sri Lanka

S A Nakandala*, K D N Weerasinghe, P Senevirathne*, S M M Iqbal*, W G D Lakmini*** and P M P S Vijithasiri*****

* *Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta*
** *Department of Agric. Engineering, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya*
*** *Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya*

Abstract

Drought is considered as one of the main environmental threats for plants that limits the growth and development. In the present study, salicylic acid (SA) was applied to mitigate the drought stress of rubber nursery plants. The study was conducted at Monaragala Sub Station, Rubber Research Institute of Sri Lanka during the dry months from May to September in 2015. The experimental design was Randomized Completed Block Design (RCBD) with five replicates. Treatments were drought-stressed (withholding of water), three concentrations of SA (0.1mM, 0.3mM and 0.5mM) and control(C) kept under regular irrigation. Drought was imposed by withholding water by weekly and lifesaving irrigation was done throughout the nursery period. Measurements were taken on morphological and physiological characters of seedling plants.

Plants which were treated with SA as a soil drench at 0.1 mM, 0.3 and 0.5mM concentrations showed significant difference on drought stress plants when compare the stressed plant. Chlorophyll content and stomatal conductance (g_s) in seedlings reduced drastically under moisture stressed conditions. Dry matter accumulation also decreased in drought stressed plants as compared to those treated with SA at 0.3 and 0.5mM concentrations. This indicates that the application of SA had a positive effect on stomatal conductance and biomass accumulation under water stressed conditions.

In conclusion, the drought-stress decreased the growth of rubber nursery plants to a greater extent and exogenous application of SA at 0.3 mM and 0.5 mM concentrations found to be effective in alleviating drought stress under sub optimal conditions in the Intermediate zone in Sri Lanka.

Key words: concentration, diameter, drought stress, rubber, Salicylic Acid

Introduction

Drought, the absence of rainfall for a period of time resulting in depletion of soil water, is considered as one of the main environmental constraints for plant growth and development. The permanent or temporary water deficit during drought severely reduces the plant growth and development than any other abiotic stresses (Anjum *et al*., 2011).

Drought induces oxidative stress in plants by generating reactive oxygen species (ROS) such as oxygen ions, free radicals, and peroxides (Farooq *et al*., 2009). ROS play an important role in cell signaling by activating defensive system in plants under abiotic stresses (Anjum *et al*., 2011).

This defense system consists of enzymatic and non-enzymatic antioxidant system, such as low-molecular mass antioxidants (glutathione, ascorbate, carotenoids) and ROS scavenging enzymes (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX). Combination of these systems acts to minimize the affections of oxidative stresses (Anjum *et al*., 2011).

Salicylic acid (SA) is known as a non-enzymatic and an anti-oxidative plant growth regulator present in plants which can play an important role in environmental stress by activating its inter defense mechanism and multiple stress tolerance (Senaratne *et al*., 2000). The SA regulates various physiological processes in plants such as seed germination, fruit yield, glycolysis, flowering and heat production in thermogenic plants (Klessing and Malamy, 1994; Senaratne *et al*., 2000), ion uptake and transport (Harper and Balke, 1981), photosynthesis rate, stomatal conductance and transpiration (Khan *et al*., 2003).

Exogenous application of SA has been shown to induce plant stress tolerance. For example, treatment of common bean (*Phaseolus vulgaris* L) and tomato (*Solanum lycopersicum* L.) plants with SA increased their drought tolerance (Senaratne *et al*., 2000). Exogenous application of SA also modulates activities of intracellular antioxidant enzymes SOD and POD and increases plant tolerance to environmental stresses.

Therefore, the present study was aimed at exploring the effect of salicylic acid in alleviating drought stress. This is the considered to be first report on the drought alleviation in rubber nursery plants by exogenous application of salicylic acid.

Materials and method

Site selection

A young budding rubber nursery at Monaragala Sub Station of Rubber Research Institute of Sri Lanka was selected for the experiment. The area belongs to the agro-ecological region; IL1c (Wijesuriya *et al*., 2010). The long dry spells prevailed in the Intermediate Zone from February to March and from May to September every year and therefore, soil moisture stress occurs due to lack of soil moisture, high evaporation, low relative humidity, high wind velocity and high temperatures. Hence the present study was conducted during the dry period from May to end of September in the year 2015.

Experiment design and treatments

The experimental design was Randomized Completed Block Design (RCBD) with five replicates. Treatments were drought-stressed plants with no application of salicylic acid and three treatments with different concentrations of salicylic acid applications (*i.e.*; 0.1mM, 0.3mM and 0.5mM). Control plants were kept under regular irrigation. A seeding nursery was established in poly bags (7"x18") according to the above design in the field and irrigated initially until the soil in the poly bag reached field capacity. Salicylic acid at the concentrations of 0.1, 0.3 and 0.5 mM was prepared and 100ml of the solution was applied to each one-month-old plant in two consecutive applications within a day as a soil drenching. Drought was imposed by withholding of irrigation for five months of study period. Life saving irrigation was done for the treatment plants once in five days throughout the study. The control plants were kept under daily irrigation up to field capacity. A repeated application of salicylic acid was done three months after the first application before bud grafting of the seedling plants. Recommended agro-management practices were done throughout the nursery period. Measurements on morphological and physiological characteristics of seedling plants were taken throughout the nursery period.

