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PLANT WATER RELATIONS AS INDICATORS FOR *EUCALYPTUS* SPP. SELECTION FOR NEW PLANTING SITES IN NUWARA ELIYA DIVISION OF SRI LANKA

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ABSTRACT

Plant water potential and osmotic potential are important physiological parameters to quantify the water and salt condition both in the plant and soil. In this study, plant water potential and osmotic potential were measured during midday and pre-dawn in three *Eucalyptus* species (*E. grandis*, *E. robusta* and *E. microcorys*) grown in the Nuwara Eliya region by using a "scholander" apparatus and a semi-micro "osmometer" respectively. Among the three species, *E. microcorys* had a higher mean water potential (-24.4 bars) than that of *E. grandis* and *E. robusta*. This could be partly due to the subgenus variation. Subgenus *Symphyomyrtus* contains species *E. grandis* and *E. robusta* where as *E. microcorys* was not clearly defined at subgenus level. Since subgenus *Symphyomyrtus* had narrow and thick-walled vessel elements, it can have much lower water potentials. Based on the results on plant water potential, *E. microcorys* has a much lower water potential than subgenus *Symphyomyrtus*. To compare the osmotic potential of *E. grandis*, *E. microcorys* and *E. robusta*, a 15-year-old stand in beat Pattipola was selected and their mean osmotic potential values (midday) were 13.5 bars, -16.2 bars and 13.5 bars respectively. Roots of *E. grandis* and *E. microcorys* penetrate deeply into the soil compared to *E. robusta*. The difference in osmotic potential of these three species may be partly due to variation in anatomy and rooting depth. Since the salt concentration of the top layer was less, this could be attributed to the low osmotic potential exhibited by *E. robusta* than the other two. Therefore, by measuring the osmotic potential in plants, salinity condition in the soils could be evaluated. Based on this criterion, suitable species could be recommended for the following rotation.

INTRODUCTION

The internal water potential indicates the amount of suction created by a tree in order to extract water from the soil. According to Mitlöhner (1990) the value of WP can go to –80 bars (800 hPa) in arid regions and is as low as 1 bar when the leaves are fully saturated with water. During the daytime the WP increases and it reaches the maximum when the maximum stomata are open. Similarly it reaches the minimum at night (pre-dawn) when most of the stomata are closed.

The internal osmotic potential reflects the concentration of dissolved salts, sugars, organic acids and inter alia in the cells (Mitlöhner, 1997a). In the diffusion principles the plant must have a higher concentration of solutes to be able to absorb the water from the soil. Therefore the OP of the cells (in the state of full water saturation of the plant) is directly proportional to the amount of salts in the soil. Generally the OP of the leaves heads towards its maximum during the midday because of the high water loss and in the night it goes to minimum due to the saturation of the leaves.

Plant water potential is one of the key factors to understand the water status within the soil-plant continuity. By perceiving the free energy of water in different parts of a system, it is possible to predict its behaviour quantitatively. Conductivity of water within the plant, and from

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soil to air via plant, is driven by gradients in water potential. The water potential (Ψ_W) has + or – value, depending on the amount of free energy of water altered (Jeffrey, 1987). Generally the free energy of pure water at standard temperature and pressure is zero MPa (megapascal). In plants, three major factors contribute to the water potential; pressure potential (turgor pressure, Ψ_P), osmotic potential (Ψ_π) and matric potential (Ψ_τ). In practice matric potential is not readily separable from osmotic potential and also its magnitude is very low. Due to these reasons, in most cases, it is considered as a negligible parameter. Therefore, the water potential has mainly two components namely pressure potential and osmotic potential.

$$\Psi_W = \Psi_P + \Psi_\pi$$

The osmotic potential, which is also referred to as chemical potential, is generated by organic and inorganic solutes in cell cytoplasm and vacuoles. The chemical potential of the solvent is higher in the pure phase than in the solution. If one separates a solution from a pure solvent by a semi-permeable membrane, there tends to be a net flux of solvent into the solution. This process is called osmosis and the pressure difference which must be applied to the solution to prevent a net flux of solvent is called osmotic pressure or osmotic potential (Slatyer, 1967), which always has a negative value.

