Physiological and biochemical traits of adaptability in *Calophyllum inophyllum* (L.)

Anandalakshmi R, Anantha Kumar M, Bharath T, Rajesh C and Suresh Kumar K

DOI: https://doi.org/10.22271/chemi.2020.v8.i1ab.8543

Abstract

*Calophyllum inophyllum* L. is a potential tree borne oilseed gaining popularity for both medicinal and biofuel properties. Clonal variations with respect to physiological and biochemical traits in this species across three different locations such as Neyveli, Chennai and Salem was studied. The results indicated that *C. inophyllum* can withstand moderately harsh climatic conditions. It was evinced that to screen the adaptability of this species to dry climatic conditions, analysis of physiological traits such as Chlorophyll Stability Index (CSI) and Membrane Injury Index (MII) and biochemical traits such as Chlorophyll contents, Proline and Phenols stand feasible. The present study indicated that clones C6 and C62 can withstand harsh climate compared to other clones studied. Clones C6 and C62 can therefore be deployed for vegetative multiplication for raising plantations with high productivity by farmers and forest departments.

Keywords: *Calophyllum inophyllum*, tree borne oilseed, adaptability, chlorophyll stability, membrane integrity, proline

Introduction

Low productivity of forest and considerable land use lead to the change from forest to agriculture in the past has resulted into enhanced biotic pressure on the existing forests. Therefore, it is crucial to increase the productivity of forest and also the area under tree cover to meet the growing demand for all kinds of timber, fuel, food, fodder, fibre within the country (Lal, 2007). Clonal technology is one such viable option to improve productivity especially in native tree species like *Calophyllum inophyllum*. *C. inophyllum* is a medium to large sized tree that belongs to Clusiaceae family. It is called as Alexandrian Laurel in English, and its vernacular names are Punnai (Tamil), Pouna (Telugu), Sultanachampa (Hindi), Punna (Malayalam) and Surahonne (Kannada). It is native to tropical Asia, east Africa and extends to Australia. It is widespread in countries like India, Malaysia, Indonesia, Sri Lanka, Philippines, Myanmar, Taiwan, Thailand, Hawaii and other Pacific Islands (Anandalakshmi, 2012) [2]. It grows well in sandy and well drained soils distributed up to an elevation of 800m and withstands temperature range of 18 to 33 °C and rainfall of 1000 to 5000mm. In India, it is found along the sea coasts of the Indian Peninsula and the Andaman and Nicobar Islands. On the West Coast, it is found from Mumbai southwards to Southern Kerala and along the East Coast, from Orissa southwards (Anandalakshmi, 2014) [1].

*C. inophyllum* is known for its oil that possesses medicinal values and is also a potential biofuel (Sahoo et al., 2009) [27]. Presently the Calophyllum oil, even without transesterification, has been proved very useful in running small motor engines, pumps etc. by farmers in Tamil Nadu. It is reported that the typical yield of an adult tree is around 5kg of cold pressed oil is produced for every 100kg of fruit (Friday and Okano, 2006) [13]. Its benefits and versatility are now increasingly known and accepted in the modern world. *C. inophyllum* is renowned for its remarkable healing properties and its oil has been used to treat diabietic sores, psoriasis, herpes and hemorrhoids (Dweck and Meadows, 2002) [12]. The seed kernel oil finds wide applications, such as luminant, lubricant, soap making apart from use as a medicated oil. The timber is used for beams, furniture, railway carriages and ship building (Shetty et al., 2002) [29].

Realising the value of the multipurpose tree, at global level South
Pacific Regional Initiative on Forest Genetic Resources (SPRIG) has identified *C. inophyllum* as a priority species for genetic improvement in South East Asian countries (Pourru, 2000). In India, studies on distribution, genetic variation, selection and germplasm bank establishment can be taken up to initiate genetic improvement of the species (Krishnakumar et al., 2010) [30].