Plant stem diameter, plant height, leaf area and number of leaves were measured as morphological parameters. Plant diameter at 1 cm above the base of the seedling was measured by using digital venire caliper at two weeks intervals. The plant height was measured by using a measuring tape in every two weeks. Leaf area was determined once a month as on site measurement by using a leaf area meter (Delta T MK2). Fifteen plants in each treatment in three replicates were taken to measure all leaves of the plants. Total number of leaves of the above experimental plants was counted once in two weeks.

Plant physiological parameters such as stomatal conductance, chlorophyll content and leaf temperature were measured every two weeks throughout the study period. Stomatal conductance and leaf temperature were measured by using a portable porometer (Delta T AP4). Three plants from each replicate of each treatment were measured mid day and completed within an hour under same light condition. Measurements were taken in top mature leaf and right leaflet of each treatment plant once in two weeks.

Leaf chlorophyll content was measured by non-destructive method, using a chlorophyll meter (SPAD 502DL Plus). The meter gives chlorophyll content as an index value (SPAD value) that is proportional to the amount of chlorophyll content of leaves. Average measurement was taken on one of top mature leaf.

Plants were uprooted after four months and measured tap root length, secondary root lengths and total plant dry matter content. Shoot:root ratio was calculated. Destructive samples were taken to analyze dry matter content and shoot: root. Samples were oven dried at 85^0C for constant weight.

Statistical analysis was done by analysis of variance followed by a mean separation procedure, Duncan's Multiple Range test (DMRT), at a probability level of 0.05. SAS statistical software package – version 9.0 (SAS Inc., USA) were used to analyze data.

Results

Morphological responses of plants

Table 1 shows the effect of different salicylic acid treatment on stem diameter of rubber seedlings plants up to eight weeks after application. A significant variation in diameter increment was shown under different SA concentrations from 4th weeks of application. Values in the Table 1 shows that the salicylic acid concentrations 0.1 to 0.5 mM positively effect on stem diameter increment on plants after four weeks of application and after 8 weeks, effect was persist in 0.3 and 0.5 mM concentrations. A lower concentration of SA that is 0.1 mM showed a lower rate of diameter increment in 8^{th} weeks when compared with other two concentrations.

The effect of salicylic acid concentrations on plant height, leaf area, number of leaves and root characters are shown in Table 2. Restricted plant height was shown when the plants were drought stressed and there were no significant difference in plant height among treatment in relation to SA concentrations. Leaf area values of treatment plants showed that the plants which were treated with salicylic acid at 0.5 mM concentration remained a significant higher value of leaf area when compare of 0.1 and 0.3 mM concentrations.

Table 1. *Effect of exogenous application of salicylic acid concentrations on diameter of rubber seedlings in millimeters (C – normal irrigation, DR - drought-stressed (withholding of water), DR + 0.1mM, DR + 0.3mM and DR + 0.5mM – drought stressed + three concentrations of SA*

Treatment	**Weeks after application of salicylic acid**				
	Initial	**2**	**4**	**6**	**8**
C	3.70±0.04	5.15±0.09^a	5.64±0.07^a	6.75±0.10^a	7.84±0.11^a
DR	3.77±0.04	5.08±0.06^a	5.44±0.05^b	6.32±0.07^b	6.99±0.06^b
DR + 0.1mM	3.84±0.04	5.17±0.05^a	5.66±0.05^a	6.47±0.05^b	7.02±0.08^b
DR + 0.3 mM	3.78±0.03	5.12±0.07^a	5.54±0.07^{ab}	6.33±0.10^b	7.50±0.10^a
DR + 0.5 mM	3.66±0.04	5.08±0.08^a	5.48±0.07^{ab}	6.33±0.07^b	7.58± 0.03^a

(Means with the same letter in a column are not significantly different)

Table 2. *Effect of salicylic acid application on plant height, leaf area and root growth of rubber seedlings after eight weeks of SA application (C – normal irrigation, DR - drought-stressed (withholding water), DR + 0.1mM, DR + 0.3mM and DR + 0.5mM – drought + three concentrations of SA)*

Treatment	**plant height (cm)**	**leaf area (cm^2)**	**No. of Leaves**	**Root length (cm)**	
				Tap root (cm)	**Secondary roots (cm)**
C	89.05±1.51^{a}	1098.57±40.20^{a}	14±0.44^{a}	43.0±2.31^{b}	427.6±15.88^{bc}
DR	77.24±1.40^{b}	830.04±54.16^{b}	08±0.45^{b}	52.7±5.36^{a}	619.2±19.78^{a}
DR+0.1mM	79.59±1.32^{ab}	933.82±63.01^{ab}	10±0.57^{a}	47.0±1.35^{ab}	441.6±31.84^{bc}
DR+0.3 mM	77.85±1.37^{ab}	952.66±70.82^{ab}	12±0.45^{a}	48.5±4.11^{ab}	404.3±50.33^{c}
DR+0.5 mM	79.66±1.34^{ab}	1052.36±62.72^{a}	11±0.37^{a}	49.2±5.14^{ab}	527.7±42.86^{b}