There are various methods available to quantify the soil factors, which affect the growth and the development of the plants. As mentioned earlier, the osmotic potential of a plant is equivalent to the concentration of dissolved solutes in the cells. To satisfy the fundamental theory for the osmotic transportation of water, the internal osmotic potential or the internal concentration in the plant must correspond at least to the concentration of the soil solution. Based on this theory, the osmotic potential could be used to measure the plant adaptability for salts in different sites either quantitatively or qualitatively (Mitlöhner, 1997a). By using a sensitivity analysis, Thorburn *et al.*, (1995) showed that ground water depth and salinity were the main components of uptake of groundwater, while soil properties appeared to have a lesser effect.

MATERIALS AND METHODS

Study area

The study area is confined to the Nuwara Eliya Division, where *Eucalyptus* spp. are the dominant crop in the forest plantation. The total land area of the Nuwara Eliya Division is about 4321 km^2 and the forest area (inclusive both natural and plantation) is about 1258 km^2 (Central Bank of Ceylon, 1993). The study area covers an altitudinal range of 1300 to 2100 meters. The mountainous terrain *i.e.* deep valleys, steep slopes and ridges dominate the topography in the general area of the study. The plantations are located on a combination of foothills, plateau with rounded hills, and ridges. Slopes are usually less than 60%.

The soil most frequently encountered in the study area were Red-Yellow Podsols. These soils are of varying depth, somewhat shallow on steep slopes, but also deep especially on plateaus and in valleys. Drainage is largely influenced by topography but occasionally a gravelly or lateritic layer may impede drainage. Mottling in the soil horizon indicates poor drainage. Texture varies from sandy loam to clay. The pH value is between 4 and 6 (Forest Department,1994). Particularly at higher elevations, tea or natural montane forest (in protected reserves) is the major land use or vegetation type adjoining the sampling areas (plantations). These extensive area of tea estate and forest reserves have resulted in a shortage of land for cultivation of food. Food crops are grown to supply a rapidly developing market (both domestic and export) and for subsistence. Consequently the study area is subject to great pressure of encroachment from individuals, groups of farmers, sometimes with political support. In some cases clear felled areas were being taken over and cultivated with food crops before the Forest Department replanted with trees (Forest Department, 1994).

Measurements

In this study, both midday and pre-dawn readings of internal water potential (WP) and internal osmotic potential (OP) of three *Eucalyptus* spp. (*E. grandis*, *E. robusta* and *E. microcorys*) leaves were measured. The internal water potential was measured by using a Scholander apparatus (pressure chamber method) (Scholander *et al.*, 1965). The measurements were taken according to the procedure described by Lassoie and Hinckley (1991). Four beats in the Nuwara Eliya divisions namely Dixon Corner, Kandapola, Mahakudugala and Pattipola were taken for sampling. The sampling units within the beats were stratified according to the age and species. In each sampling unit 6 individuals were measured both at midday and at night. Three measurements were taken for each individual for each parameter separately.

In addition, the osmotic potential (OP) was measured on the same individuals, on which the WP measurements had been taken. Fresh weight of leaves (about 10 grams per tree) was taken under field conditions and then it was killed and dried with a movable gas oven at about 60°C in order to stop the enzymatic activities. Then it was taken to the laboratory and dried in the oven at 100°C until a constant weight was obtained. The leaf water content (in a dry basis) was calculated by using the following formula:

$$WC\% = \left[\frac{FW - DW}{DW}\right] \times 100$$

Where WC = Water content
FW = Fresh weight
DW = Dry weight

Leaves were subsequently ground using a grinder (coffee grinder) and, 1 g of sample was mixed with 8 ml of distilled water and kept in the water bath at 60°C over night. The samples were centrifuged for 15 minutes at 5000 rpm and the clear solutions were extracted and the freezing points of these solutions were measured with the help of a semi-micro osmometer as described by Kreeb (1990). A close physical relationship exists between freezing point of a solution and its osmotic potential. Based on this phenomenon, the osmotic potential was recalculated from their respective freezing points (Kreeb, 1990).

RESULTS AND DISCUSSION

In general the midday water potential value should be more than that of night. But this situation could turn vice versa if the climatic condition changes. For instance clouds during the daytime and very heavy wind in the nights could reduce the difference between midday and night values. However, chances of occurrence of such situations are very low in the tropics except in the cool and high elevations.