At the Institute of Forest Genetics and Tree Breeding, Coimbatore, high fruit and oil yielding trees of *C. inophyllum* were identified, multiplied and established multiloculation clonal trials at three places in Tamil Nadu, namely, Neyveli, Salem and Chennai in order to assess the adaptability of these clones to different sites. The variability expressed in the physiological and biochemical traits which could serve as indicators for adaptive characters to tolerate dry conditions were analysed so that superior clones of *C. inophyllum* could be screened.

**Materials and Methods**

**Collection of leaf sample**
The fresh green leaves from the trees of five *C. inophyllum* clones – C6, C30, C62, C86, C88 were collected from the six year old multi location trials laid at Neyveli, Salem and Chennai. The details of the trials are as follows,

| Trial location | Altitude (msl) | Latitude | Longitude | Mean annual Rainfall (mm) | Maximum temperature °C | Soil type | Topography |
|----------------|----------------|----------|-----------|---------------------------|------------------------|-----------|------------|
| Neyveli        | 87             | 11.61°N  | 79.44°E   | 1142                      | 40                     | Sandy loam | Level ground |
| Chennai        | 28             | 12.79°N  | 80.04°E   | 1400                      | 41                     | Sandy loam | Level ground |
| Salem          | 278            | 11.76°N  | 78.16°E   | 600                       | 39                     | Sandy loam | Level ground |

The leaves were surface cleaned and packed in polythene covers immediately after collection and used for the study.

**Physiological traits**

**a. Relative Water content (RWC)**
Fuly expanded leaf samples were taken from the middle of the plant and used 5-6cm portion for recording Relative Water Content. RWC is calculated by the formula given by Bars and Weathery (1962) and expressed in percentage.

\[
RWC = \frac{\text{Fresh weight-Dry weight}}{\text{Turgid weight-Dry weight}} \times 100
\]

**b. Chlorophyll Stability Index (CSI)**
CSI was estimated by following procedure of Koleyoreas (1958) and expressed in percentage.

\[
CSI = \frac{\text{Total chlorophyll content (treated)}}{\text{Total chlorophyll content (Control)}} \times 100
\]

**c. Membrane Injury Index (MII)**
Membrane Injury Index of leaves was calculated by the method of Blum and Ebercon (1981) [7].

\[
\text{MII} = \frac{1 - \frac{[\text{EC at 40 °C}]}{[\text{EC at 100 °C}]}}{1} \times 100
\]

**Biochemical Parameters**

**a. Estimation of Chlorophyll** (Arnon et al., 1949) [3]
Chlorophyll is extracted in 80% acetone and the absorption at 663nm and 645nm are read in a spectrophotometer. Using the absorption coefficients, the amount of chlorophyll was calculated.

\[
\text{Mg chlorophyll a/g tissue} = 12.7(A_{663}) - 2.69(A_{665}) \times \frac{V}{1000xW}
\]

\[
\text{Mg chlorophyll b/g tissue} = 22.9(A_{663}) - 4.68(A_{665}) \times \frac{V}{1000xW}
\]

\[
\text{Mg total chlorophyll/g tissue} = 20.2(A_{665}) + 8.02 (A_{663}) \times \frac{V}{1000xW}
\]

Where

- \(A\) = absorbance at specific wavelengths
- \(V\) = final volume of chlorophyll extract in 80% acetone
- \(W\) = fresh weight of tissue extracted

**b. Estimation of Total Phenols** (Malik and Singh, 1980) [24]
Phenols react with phosphomolybdic acid in folin-ciocalteau reagent in alkaline medium and produce blue coloured complex. The absorbance is noted at 650 nm. Standard curve using different concentrations of catechol was prepared, from which the amount of phenols was calculated.

**c. Estimation of Proline** (Bates et al., 1973) [6]
During selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. Other interfering materials are also presumably removed by absorption to the protein-sulphosalicylic acid complex. The extracted proline is made to react with ninhydrin in acidic conditions to form the chromophore (red colour) and read at 520nm.