(Means with the same letter in a column are not significantly different)

According to the results, the plants under water stress condition without SA treatment recorded the lowest mean values ($p<0.05$) of total number of leaves and it was significantly lower compared to all other treatments. Control plants which were irrigated regularly recorded the highest mean value for total number of leaves per plant. When the moisture stressed plants were treated with SA, number of leaves remained similar to control.

Drought-stressed plants with or without SA application recorded a significantly ($p\leq 0.05$) higher mean length of tap root compared to non-stressed control treatment. The plants treated with 0.5 mM concentration of SA increase the secondary root lengths when compare to 0.1mM and 0.3mM concentrations.

Physiological responses

Leaf chlorophyll content is a major component of the photosynthetic system. Figure 1 shows the variation of chlorophyll content of plants after 8 weeks of treatment applied. There was a significantly lower ($p \leq 0.05$) chlorophyll content in drought-stressed plants without SA application. Drought plants which were treated with 0.5mM concentration of SA (DR + 0.5SA) showed a higher chlorophyll content similar to control plants and less in 0.1 and 0.3 mM concentrations (Fig. 1).

Fig. 2 indicates the variation of stomatal conductance (g_s) and leaf temperature of treatment plants after 8th weeks of SA application. SA treated plants maintained a higher g_s value even at high temperatures indicating that the application of SA had positive effects on g_s (Fig. 2).

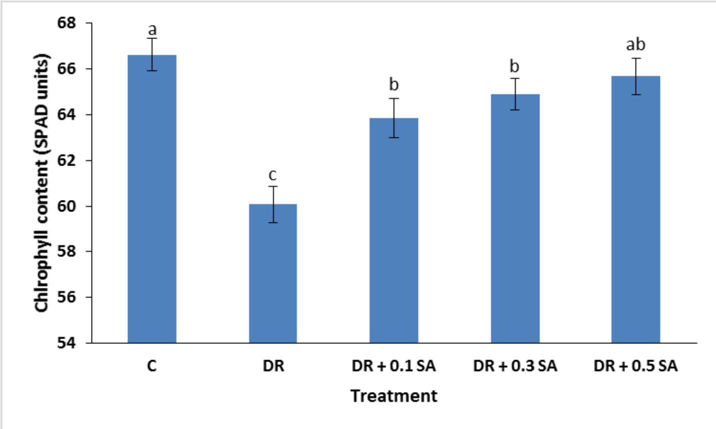

Fig. 1. Chlorophyll content of rubber seedlings at eight weeks after application (C – normal irrigation, DR - drought-stressed (withholding water), DR + 0.1mM, DR + 0.3mM and DR + 0.5mM – drought + three concentrations of SA). (Means with the same letter in a column are not significantly different)

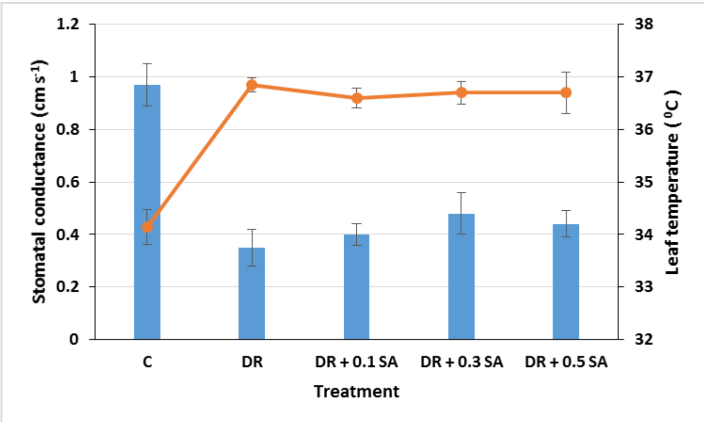

Fig. 2. Stomatal conductance and leaf temperature of plants after eight weeks of application of salicylic acid (C – normal irrigation, DR - drought-stressed (withholding water), DR + 0.1mM, DR + 0.3mM and DR + 0.5mM – drought + three concentrations of SA).

Table 3 shows the variation of dry matter content and shoot: root ratio of plants treated with different SA concentrations under drought stress condition. According to the values of the different parts of the treatment plants, there was a positive trend of increased the total dry weight in SA treated plants and drought stress plants due the dry matter increment on drought response.