The difference between midday and pre-dawn indicates the amount of stress that the plant undergoes. If this difference is very high (midday value is higher than pre-dawn), it implies that the plants undergo high water stress during the daytime and it has to spend lot of energy to adjust to that particular environment and it recovers in the night by re-saturation (relaxation phase). In case, if the pre-dawn values are very high (less negative value) it could suggest that there is hardly any water problem in these stands or sites. It is expected that the plant and soil will be in equilibrium in nights, so that pre-dawn water potential could be used to highlight the soil water potential.

The mean values and the standard deviations of water potential of the study area both midday and pre-dawn are presented in Table 1.

Table 1
Mean and standard deviation of midday and pre-dawn water potential of *Eucalyptus* spp in some selected beats in Sri Lanka (6 observations each)

Beat	Species	Age [years]	Plant water potential	
			Midday [bar]	Pre-dawn [bar]
Dixon Corner	*E. grandis*	5	-16.1 ± 1.5	-1.5 ± 0.9
	E. grandis	8	-17.2 ± 3.6	-1.8 ± 0.6
	E. grandis	9	-15.6 ± 2.4	-2.1 ± 1.5
	E. grandis	12	-18.7 ± 1.3	-4.0 ± 0.9
	E. grandis	13	-14.2 ± 2.2	-0.4 ± 0.2
	E. microcorys	13	-21.5 ± 4.0	-5.9 ± 1.0
Kandapola	*E. grandis*	1	-19.8 ± 1.9	-4.4 ± 0.7
	E. grandis	3	-18.2 ± 4.2	-1.3 ± 1.3
	E. grandis	9	-15.3 ± 2.1	-5.3 ± 1.5
	E. grandis	12	-17.7 ± 1.9	-2.8 ± 1.7
	E. microcorys	12	-24.4 ± 4.0	-7.1 ± 2.9
	E. robusta	9	-12.9 ± 3.3	-5.3 ± 1.2
Mahakudugala	*E. grandis*	3	-17.6 ± 1.8	-1.4 ± 0.7
	E. grandis	5	-17.2 ± 2.3	-1.1 ± 0.4
	E. grandis	6	-18.4 ± 2.6	-0.8 ± 0.2
	E. grandis	9	-20.0 ± 2.3	-0.5 ± 0.2
Pattipola	*E. microcorys*	14	-17.8 ± 1.5	-3.3 ± 1.6
	E. robusta	14	-15 6 ± 1.4	-1.5 ± 1.3
	E. grandis	15	-16.1 ± 2.0	-1.1 ± 1.1
	E. microcorys	15	-23.7 ± 1.9	-3.9 ± 2.1
	E. robusta	15	-15.1 ± 2.0	-2.3 ± 0.8

E. microcorys had more negative water potential than the other two species *E. grandis* and *E .robusta.* At Pattipola where all three species were found in the same stand with same age, the mean difference of midday and pre-dawn was found to be high in *E. microcorys* and followed by *E. grandis* and *E. robusta.* This could be partially explained by the subgenus variation. *E. grandis* and *E. robusta* belong to subgenus *Symphyomyrtus* but for *E. microcorys* the subgenus is not clearly defined yet. Ridge *et al.* (1984) showed that subgenus *Symphyomyrtus* had comparatively narrower and thicker-walled vessel elements where they could undergo much lower water potentials without cavitation of the conductive tissue than subgenus *Monocalyptus*. In contrast, *E. microcorys* might be superior in the above character so that it could undergo very low water potential compared to the other two species studied in the same stand.

Based on the visual observations in the study areas, the growth of *E. robusta* was comparatively less than that of other two species. The mean pre-dawn values of *E. robusta* were always more negative than *E. grandis*. This may be due to the less rooting depth, so that it cannot re-saturate completely during the night. On the other hand, the tendency towards drought tolerance increases with the decrease of pre-dawn water potential (Williams and Woinarski, 1997). Based on this criterion, *E. microcorys* and *E. robusta* are more drought tolerant than *E. grandis*. This shows the suitability of above species for these regions considering water as the main limiting factor. Depending on the water availability and the soil depth to the water table one could choose the species. *E. grandis* could be planted in areas where there is no water stress while *E. microcorys* and *E. robusta* could be used in high rainfall areas with prolonged drought periods.