**d. Estimation of Ascorbic Acid** (Harris and Ray, 1935) [14]
Ascorbic acid reduces the 2, 6-dichlorophenol indophenol dye to a colourless leuco-base. The ascorbic acid gets oxidised to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The amount of the dye consumed is equivalent to the amount of ascorbic acid.

**e. Estimation of Malondialdehyde** (Dhinsa, et al., 1981) [11]
The level of lipid peroxidation was measured following the thiobarbituric acid (TBA) test which determines malondialdehyde (MDA) as an end product of lipid peroxidation. Freshly harvested seedling sample (0.5g) was homogenized in 4 ml of 1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was added to 1.0 ml of 0.5% (w:v) thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 95°C for 30 min and then quickly cooled in an ice bath. Again centrifuge it for another 10,000 rpm for 10 min, and read the absorbance at 532nm using a UV-VIS spectrophotometer. The value for non-specific absorption at 600 nm was subtracted from the value recorded at 532 nm. The MDA-TBA complex (pink pigment) content was calculated using its extinction coefficient of 155 mM⁻¹ cm⁻¹ and expressed as nmol (MDA)g⁻¹ fresh weight.

**Statistical analysis**
The experiments were conducted in Completely Randomized Design. The effect of insect control treatments and effect of
location (seed source) were analysed by two way ANOVA at 5% level of significance using GENSTAT 5.0 software. Prior to analysis the percentage data were transformed to arc sine values.

Results and Discussion

Variability in physiological traits of the clones of Calophyllum inophyllum

The three physiological traits studied for variations among the clones of C. inophyllum were Relative water content (RWC), Membrane injury index (MII) and Chlorophyll stability index (CSI). Relative water content (RWC) did not vary significantly across clones, locations and clones x locations. However, membrane injury index (MII) varied significantly across clones, locations and clones x location. Chlorophyll stability index (CSI) did not vary across locations alone. The CSI was found very high for C6 and C62 recording 1.078 and 1.169 respectively. MII was very high for C6 and C88 recording 37.94% and 41.21% respectively, and the lowest for C62 showing 29.3%. The results indicate that C62 is the superior clone based on CSI and MII. Across locations, clones established at Salem recorded the highest RWC of 74.07% and the highest MII of 39.07% (Tables 1a, 1b & Figures 1, 2).

Table 1a: ANOVA for physiological traits of C. inophyllum clones

| Physiological Parameter | Source of variation | Sum of squares | Mean square | F probability | Significance |
|-------------------------|---------------------|----------------|-------------|---------------|--------------|
| Relative Water Content (RWC) | Clones | 82.27 | 20.57 | 0.606 | NS |
| | Location | 149.86 | 74.93 | 0.094 | NS |
| | Clones*Location | 185.02 | 23.13 | 0.630 | NS |
| Chlorophyll Stability Index (CSI) | Clones | 7689.8 | 1922.5 | <.001 | S |
| | Location | 1.6 | 0.8 | 0.996 | NS |
| | Clones*Location | 13474.2 | 1684.3 | <.001 | S |
| Membrane Injury Index (MII) | Clones | 909.29 | 227.32 | <.001 | S |
| | Location | 695.35 | 347.68 | <.001 | S |
| | Clones*Location | 1361.98 | 170.25 | <.001 | S |

Table 1b: Mean values for physiological traits of C. inophyllum clones

| Physiological Parameter | Clones | Relative Water Content | Chlorophyll Stability Index | Membrane Injury Index |
|-------------------------|--------|------------------------|----------------------------|-----------------------|
| Neyveli | C6 | 67.26 | 1.25 | 34.46 |
| | C30 | 70.82 | 1.14 | 45.23 |
| | C62 | 71.45 | 1.23 | 26.00 |
| | C86 | 74.54 | 1.21 | 38.42 |
| | C88 | 73.20 | 0.57 | 45.72 |
| Chennai | C6 | 71.94 | 0.63 | 41.39 |
| | C30 | 68.36 | 0.59 | 30.44 |
| | C62 | 71.69 | 0.63 | 23.22 |
| | C86 | 69.13 | 1.71 | 32.90 |
| | C88 | 70.30 | 1.30 | 29.31 |
| Salem | C6 | 72.93 | 1.47 | 37.99 |
| | C30 | 73.34 | 1.04 | 33.60 |
| | C62 | 76.10 | 0.75 | 38.68 |
| | C86 | 70.78 | 0.73 | 37.26 |
| | C88 | 77.19 | 0.61 | 48.58 |
| Grand mean | | 71.94 | 1.00 | 36.21 |