Table 3. *Effect of salicylic acid application on dry matter production under drought stress*

Treatment	Dry weight (g/plant)			Total dry weight (g/plant)	Shoot: root ratio
	Stem	leaves	roots		
C	6.50±1.58	4.50±0.32	4.00±0.60	15.00	2.75[a]
DR	5.66±0.25	3.36±0.52	4.58±0.25	13.60	1.97[b]
DR + 0.1 SA	6.33±0.25	3.33±0.52	4.53±0.25	14.19	2.13[ab]
DR + 0.3 SA	6.33±1.53	4.00±0.77	4.66±0.25	14.99	2.22[ab]
DR + 0.5 SA	7.5±1.06	4.25±0.43	5.75±0.43	17.50	2.04[ab]

Table 3 shows that the root development under drought condition less affected when compared to shoot growth. Thus, a decrease in shoot:root ratio is a common observation under drought stress, which results either from an increase in root growth or from a decrease in shoot growth.

Discussion

Drought stress poses adverse effects on rubber seedlings including morphological and plant physiological changes. Drought leads to substantial impairment of growth-related traits in terms of plant height, leaf area, number of leaves per plant as found in this experiment. Total leaf area of stressed plants reduces due to loss of leaf turgor and high temperature. The present study showed that water stress suppressed the expansion of leaves and resulted in reduced leaf area, causing reduced availability of assimilates for stem growth. Similar results were reported by Anosheh *et al.* (2012) indicating the negative effect of drought on plant growth. However, the present study showed that the application of salicylic acid in different concentrations have a positive effect on morphological traits of rubber seedlings (Table 1 and 2).

Extensive root systems are developed by plants in response to the drought stress. A greater percentage of fine roots, capable of penetrating smaller soil pores, presumably optimises the exploratory capabilities of the root system as a whole and may have an important role in the survival of plants under drought stress. Root signaling during drought stress supports plant growth during the early stages of crop growth and extract water from shallow soil layers that is otherwise easily lost by evaporation (Anjum *et al*., 2011). The similar rooting pattern was shown in rubber seedlings under stress conditions which help to strive nursery plants until stress conditions recovered (Table 2).

Drought stress creates numerous physiological effects on plants, including less chlorophyll content, stomatal closer and increase in leaf temperature. However, these adverse effects could be partially alleviated by applying SA. In the present study, exogenous application of SA resulted in increased of chlorophyll content when compared with the water stressed plants without SA application. Singh, (2003) reported the direct effect of chlorophyll content of plants, photosynthetic rate, and net CO_2 assimilation. Chlorophyll content as photosynthetic pigments is important to plant mainly for harvesting light and production of organic compounds. Drought stress causes large decline in both chlorophyll content and low concentrations of chlorophyll pigments can directly limit photosynthetic potential and hence, primary production (Anjum *et al*., 2011). The results obtained from the present study clearly indicated that the exogenous SA application significantly improved the level of chlorophyll content under 0.3 to 0.5 mM SA concentrations (Fig. 1).

Stomatal regulation is another key process involving in the photosynthesis process. Results obtained for stomatal regulation under in each concentration are in conformity with the work reported by Habbi, (2012). Stomatal conductance was reduced strongly under water stress conditions but increased with SA application. Results showed that application of salicylic acid indicates a positive effect on stomatal conductance under higher leaf temperatures (Fig. 2).

The water deficit also increased leaf temperature of stressed plants in all treatments. Anosheh *et al.* (2012) reported that soil water content had a direct effect on leaf temperature and the effect of water deficit was more pronounced. However, high leaf temperature induces defense mechanisms and gene expression to modulate plants responses (Habbi, 2012). Salicylic acid-mediated improved plant tolerance to heat stress has also been reported (Larkindale *et al*., 2005). Therefore, the present study indicated that SA plays an important role in inducing heat stress tolerance by increasing the activity of the antioxidant system of plants.

The overall study highlights the drought stress decreased the plant growth severely and application of salicylic acid in the range of 0.3 to 0.5 mM as soil drench enhanced plant growth by adjusting various morphological and physiological characters of stressed plants which are badly affected by drought stress. Therefore, an exogenous application of salicylic acid is found to be a promising and feasible option in alleviating negative effects of rubber nursery plants under drought conditions at the Intermediate zone in Sri Lanka.

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Address for correspondence: Mrs S A Nakandala, Research Officer, Plant Science Dept., Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka.
e-mail: *sashika_75@yahoo.com*

Effect of using sub soil with organic materials as a substitute to top soil in potting medium of rubber nurseries

R P Hettiarachchi*, V Edirimanna*, A Thewarapperuma*, T Gunathilake*, E de Silva*, J A S Chandrasiri* and G C Malawaraarachchi*