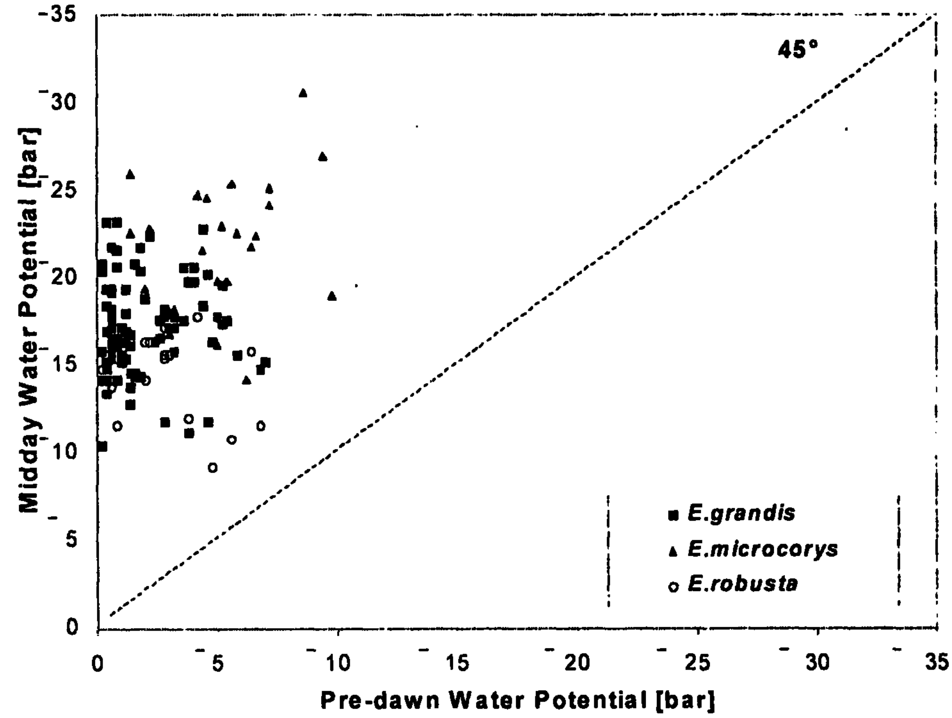


Figure 1. Water potential of three *Eucalyptus* species in Nuwara Eliya division.

In Figure 1, the 45° line is the limit line where both midday and pre-dawn water potential are found to be same and this is referred to as permanent wilting point of a plant species (when the relaxation value at night becomes the same with the stress value at noon). The values of the samples of the study area were well above this line i.e. water was not a limiting factor for these species in the sampled area. A study by Louw (1997), showed that the growth of *E. grandis* was mainly influenced by factors controlling available soil moisture, together with the organic carbon content of the topsoil. From these results it can be concluded that there is no water problem for above three species in the studied areas where the rainfall is above 2000 mm per annum.

The mean values and the standard deviations of osmotic potential for midday and pre-dawn as well as standardised osmotic potentials are presented in Table 2. The mean midday osmotic potential was always greater than the mean pre-dawn osmotic potential except in a single stand of *E. grandis* in Dixon Corner. Both minimum and maximum osmotic potential for *E.*

grandis (n = 168) was obtained from Dixon Corner and the values were -20.5 and -4.1 bar, respectively. The observed values for *E. microcorys* (n = 48) were -21.4 and -11.3 bar and for *E. robusta* (n = 36), -18.9 and -10.3 bar, respectively. According to Mitlöhner (1997b), in Paraguayan Chaco, the mean osmotic potential value for *E. robusta* both midday and pre-dawn was found to be greater than -10 bar.

Differences in salinity tolerance have been documented within a single provenance (Van der Moezel and Bell, 1987; Van der Moezel *et al.* 1991). In eucalypts, a range of physiological mechanisms has evolved to cope with the effects of excessive soil ion concentrations (Williams and Woinarski, 1997).