Mean values for Clones

| | C6 | C30 | C62 | C86 | C88 |
|-------------------|---------|---------|---------|---------|---------|
| Neyveli | 70.71a | 70.84a | 73.08a | 71.48a | 73.56a |
| Chennai | 70.84c | 70.84c | 70.84c | 70.84c | 70.84c |
| Salem | 71.48b | 71.48b | 71.48b | 71.48b | 71.48b |

Mean values for Location

| | Neyveli | Chennai | Salem |
|-------------------|---------|---------|---------|
| Neyveli | 71.46a | 70.29b | 74.07a |
| Chennai | 71.46a | 70.29b | 74.07a |
| Salem | 74.07a | 74.07a | 74.07a |

Standard error of differences (s.e.d.)

| Source of variation | Clones | Location | Clones*Location |
|---------------------|--------|----------|-----------------|
| Neyveli | 2.237 | 0.057 | 1.962 |
| Chennai | 1.732 | 0.044 | 1.520 |
| Salem | 3.874 | 0.095 | 3.952 |

Least significant differences (l.s.d.)

| Source of variation | Clones | Location | Clones*Location |
|---------------------|--------|----------|-----------------|
| Neyveli | 4.505 | 0.115 | 3.952 |
| Chennai | 3.489 | 0.089 | 3.061 |
| Salem | 7.802 | 0.199 | 6.844 |

NS – Not Significant; S – Significant; Means with the same letter do not differ significantly at p<0.05%
From the above study it is evident that Chlorophyll Stability Index (CSI) and Membrane Injury Index (MII) stand as dependable traits to evaluate the adaptive capacity of *C. inophyllum* clones. Chlorophyll stability is a function of temperature and it is found to correlate with drought tolerance. Chlorophyll stability index is a measure of integrity of membrane or heat stability of the pigments under stress conditions (Kaloyereas, 1958). The CSI is a single parameter used to measure frost or drought resistance of a plant. Sairam *et al.* (1996) reported that both drought stress and temperature stress decreased membrane stability, chlorophyll content and chlorophyll stability index in all wheat genotypes. The high chlorophyll stability indices help the plants to withstand stress through better availability of chlorophyll. This leads to increased photosynthetic rate and more dry matter production (Madhan Mohan *et al.*, 2000)\(^2\). Relative water content of leaf (RWC) indicates the actual water content to its maximum turgidity. It was observed that, plants under zero stressed condition had maintained higher RWC throughout the course than strong stressed and severe stressed plants (Kardile, 2018)\(^3\). Upreti *et al.*, (1997)\(^4\) noted changes in RWC under stress and normal conditions, the reduction being significant under stress condition. The higher CSI, higher RWC and lower MII values in the present study reveals that this species is able to sustain under stressed conditions like drought and high temperatures.

**Variability in biochemical traits of the clones of *Calophyllum inophyllum***

Among the various biochemical parameters, phenols, ascorbic acid and malondialdehyde (MDA) contents did not vary significantly across the clones, whereas chlorophyll contents and proline varied for the clones. Chlorophyll b alone did not vary significantly across the locations. The interactive effect of clones and locations were found varying for all the biochemical parameters studied except for ascorbic acid and MDA (Tables 2a, 2b & Figures 3-8).