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

Abstract

The study examined the influence of sub soil with organic material or materials compared to top soil on the growth of Hevea seedlings. Substitution of top soil with sub soil and five locally available organic materials on growth and nutrient contents of the rubber plants (Hevea brasiliensis) during nursery stage were studied. Only top soil was used as control treatment (T1). The experiment was included nine treatments and the combinations of the treatments were arranged in a completely randomized block design with twenty replicate plants. Plant growth parameters viz; diameter, height, leaf area and plant dry matter accumulation were measured periodically at the end of before and after budgrafting stages. Macro nutrient concentrations in leaves of the after budgrafting plants were determined to assess the nutritional status of the plant. Improved growth assessments; plant height, leaf area, plant dry matter accumulation and enhanced leaf nutrient contents could be observed with the treatment having sub soil and organic materials such as coir dust, poultry and refuse tea. Almost all growth assessments (94%) and all leaf nutrient contents (100%) related to sub soil with organic materials; coir dust or poultry or refuse tea or coir dust and poultry treatments gave significantly higher or no significant effect compared to top soil (T1). Deterioration of growth assessments; plant height, leaf area and plant dry matter accumulation could be observed with the treatments having sub soil and organic materials such as paddy husk and saw dust. All growth assessments (100%) related to sub soil with organic materials; paddy husk or saw dust or poultry and paddy husk or poultry and saw dust treatments gave significantly lower or no significant effect compared to top soil (T1). In general, many occasions (93%) leaf nutrient contents in sub soil + organic material or materials gave significantly higher or no significant effect compared to top soil (T1). It is evident from results that significantly higher many growth parameters associated with sub soil with coir dust could be accepted as a superior medium for polybag plants compared to the RRI recommended top soil only potting medium. Moreover, sub soil with refuse tea or poultry could be used as a substitute for top soil only medium without any failure.

Key words: dry matter, *Hevea brasiliensis*, leaf nutrients, organic materials, seedling plants

Introduction

Rubber is a perennial crop grown for latex which is the economically important product given by the plant. Thirty years life cycle is remaining this tree crop including three growth stages named nursery, immature and mature. Nursery stage is very important and the production of healthy planting material is an important aspect for the plantation industry in Sri Lanka. Currently this nursery plants are maintained in polybag at about eight months and after that stage it has to be planted under field conditions. Rubber Research Institute of Sri Lanka (RRISL) recommends to fill polybags of rubber nursery plants with top soil and this condition may help to manage optimum soil fertility throughout the nursery stage of rubber. Presently, the unavailability of fertile top soil directly affects the fertility management of polybag nursery plant. Fertile top soil can be substituted by sub soil with organic material is one of the best option to cater this problem in an environmental friendly manner. Hettiarachchi (2002) observed that the less fertile subsoil could be improved by the application of organic materials. Locally available organic materials are mainly originated from the wastes and residues of plant and animal life. Yogaratnam and Silva (1987) observed the importance of the use of organic manures in rubber fields and some locally available organic sources such as cow dung, poultry and pig dung were identified as fertilizers. Organic manures play an increasingly important role in sustainable cultivation of *Hevea* and optimize the productivity of the soil for *Hevea* cultivation, and consequently obtain higher growth and yield (Yogaratnam, 2000). Numerous workers observed that the performances of rubber plants could be improved as results of improvement of soil fertility by the application of organic sources (Samarappuli *et al*., 1998; Samarappuli, 1995; Amarasiri and Wickramasinghe, 1978; Amarasiri and Wickramasinghe, 1977). However, information in this aspect is not well documented.

The aim of this study was to evaluate the effectiveness of organic materials with sub soil as a substitute for top soil on growth of rubber seedling plants in polybags.

Material and Methods

Top soil and sub soil were collected from the upper 15 cm and 15 cm below from the surface of the soil respectively. These soils represent a great soil group of red yellow podzolic (RYP) soils classified as Ultisols according to the FAO-USDA system. Soil was air dried, stubbles and root particles were removed by hand and crushed gently to pass through 2 mm sieve. Basic properties of such soils were given in Table 1. Five locally available organic materials which were used in the experiment were air dried and passed through 2 mm sieve. This experiment was included nine treatments and the combinations of the treatments are given in Table 2 and were arranged in a completely randomized block design with twenty replicate plants.

Potting mediums were prepared according to the experimental design in Table 2 and was thoroughly mixed with 50 g of higher grade Eppawala Rock Phosphate (HERP) according to the RRISL recommendation for rubber nursery plant. These bags were placed in shallow trenches. Germinated seeds were placed one seed per bag and watered daily in dry weather to keep the soil moist. The systemic fungicides were sprayed regularly to keep the plants disease free (Fig. 1). Plants were fertilized according to the RRISL recommendation for rubber nursery plants (Advisory circular, fertilizer to rubber 2009). Recommended quantities of N, P, K, Mg fertilizer mixtures were dissolved in water and applied as 50 ml of the solution per bag at 2 weeks intervals. Compared to inorganic fertilizers, organic materials which were used the experiment had low nutrient values.