Table 2
Mean and standard deviation of midday and pre-dawn osmotic potential of *Eucalyptus* spp. in some selected beats in Sri Lanka

Beat	Species	Age [years]	Plant osmotic potential		Standardised osmotic potential [bar]
			Midday [bar]	Pre-dawn [bar]	
Dixon Corner	*E. grandis*	5	-15.7 ± 2.7	-15.5 ± 1.0	-10.0 ± 1.4
	E. grandis	8	-18.0 ± 0.8	-13.1 ± 1.7	-8.5 ± 2.0
	E. grandis	9	-14.7 ± 2.7	-13.8 ± 1.5	-9.8 ± 0.8
	E. grandis	12	-14.2 ± 3.8	-11.2 ± 3.9	-7.9 ± 2.1
	E. grandis	13	-10.1 ± 3.2	-11.7 ± 1.9	-8.3 ± 2.2
	E. microcorys	13	-16.6 ± 2.9	-14.8 ± 2.6	13.3 ± 1.6
Kandapola	*E. grandis*	1	-16.3 ± 1.8	-16.0 ± 1.6	-11.2 ± 0.9
	E. grandis	3	-15.0 ± 1.5	-14.3 ± 1.9	-9.7 ± 1.2
	E. grandis	9	-15.1 ± 2.4	-13.3 ± 2.7	-10.3 ± 1.3
	E. grandis	12	-14.9 ± 0.8	-14.6 ± 0.9	-11.6 ± 0.9
	E. microcorys	12	-18.3 ± 1.5	-16.2 ± 2.2	-14.2 ± 1.5
	E. robusta	9	-16.6 ± 2.0	-16.3 ± 0.9	14.2 ± 1.0
Mahakudugala	*E. grandis*	3	-12.7 ± 2.6	-10.8 ± 1.5	-7.7 ± 1.4
	E. grandis	5	-12.4 ± 1.9	-11.0 ± 2.4	-8.7 ± 2.0
	E. grandis	6	-14.2 ± 1.3	-10.8 ± 1.7	-8.3 ± 0.6
	E. grandis	9	-14.1 ± 2.1	-13.5 ± 2.7	-7.8 ± 1.3
Pattipola	*E. microcorys*	14	-16.7 ± 1.7	-15.2 ± 2.8	-12.9 ± 1.1
	E. robusta	14	-13.4 ± 1.1	-12.0 ± 1.1	-10.4 ± 1.2
	E. grandis	15	-13.5 ± 1.3	-12.9 ± 1.1	-11.5 ± 0.9
	E. microcorys	15	-16.2 ± 1.5	-15.5 ± 1.5	-12.8 ± 1.1
	E. robusta	15	-13.5 ± 0.5	-10.7 ± 0.4	-9.7 ± 0.7

Van der Moezel and Bell (1990) observed a gradual stomatal closure with salt uptake and tissue tolerance in *E. robusta*. They also found out that in species like *E. camaldulensis*, with combined rapid stomatal closure and salt exclusion; the stomatal closure appeared to preceed salt uptake.

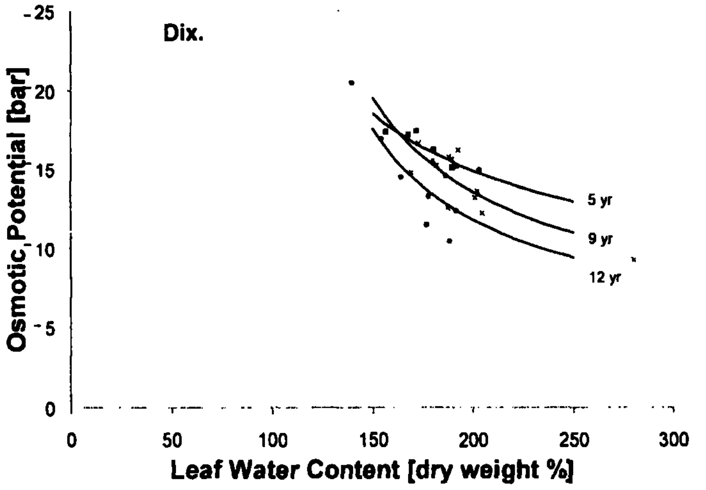
The relationship between osmotic potential and leaf water content gave a "J shaped" curve. This has been proved outside the plant with ideal sucrose and NaCl solutions (Mitlöhner, 1998). He proved this relationship as true in trees (within their natural environment) showing *Ruprechtia triflora* as an example. He has explained this with an "inversely proportional simple liner regression" equation. In this study, different inversely proportional equations were used to explain the relationship between osmotic potential and leaf water content. A modified version of the above equation was found to be the best fitting for this relationship. Here the independent variable (leaf water content) was substituted with their natural logarithm. The equation is as follows:

$$\Psi_\pi = \frac{1}{[a + b \cdot ln(LWC)]}$$

Where Ψ_π = Osmotic potential (bar)
LWC = Leaf water content (dry weight %)
a & b = Parameters