In this study, based on the interactive effects of clones x locations Chlorophylls A and B, Total Chlorophyll, Phenols and Proline could be used to screen adaptive clones. Most of these biochemical traits were found high for clones C62 and C6 which could be ranked superior for adaptability across locations among the five clones taken up for the study.
Table 2a: ANOVA for biochemical traits of C. inophyllum clones

| Biochemical parameter | Source of variation | Sum of squares | Mean square | F probability | Significance |
|-----------------------|---------------------|----------------|-------------|---------------|--------------|
| Chlorophyll a         | Clones              | 0.68459        | 0.17115     | <.001         | S            |
|                       | Location            | 1.69156        | 0.84578     | <.001         | S            |
|                       | Clones*Location     | 1.11899        | 0.13987     | <.001         | S            |
| Chlorophyll b         | Clones              | 0.13576        | 0.03394     | 0.007         | S            |
|                       | Location            | 0.03796        | 0.01189     | 0.114         | NS           |
|                       | Clones*Location     | 0.28875        | 0.03699     | <.001         | S            |
| Total chlorophyll     | Clones              | 1.25851        | 0.31463     | <.001         | S            |
|                       | Location            | 2.09396        | 1.04698     | <.001         | S            |
|                       | Clones*Location     | 2.30352        | 0.28794     | <.001         | S            |
| Phenols               | Location            | 155.18         | 38.79       | 0.052         | NS           |
|                       | Clones              | 211.12         | 105.56      | 0.002         | S            |
|                       | Clones*Location     | 262.42         | 32.80       | 0.049         | S            |
| Proline               | Clones              | 0.00284        | 0.00070     | 0.002         | S            |
|                       | Location            | 0.02672        | 0.01136     | <.001         | S            |
|                       | Clones*Location     | 0.00742        | 0.00093     | <.001         | S            |
| Ascorbic acid         | Location            | 4570.4         | 1142.6      | 0.193         | NS           |
|                       | Clones              | 22161.2        | 11080.6     | <.001         | S            |
|                       | Clones*Location     | 9927.6         | 1241.0      | 0.118         | NS           |
| Malondialdehyde       | Location            | 43.66          | 10.91       | 0.780         | NS           |
|                       | Clones              | 221.63         | 110.82      | 0.017         | S            |
|                       | Clones*Location     | 324.96         | 40.62       | 0.142         | NS           |

NS – Not Significant  S - Significant

Table 2b: Mean values for biochemical traits of C. inophyllum clones

| Biochemical parameter | Clones | CHL. A mg/g | CHL. B mg/g | Tot. CHL mg/g | Phenols mg/g | Proline mg/g | Asc. acid mg/100g | MDA nmol/g |
|-----------------------|--------|-------------|-------------|---------------|--------------|---------------|-------------------|-------------|
| Neyveli               | C6     | 1.10        | 0.52        | 1.62          | 18.14        | 0.083         | 58.33             | 11.05       |
|                       | C30    | 0.48        | 0.32        | 0.81          | 19.64        | 0.076         | 53.33             | 15.75       |
|                       | C62    | 1.19        | 0.66        | 1.85          | 14.03        | 0.088         | 75.00             | 13.13       |
|                       | C86    | 0.49        | 0.43        | 0.92          | 18.45        | 0.055         | 61.67             | 11.65       |
|                       | C88    | 0.94        | 0.56        | 1.50          | 13.30        | 0.059         | 61.67             | 11.44       |
|                       | C6     | 0.53        | 0.52        | 1.04          | 11.57        | 0.057         | 56.67             | 18.02       |
|                       | C30    | 0.55        | 0.60        | 1.15          | 9.32         | 0.027         | 53.33             | 12.06       |
|                       | C62    | 0.52        | 0.53        | 1.04          | 10.25        | 0.016         | 36.67             | 16.09       |
|                       | C86    | 0.49        | 0.50        | 0.99          | 14.32        | 0.055         | 35.00             | 18.85       |
|                       | C88    | 0.49        | 0.42        | 0.91          | 16.88        | 0.020         | 56.67             | 16.84       |
| Chennai               | C6     | 0.48        | 0.50        | 0.90          | 14.10        | 0.019         | 123.33            | 13.00       |
|                       | C30    | 0.41        | 0.36        | 0.76          | 13.38        | 0.018         | 100.00            | 12.52       |
|                       | C62    | 0.47        | 0.52        | 0.99          | 8.80         | 0.032         | 50.00             | 9.27        |
|                       | C86    | 0.45        | 0.51        | 0.96          | 12.31        | 0.024         | 93.33             | 8.25        |
|                       | C88    | 0.47        | 0.38        | 0.86          | 16.74        | 0.018         | 101.67            | 17.17       |
| Salem                 | C6     | 0.702a      | 0.512b      | 1.187a        | 14.60a       | 0.0528a       | 79.4a            | 14.02a      |
|                       | C30    | 0.479a      | 0.432b      | 0.903a        | 14.12a       | 0.0403a       | 68.9a            | 13.44a      |
|                       | C62    | 0.725a      | 0.569a      | 1.296a        | 11.03b       | 0.0456a       | 53.9b            | 12.83a      |
|                       | C86    | 0.477b      | 0.479b      | 0.958b        | 15.03b       | 0.0446b       | 63.3b            | 12.92b      |
|                       | C88    | 0.634a      | 0.458b      | 1.090a        | 15.64a       | 0.0320b       | 73.3a            | 15.15a      |
| Grand mean            | C6     | 0.60        | 0.49        | 1.09          | 14.08        | 0.043         | 67.78             | 13.67       |