Table 1. *Properties of top soil and sub soil used in the experiment*

Property	Top soil	Sub soil
pH	4.5	4.8
Organic carbon (%)	1.35	1.15
Total N (%)	0.15	0.097
Available P (ppm)	28	19
Exchangeable K (ppm)	58	20
Exchangeable Ca (ppm)	55	45
Exchangeable Mg (ppm)	15	10

Table 2. *Treatment combination of the experiment*

Treatment	Design of potting mediums	Ratio
1	Top soil only	-
2	Sub soil + Coir dust	1 : 1
3	Sub soil + Paddy husk	1 : 1
4	Sub soil + Poultry	2 : 1
5	Sub soil + Saw dust	1 : 1
6	Sub soil + Saw dust + Poultry	1 : 1 : 1
7	Sub soil + Paddy husk + Poultry	1 : 1 : 1
8	Sub soil + Coir dust + Poultry	1 : 1 : 1
9	Sub soil + Refuse tea	1 : 1

Fig. 1. Rubber seedlings were grown in polybags under field conditions

Growth assessments

Height and diameter assessments were made at 13 weeks after planting and 16 weeks after budgrafting. At the time of 13 weeks after planting and 16 weeks after budgrafting randomly selected four replicates from each treatment, were harvested and separated into components and their dry weights were recorded by drying the components at 105^{0}C in an oven for constant weight except leaves and leaves were dried at 60^{o}C for constant weight. Before drying the components leaf area measurement was made using a portable type leaf area meter.

Plant analysis

Fully matured, all leaves from each plant were collected for assessment of mineral nutrient concentration of leaves (RRIM 1971b).

Statistical analysis

Statistical analysis of the experimental data was done by analysis of variance followed by a mean separation procedure, Duncan's Multiple Range test (DMRT), at a probability level of 0.05.

Results and Discussion

The assessments of plant diameter, height, leaf area, dry matter accumulation of seedlings were made before budgrafting at the end of 13 weeks after commencement of the experiment is given in Table 3. Plant height, leaf area, root and shoot dry matter accumulations were significantly higher at before bud grafting stage in sub soil + coir dust treatment (T2) compared to those in top soil (T1). Moreover, only the plant height and shoot dry matter accumulation were significantly higher in sub soil + poultry treatment (T4) compared to those in top soil (T1). All growth parameters were measured at before bud grafting stage in sub soil + coir dust + poultry treatment (T8) and sub soil + refuse tea treatment (T9) did not show any significant differences compared to those in top soil (T1). The shoot and root dry matter accumulations were significantly lower at before bud grafting stage in sub soil +

paddy husk treatment (T3), sub soil + saw dust treatment (T5), sub soil + saw dust + poultry treatment (T6), sub soil + paddy husk + poultry treatment (T7) and another growth parameter; plant height was significantly lower only in sub soil + saw dust + poultry treatment (T6) compared to those in top soil (T1).

The assessments of plant height, leaf area, root and shoot dry weight of scion were made at the end of 16 weeks after budgrafting is given in Table 4. All growth parameters, plant height, leaf area, root and shoot dry matter accumulations which were measured at after bud grafting stage were significantly higher in sub soil + coir dust treatment (T2) compared to those in top soil (T1). Except plant height other growth parameters were significantly higher in sub soil + refuse tea treatment (T9) compared to top soil (T1). All growth parameters in sub soil + paddy husk + poultry treatment (T7); shoot and root dry matter accumulation in sub soil + saw dust + poultry treatment (T6), leaf area and root dry matter accumulation in sub soil + poultry treatment (T4), shoot dry matter accumulation in sub soil + saw dust (T5) and sub soil + paddy husk treatment (T3) were significantly lower compared to those in top soil (T1) (Table 4).

Table 3. *Effect of different soil combinations on growth of Hevea seedlings at before budgrafting stage*

Treatment	Dia. (mm)	Height (cm)	Leaf area (cm^2)	Root Dry Wt.(g)	Stem Dry Wt.(g)
(T1)Top soil only	6.6^{ab}	62.1^{b}	1170^{bcd}	4.27^{bc}	6.27^{b}
(T2)Sub soil + Coir dust	7.2^{a}	68.4^{a}	1464^{a}	5.4^{a}	7.77^{a}
(T3)Sub soil + Paddy husk	6.35^{b}	59.88^{bc}	1287^{abc}	3.12^{e}	5.47^{cd}
(T4)Sub soil + Poultry	6.85^{ab}	67.93^{a}	1415^{ab}	4.7^{b}	7.55^{a}
(T5)Sub soil + Saw dust	6.475^{ab}	59.57^{bc}	1086^{cd}	3.9^{d}	5.27^{c}
(T6)Sub soil + Saw dust + Poultry	6.225^{b}	55.67^{c}	1104^{cd}	3.35^{de}	4.77^{d}
(T7)Sub soil + Paddy husk + Poultry	6.55^{ab}	57.75^{bc}	954^{d}	3.07^{e}	3.82^{e}
(T8)Sub soil + Coir dust + Poultry	6.825^{ab}	68.6^{bc}	1296^{abc}	4.52^{bc}	6.5^{b}
(T9)Sub soil + Refuse tea	6.75^{ab}	68.6^{bc}	1258^{abc}	4.67^{b}	6.77^{b}

Values in the same column followed by the same letter are not significantly different at p=0.05.