To compare the sites (beats), two beats were chosen namely Dixon Corner and Mahakudugala. In each site, 3 stands of different age were selected for comparison. Figure 2 shows the fitted lines for the above two beats.

According to Figure 2, both sites behaved differently in this relationship. Here, it is clearly seen that there is no influence of age on osmotic potential. The age does not affect the internal osmotic potential under similar soil conditions. In general, young plants absorb the resources from the top layer of the soil and as the age increases they try to expand the rooting depth not only for absorption but also for anchorage. If we apply this phenomenon for Figure 2, in beat Dixon Corner, 12-year-old stand shows lesser osmotic potential than young stands. Thus the bottom layer seems to be with a less concentration of osmotic active substances than the top layer. The reverse situation is evident in beat Mahakudugala, where the young stands show lower osmotic potential than the 9-year-old stand, which indicates the high salt concentration in the bottom layer. On the other hand, visual observations revealed that the undergrowth in Dixon Corner is low compared to Mahakudugala. Since the area receives high rainfall, the erosion could have removed the top layer (humus). This warns the managers to take care during the primary stages of crop establishment in Dixon Corner to reduce the mortality of seedlings. Table 3 gives the parameter values for the fitted curves in Figure 2.



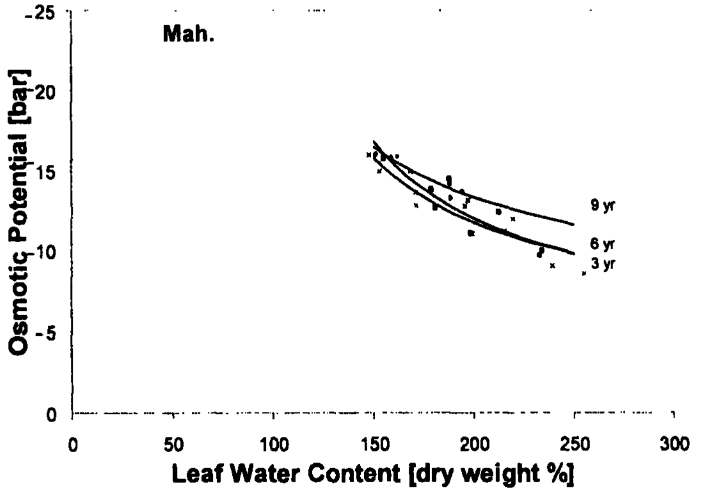


Figure 2. The relationship between leaf water content and osmotic potential for different age stands for two selected beats of *E.grandis*: Dixon Corner (Dix.) and Mahakudugala (Mah.)

To examine the difference in osmotic potential between the species, a mixed stand where *E. grandis*, *E. microcorys* and *E. robusta* grown together was selected. It was a 15-year-old stand, situated in Pattipola. The same model was found to be applicable for all three species in this stand. The fitted curves and their parameter values are given in Figure 3.

The roots of *E. grandis* and *E. microcorys* penetrate into a deeper layer than those of *E. robusta*. The osmotic potential difference among the species in Figure 3 is obviously due to difference in anatomy and rooting depth. Since the top layer has a lesser concentration of osmotically active substances, species *E. robusta* has less osmotic potential value than the other two species. Limcoff *et al.* (1994) showed that the seedlings of *E. grandis* and *E. camaldulensis* had the lowest osmotic adjustment compared to *E. viminalis* and *E. tereticornis*. Based on these phenomena it appears that osmotic potential in plants could be used to monitor the "salting" of soils and to choose most suitable species for different sites considering the rotation cycle.