Mean values for Clones

Neyveli

| Location             | Mean values for Location |
|----------------------|--------------------------|
| Neyveli              | 0.839a                   | 0.501a                   | 1.338a       | 16.71a       | 0.0719a       | 62.06b       | 12.60b       |
| Chennai              | 0.156c                   | 0.514a                   | 1.028b       | 12.47b       | 0.0350b       | 47.7b        | 16.37b       |
| Salem                | 0.457b                   | 0.455c                   | 0.892c       | 13.06b       | 0.0221c       | 93.7a        | 12.04b       |

Standard error of differences (s.e.d.)

| Clones               | Location                  | Location*Clone |
|----------------------|---------------------------|----------------|
| Neyveli              | 0.03473                   | 0.0372         | 0.0692       |
| Chennai              | 0.0366                    | 0.0288         | 0.0536       |
| Salem                | 0.0819                    | 0.0645         | 0.1199       |

Least significant differences (L.s.d.)

| Clones               | Location                  | Location*Clone |
|----------------------|---------------------------|----------------|
| Neyveli              | 0.0052                    | 0.0750         | 0.1394       |
| Chennai              | 0.0738                    | 0.0581         | 0.1080       |
| Salem                | 0.1649                    | 0.1298         | 0.2414       |

Means with the same letter do not differ significantly at p<0.05%
Fig 3: Chlorophyll A in leaves of C. inophyllum clones across locations

Fig 4: Chlorophyll B in leaves of C. inophyllum clones across locations

Fig 5: Total Chlorophyll in leaves of C. inophyllum clones across locations
In *C. inophyllum* Hathurusingha et al., (2011) [15] has reported variations in oil content across various seed sources. However, studies on clonal variations in biochemical traits is very scanty. Chlorophyll is an essential pigment for photosynthesis, utilizing the energy of photons for redox reactions (Baker, 2008) [4]. Thus, leaf chlorophyll concentration (Chl) may directly influence the photosynthetic capacity of plants (Croft et al., 2017) [9]. From the table 3a and 3b it was evident that highest chlorophyll contents were recorded for C62. Similarly, across locations, Neyveli recorded the better variation compared to other two locations. From the result it can be suggested that C6 and C62 show good performance.

Phenolic compounds play important roles in plant growth and development, particularly in defense mechanisms. Most of the phenolic compounds have potent antioxidant properties, neutralizing the effects of oxidative stress. Some of them exhibit ability to chelate heavy metal ions (Kamila Kulbat, 2016). C62 contained comparatively less phenolic compounds across the clones and Neyveli recorded high phenols across locations.