Table 4. *Effect of different soil combinations on growth of Hevea seedlings at after bud grafting stage*

Treatment	Height (cm)	Leaf area (cm^2)	Root Dry wt.(g)	Stem Dry Wt.(g)
(T1) Top soil only	57.05^{bc}	2544^{bc}	16.07^{cd}	9.65^{b}
(T2) Sub soil + Coir dust	63.4^{a}	2973^{a}	21.35^{a}	12.35^{a}
(T3) Sub soil + Paddy husk	57.15^{bc}	2440^{bcd}	15.87^{cd}	8.15^{cde}
(T4) Sub soil + Poultry	54.85^{cd}	2050^{e}	13.07^{e}	8.7^{bcd}
(T5) Sub soil + Saw dust	60.25^{abc}	2526^{bc}	16.5^{bc}	7.47^{e}
(T6) Sub soil + Saw dust + Poultry	54.75^{dc}	2284^{cde}	10.77^{f}	5.02^{f}
(T7) Sub soil + Paddy husk + Poultry	49.95^{d}	2196^{de}	13.3^{e}	7.12^{e}
(T8) Sub soil + Coir dust + Poultry	62.4^{ab}	2322^{cd}	16.3^{bc}	9.2^{bc}
(T9) Sub soil + Refuse tea	58.0^{abc}	2922^{a}	17.45^{b}	11.52^{a}

Values in the same column followed by the same letter are not significantly different at p=0.05.

Several workers observed that the performance of rubber plants could be improved as a result of improvement of soil fertility by the application of organic residues as a mulching material (Samarappuli, 1995; Samarappuli *et al*., 1998; Amarasiri and Wickramasinghe, 1988; Amarasiri and Wickramasinghe, 1977). The better establishment of seedlings and vigorous growth of plants could be attributed to the improvement of the environment around the root zone as a result of addition of organic sources (Joshi *et al*., 1982). Allmaras & Nelson, 1971 and Chaudhary & Prihar (1974) reported that organic mulching induced density of rooting and greater lateral spread of roots. Varying degrees of significance of correlations were obtained between the application of different organic materials and their response of growth of seedling plants (Dharmakeerthi *et al*., 2009; Meng *et al.,* 2018; Goswami *et al*., 2017; Kadoglidou *et al*., 2014; Maja *et al*., 2017; Adekiya and Agbede, 2017).The beneficial effect of organic amendment for seedling might have helped in uptake of water and nutrients and thereby improving the growth of seedling plants.

Similar effects such as improved growth assessments; plant height, leaf area and plant dry matter accumulation could be observed with the treatments which were having sub soil and organic materials such as coir dust, poultry and refuse tea. Almost all growth assessments (94%) except only two occasions, sub soil with organic material; coir dust or poultry or refuse tea or coir dust and poultry treatments gave significantly higher or no significant effect compared to top soil.

Nutrient contents of leaves were measured at after bud grafting stage in Table 5. Results showed that leaf Mg contents were significantly higher in all treatments which were included sub soil and organic material or materials compared to top soil (T1). Significantly higher growth parameters were observed in sub soil + coir dust treatment (T2), sub soil + poultry treatment (T4) and sub soil + refuse tea treatment (T9) gave significantly higher leaf N, P and Mg contents compared to top soil (T1). Only the sub soil + paddy husk treatment (T3) and sub soil + saw dust treatment (T5) gave significantly lower values of leaf N compared to top soil (T1). In general, many occasions (93%) leaf nutrient contents in sub soil + organic material or materials gave significantly higher or no significant effect compared to top soil (T1). It has been reported that soil nutrient levels for N, P, K and Mg in *Hevea* are reflected by the leaf nutrient levels (Yew and Pushparajah, 1984; Guha and Yew, 1966; Tan, 1972). Accordingly application of organic material or materials with subsoil enhanced nutrient availabilities in soils and their effect could be observed as enhancement of leaf nutrient contents in different treatments. Organic materials differ considerably in their ability to supply nutrients to the soil and crop. These differences are related to the decomposition and nutrient release rates and patterns, which are in part controlled by resource quality of the materials (Paul, 2016). Organic material contains above the critical level C:N ratio of about 20:1, microorganisms absorb nutrients from the soil solution, occur net immobilization. When the C:N ratio falls below the critical 20:1 ratio, nutrients are excreted by microorganisms and become available to the plant (Paul and Clark, 1989). Organic materials with low lignin and poly phenol contents decompose rapidly, have high direct nutrient effect (Xu *et al*., 2017; HE *et al*., 2016; Fox *et al*., 1990; Palm and Sanchez, 1991).