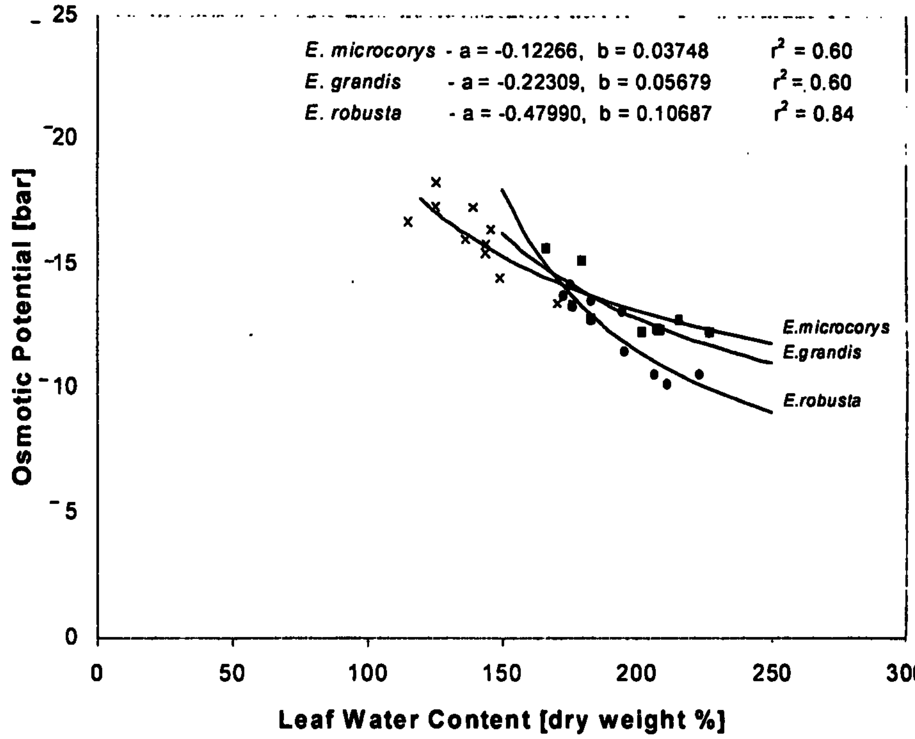


Figure 3. Leaf water content - osmotic potential relationship for three *Eucalyptus* species of 15-year--old mixed stand grown in Pattipola, Sri Lanka.

Table 3
The parameter values of leaf water content - osmotic potential relationship for two selected beats: Dixon Corner and Mahakudugal

Beat	Age	Parameter Value		N	R^2
		A	B		
Dixon Corner	5	-0.17314	0.04532	6	0.79
	9	-0.33914	0.07790	12	0.67
	12	-0.42480	0.09615	9	0.66
Mahakudugala	3	-0.35580	0.08287	8	0.81
	6	-0.31842	0.07617	12	0.85
	9	-0.18741	0.04955	8	0.90

Since *E. microcorys* has the most negative water potential than that of *E. grandis* and *E. robusta*, it is superior in undergoing much lower water potential than subgenus *Symphyomyrtus*. Drought tolerance increases with the decrease of pre-dawn water potential. Therefore, *E. robusta* and *E. microcorys* have ability to tolerate drought conditions than *E. grandis*. These three species can be used in Nuwara Eliya and Badulla divisions, depending on the water availability of the site. Since the rainfall is high, the sampled area (study area) does not have a limitation with regards to water. There is no influence of age on osmotic potential. In beat Dixon Corner, old stands had less osmotic potential than the young stands. On the other hand the situation in Mahakudugala is very different, i.e. here the young stands had less osmotic potential than the old stands. Considering this phenomenon it could be concluded that in beat Dixon Corner the top layer has higher concentration of osmotically active substances than that of the bottom layer whereas in beat Mahakudugala the bottom layer has high concentration of osmotically active substances than the top layer.

In same age stands, compared to *E. robusta*, roots of *E. grandis* and *E. microcorys* penetrate deep into the soil. Since the top layer has a lesser concentration of osmotically active substances, *E. robusta* has less osmotic potential than the other two species. As a general conclusion it could be stated that by measuring osmotic potential in plants, salinity condition in the soils could be evaluated, and thereby suitable species could be recommended, at least, for the next rotation.

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