Water deficit stress often causes an increase in proline accumulation in plant leaves (Dashek and Ericson, 1981) [10]. The enhancement level of proline is believed to be of adaptative significance. Resistance to water deficit stress occurs when plants withstand the imposed stress, and may arise from either tolerance or avoidance of dehydration (Levitt, 1980) [22]. Proline is a non-toxic compatible osmolyte which may alleviate the deleterious effects of stress on enzyme activity and the structure of cell membranes. It has been indicated that proline lowers the generation of highly destructive free radicals species (Smirnoff and Cumbes, 1989) [30]. C6 recorded high proline content across clones and similarly, Neyveli location recorded contained high quantities of proline.

L-Ascorbic acid is a highly abundant metabolite and has important roles in plant stress physiology as well as growth and development. In the detoxification of reactive oxygen species, it is a key antioxidant. As an enzyme cofactor, it...
plays significant parts in photoprotection, the wounding response, and insect herbivory as well as cell expansion and division (Conklin, 2001) [1]. The *C. inophyllum* clones did not show any significant variation in ascorbic acid content. However, Salem has recorded higher content of ascorbic acid across locations.

Lipid peroxides are disintegrated quickly and form reactive carbon compounds. MDA is an important reactive carbon compound which is used commonly as an indicator of lipid peroxidation (Jacob and Burri, 1996) [16]. MDA in the samples is important for the evaluation of oxidative stress in biological systems (Nordberg and Arner, 2001) [25]. Though Chennai showed high quantities of MDA across the locations, there was no significant variation in MDA contents across the clones. It could also be observed that Neyveli, Chennai and Salem record high temperatures up to 40 °C during summers and the mean rainfall is also not very luxuriant making the environment in these locations moderately harsh and dry. Despite these conditions some clones of *C. inophyllum* could establish and perform well indicating that this species can adapt to moderately harsh climatic conditions and can be domesticated.

**Conclusion**

From the study it could be evinced that screening *Calophyllum inophyllum* clones for traits such as Chlorophyll Stability Index (CSI), Membrane Injury Index (MII), biochemical traits such as Chlorophyll, Proline and Phenols could help us screen climate hardy clones that can adapt well to harsh climatic conditions. In general, based on the physiological and biochemical variability studies, C6 and C62 are superior compared to other clones and their performance across locations are fairly good. Hence, these two clones can be recommended for vegetative propagation on large scale and used for establishing plantations by State Forest Departments and farmers in agroforestry mode for better productivity.

**Fig 8:** Calophyllum inophyllum trial plot at Neyveli

**References**

1. Anandalakshmi R, Cultivation techniques- *Calophyllum inophyllum* C. Buwaneswaran V, Sivakumar RS. Prasanth and N. Krishnakumar (eds.) Transfer of tree cultivation technologies, IFGTB Publication, 2014, 33-35.

2. Anandalakshmi R, Vamadevan T, Suresh Kumar K, Sivakumar V, Krishna Kumar N. Tree improvement of *Calophyllum inophyllum* L. Madras Agricultural Journal. 2012; 99:9-11.

3. Arnon DI. Estimation of Chlorophyll. Plant Physiology. 1949; 24:1.

4. Baker NR. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant. Biol. 2008; 59:89-113.

5. Bards HD, Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Aust. J. Biol. Sci. 1962; 15:413-428.

6. Bates LS, Waldeen RP, Teare ID. Plant Soil. 1973; 39:205.

7. Blum A, Ebercon A. Cell Membrane Stability as a measure of drought and heat tolerance in Wheat. Crop Science. 1981; 21:43-47.

8. Conklin PL. Recent advances in the role and biosynthesis of ascorbic acid in plants. Plant, Cell and Environment. 2001; 24:383-394.

9. Croft H, Chen JM, Luo X, Bartlett P, Chen B, Staebler RM. Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. Glob. Change Biol. http://dx.doi.org/10.1111/gcb.13599, 2017.

10. Dashek WV, Ericson S. Isolation, assay, biosynthesis, metabolism, uptake and translocation and function of proline in plant cells and tissues. Bot. Rev. 1981; 47:349-385.