Table 5. *Effect of different soil combinations on leaf nutrient contents of rubber seedling plants*

Treatment	N%	P%	K%	Mg%
(T1) Top soil only	3.61^{b}	0.268^{cd}	1.102^{c}	0.181^{d}
(T2)Sub soil + Coir dust	3.72^{a}	0.313^{b}	1.132^{c}	0.244^{b}
(T3)Sub soil + Paddy husk	3.172^{d}	0.28^{cd}	1.144^{c}	0.217^{c}
(T4)Sub soil + Poultry	3.83^{a}	0.315^{b}	1.095^{c}	0.261^{a}
(T5)Sub soil + Saw dust	3.42^{c}	0.264^{d}	1.104^{c}	0.226^{c}
(T6)Sub soil + Saw dust + Poultry	3.69^{b}	0.309^{b}	1.196^{b}	0.221^{c}
(T7)Sub soil + Paddy husk + Poultry	3.85^{a}	0.352^{a}	1.199^{b}	0.223^{c}
(T8)Sub soil + Coir dust + Poultry	3.68^{b}	0.341^{a}	1.301^{a}	0.249^{ab}
(T9)Sub soil + Refuse tea	3.76^{a}	0.292^{b}	1.111^{c}	0.246^{ab}

Values in the same column followed by the same letter are not significantly different at p=0.05.

Considering all growth assessments and leaf nutrients, its seems to suggest that organic materials such as coir dust, poultry and refuse tea were decomposed rapidly and gave favourable effect similar or better to good fertile top soil for growing seedling plants in the polybags. However, organic materials such as saw dust and paddy husk were resistant to decompose and failed to give favourable effect similar to above. Coir dust also has high C:N ratio and decomposition of coir dust might accelerate with application of chemical fertilizers.

Successful establishment of rubber in the field is achieved by young budding poly bag plants. Top soil of clay loamy mixture should be used for filling these polybags. In Sri Lanka about 5000 ha is replanted annually with new planting materials, in order to maintain the 30 year planting cycle. For this purpose 12000 MT of top soil is required annually for filling bags. Scarcity of fertile top soil for filling purposes and continuous application of same soil in their surrounding areas may create less fertile conditions in the potting medium of polybag plants. These conditions may lead the production of unhealthy planting materials. Accordingly, selected some of the organic materials could be used advantageously with sub soil as a substitute for top soil for filling bags.

Conclusion

In this study it was observed comparable or better plant parameters with sub soil accompanied with selected organic materials compared to top soil. It can therefore be concluded there is a possibility of using organic materials such as coir dust, poultry and refuse tea with sub soil as a substitute for top soil for the filling medium of rubber seedling plants.

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Address for correspondence: Dr (Mrs) R P Hettiarachchi, Senior Research Officer, Soils & Plant Nutrition Dept., Rubber Research Institute of Sri Lanka, Dartonfield, Agalawatta, Sri Lanka.
e-mail: rasikarri@yahoo.com

Journal of the Rubber Research Institute of Sri Lanka
Instructions to authors

Aims and scope: The present policy of the Institute publications is research papers of a very high standard are generally published in the Journal whilst semi-technical, readable articles of topical interest will be considered for the Bulletin. As a rule, all papers scheduled to be included in the Journal are reviewed by at least two subject specialists in the Editorial Committee. The Director, Additional Director, Deputy Director (Biology) and Deputy Director (Technology) who functions as the Editors have the discretion to decide ultimate suitability of the paper for the Journal.

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Title Page: A concise and informative title (authors should not underline titles unless certain words are desired in italics), author's full name, to whom correspondence should be addressed, addresses, a short running title not more than **six** words.

Articles are subdivided into Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgement and References.

Abstract: Abstract should not exceed 250 words. This should provide a clear and factual synopsis of the paper, complete in itself without reference to the paper, in that it should not be a collection of sentences from the paper.

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Articles from Journals:

1. Seneviratne, P., Flegman, A.W. and Wijesekera, G.A.S. (1995). The problem of surface sterilization of shoot materials of *Hevea. Journal of the Rubber Research Institute of Sri Lanka* **75**, 51-60.
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1. Domsch, K.H., Gams, W. and Anderson, T.H. (1980). *Compendium of Soil Fungi.* Vol.1. Academic Press, New York. 89 pp.
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Thesis/Dissertations

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Plantation agriculture towards national prosperity

Scientists in four major plantation crop research institutes mandated to conduct research on tea, rubber, coconut and sugarcane industries in Sri Lanka assemble biennially to hold "Plantation Crop Research Symposium" in view of discussing their research findings with stakeholders. This began in 2004 and the 6th Symposium was held in Colombo in 2016 under the leadership of Coconut Research Institute. Since these symposia bring Scientists, Academics, Industrialists, Inventors, Plantation Managers and Policy makers to a common platform, the scientists in the above mentioned institutes tend to present the best of their research for extended benefits. Therefore, the Chairmen's and Directors' Forum of four Plantation Crop Research Institutes decided to publish high quality papers of these symposia in the peer reviewed journals published by respective research institutes. In the 6th Symposium, eight papers were presented by the scientists of the Rubber Research Institute under different disciplines in line with the theme "Plantation agriculture towards national prosperity". Out of those, four were found suitable for this journal after peer reviewing again. Along with two more peer reviewed manuscripts, 96th volume of the Journal of the Rubber Research Institute has been published. With this background, editors believe this volume would facilitate the stakeholders to bring the rubber plantation agriculture towards national prosperity.