11. Dhinsa RA, Plumb Dhinso P, Thorpe PA. Leaf senescence: Correlated with increased permeability and lipid peroxidation, and decreases levels of superoxide dismutase and catalase. J Exp. Bot. 1981; 126:93-101.

12. Dweck AC, Meadowys T. Tamanu (*Calophyllum inophyllum*) – the African, Asian, Polynesian and Pacific Panacea. International Journal of Cosmetic Science. 2002; 24:341-348.

13. Friday JB, Okano D. *Calophyllum inophyllum* (Kamani), ver. 2.1. In: Elevitch CR. (Eds.). Species Profiles for Pacific Island Agroforestry. Permanent Agriculture Resources (PAR), Hualoa, Hawai’i. http://www.traditionalaltree.org, 2006.

14. Harris LJ, Ray SN. Estimation of Ascorbic acid. Lancet. 1935; 1:462.

15. Hathurusingha S, Ashwath N, Midmore D. Provenance variations in seed-related characters and oil content of *Calophyllum inophyllum* L. in Northern Australia and Sri Lanka. New Forests. 2011; 41:89-94.

16. Jacob RA, Burri BJ. Oxidative damage and defense. Am. J Clin. Nutr. 1996; 63:985-990.

17. Kamila Kulbat. The role of phenolic compounds in plant resistance. Biotechnol Food Sciences. 2016; 80(2):97-108.

18. Kardile PB, Dahatonde KN, Raksh MV, Burondkar MM. Effect of Moisture Stress on Leaf Relative Water Content (RWC) of Four Cowpea (*Vigna unguiculata* L. walp.) Genotypes at Different Stages of Growth. Int. J Curr. Microbiol. Appl. Sci. 2018; 7(04):2645-2649.

19. Koleyoreas SA. A new method for determining drought resistance. Plant Physiol. 1958; 33:22.

20. Krishnakumar N, Palanisamy K, Maheshwar H, Kannan CS, Krishnamoorthy M. Manual of Economically Important Forestry Species In South India, Institute Of Forest Genetics And Tree Breeding, ICFRE, 2010, 169-178.

21. Lal P. Productivity of clonal plantations in northern India. Indian Forester, 2007, 1014-1018.

22. Levitt J. Responses of plants to environmental stresses, Water, radiation, salt and other stresses. Acad Press New-York. 1980; II: 3-211.
23. Madhan Mohan M, Lakshmi Narayanan S, Ibrahim SM. Chlorophyll Stability Index (CSI): Its impact on salt tolerance in rice. Int. Rice Res. Notes. 2000; 25:38-39.
24. Malik CP, Singh MB. In: Plant Enzymology and Histo Enzymology. Kalyani Publishers New Delhi, 1980. 286.
25. Nordberg J, Arner ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. J Free Radic Biol Med. 2001; 31:1287-1312.
26. Pouru K. Pacific sub-regional action plan for conservation, management and sustainable use of forest and tree genetic resources. FAO publication. http://www.fao.org/3/x9662e/x9662e06.htm#top, 2000.
27. Sahoo PK, Das LM. Combustion analysis of Jatropha Karanja and Polanga biodiesel as fuel in diesel engine. Fuel. 2009; 88:994-999.
28. Sairam RK, Shukla DS, Deshmukh PS. Effect of homobrassinolide seed treatment on germination, α-amylase activity and yield of wheat under moisture stress condition. Indian J Plant Physiol. 1996; 1:141-144.
29. Shetty BV, Kaveriappa KM, Bhat GK. Plant resources of Western Ghats and lowlands of Dakshina Kannada and Udipi districts. Plikula Nisarga Dhama Society, Moodushedde Mangalore, 2002, 48-66.
30. Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. 1989; 28:1057-1060.
31. Upreti KK, Murthi GSR, Bhatt RM. Response of French bean cultivars to water deficits: Changes in endogenous hormones, proline and chlorophyll. Biol. Plant. 1997; 40:381-388.