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ORIGINAL ARTICLE

Gas Exchange Characteristics and Water Use Efficiency in Eucalyptus Clones

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The Institute of Forest Genetics and Tree Breeding, Coimbatore, India functioning under the Indian Council of Forestry Research and Education, Dehara Dun, has a long term systematic tree improvement programme in *Eucalyptus* spp. aimed to enhancing productivity and screening of clones for site specific. In the process, twenty four clones of *Eucalyptus* spp. were studied for the physiological parameters and water use efficiency from the established clonal trials. It also provides valuable information for establishing plantations at different geographic locations. Considerable variations were observed when the selected 24 clones of *Eucalyptus* spp. were subjected to physiological and WUE studies. The clones of C-188, C-186, C-14, C-10, C-123 and C-19 are falls in one cluster and the water use efficiency values are lower when compared to other clones and these clones are ranked first for high water use efficient clones for better productivity. Further, the clonal variation in physiological parameters and water use efficiency are discussed in detail in this article and clones suitable for large scale planting with higher productivity and WUE.

Key words: Physiological parameters, Water use efficiency, Eucalyptus clones, Productivity

Eucalypts are among the most widely cultivated forest trees in the world. The major *Eucalyptus* growing countries are China, India and Brazil and the growth rates that routinely exceed 35 m^3 ha^{-1} $year^{-1}$. *Eucalyptus* shows a broad productivity response depending on species, clones and soils factors (Onyekwelu *et al*., 2011). *Eucalyptus sp*. has some of the highest net primary productivity rates up to 49 $m^3ha^{-1}year^{-1}$ (Hubbard *et al*., 2010). The high biomass accumulation potential makes *Eucalyptus* sp. a good prospect for timber, wood products and carbon sequestration projects. The pulp and paper industry is one of the key industrial sectors contributing to the Indian economy. There are 759 paper mills in India with an operating capacity of 12.7 million tonnes and consumption at 11 million tonnes with 9.3 kg per capita consumption of paper. The demand of wood from forest or commercial plantation for timber, fuel wood, pulp and paper production is increasing each year at an alarming rate. Therefore, there is an urgent need for improvement in production of forest resources to meet the needs. Large scale *Eucalyptus* plantations have been raised on forest and farm lands, community lands and road / rail / canal strips in India. However, most of these past plantations had very large genetic variation, low productivity ranging from 6 to 10 $m^3ha^{-1}yr^{-1}$ and poor returns because inferior seed used for raising most of the target oriented plantations (Lal, 1993). Clonal selection and deployment in *Eucalyptus* is receiving attention as an intensive forest management tool for increased wood production. Many wood based industries in particular, pulp and paper industries are involved in plantation establishment program using clonal forestry approaches in the recent past. Over 5 million hectares of *Eucalyptus* plantations have been established throughout India. As a fast growing, remunerative and consistently demanded industrial wood, *Eucalyptus* has witnessed an unfettered support in India. *Eucalyptus* clonal planting has been said to have advantages includes quick provision of benefits associated with fast growth, short rotation for production of pulp wood (about 70 t ha^{-1}), ready marketing and other reasons. In the present day scenario, research organizations and paper and pulp industries are involving in developing new *Eucalyptus* clones for higher productivity with wide range of adaptability. Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore also started the tree improvement program in *Eucalyptus* and shortlisted clones based on productivity. But there is a gap in knowledge on physiological and nutritional aspects especially the water and nutrient use efficiency of productive clones. Therefore the present study was undertaken to assess and rank the clones through gas exchange characteristics and water use efficiency of *Eucalyptus* clones along with the commercial clones available in the market at present and the seed origin seedlings for comparison purpose. The findings of the study will help in screening the clones for higher water use efficiency to specific site suitability and also adds value for the particular clone at the time of commercial release.

MATERIALS AND METHODS

To carry out the gas exchange characteristics and water use efficiency study, *Eucalyptus* clones are selected as the experimental material. This includes 24 clones and two seed origin seedlings. Among the 24 clones, 16 clones are shortlisted by IFGTB and these clones are numbered from C-7 to C-196. For comparison purpose, 8 clones (6 ITC clones and 2 TNPL clones) and two seed origin seedlings (each one from Tamil Nadu Forest Plantation Corporation and IFGTB) are selected and named as check clone 1 to 10. The physiological parameters and derived parameters like intrinsic and instantaneous WUE, etc. in different *Eucalyptus* clones from the established clonal trials, the Portable Photosynthetic System (PPS) model LiCOR – 6400XT was used to measure the physiological parameters like net photosynthetic rate, stomatal conductance, transpiration rate, etc. from the *Eucalyptus* clones in the established clonal trials The collected wood samples are dried and powdered for water use efficiency study through the Isotope-ratio mass spectrometry (IRMS). The Gas exchange parameters measurements were taken between 9.30 am and 12.00 noon under cloud free conditions. Intrinsic water use efficiency (Pn/gs), instantaneous water use efficiency (Pn/E) and intrinsic carboxylation efficiency (Pn/Ci) were also determined using the primary data. Observations from 25 ramets per clone in 3 replications were recorded for all the physiological parameters in the *Eucalyptus* clonal trials established in 4 locations to study the WUE. The data obtained on gas exchange characteristics *viz.,* net photosynthetic synthesis, stomatal conductance, transpiration rate, instantaneous and intrinsic water use efficiency and the soil nutrients were used to perform correlation, regression and other statistical analysis using SPSS® 21.0 version and Microsoft® Excel 2007 (Panse and Sukhatme, 1985).

RESULTS

I. Physiological parameters

Observations on physiological parameters *viz.,* net photosynthetic rate, transpiration rate, stomatal conductance, intercellular CO_2 level, intrinsic carboxylation rate, intrinsic water use efficiency and instantaneous water use efficiency were recorded by using portable photosynthetic system (LiCOR). The data were analysed for statistical significance by using the software SPSS version 21. The results showed that, there is a significant difference among the clones with reference to the all the physiological parameters in different clones and the seed origin seedlings.

A. Net Photosynthetic rate (μ $molm^{-2}s^{-1}$)

The highest net photosynthetic rate was recorded in the clone C-188 (16.59) followed by C-186 (15.85) and the clone check clone 4 registered the lowest rate of net photosynthetic rate (6.21) followed by check clone 8 (7.29) compared to the mean 11.53. In the case of net photosynthetic rate, seed origin seedlings are registered lower rate when compared to the clones of *Eucalyptus*. The seed origin seedlings registered an average rate of 7.50 when compared to the clonal rate of 12-13.

B. Stomatal conductance ($molm^{-2}s^{-1}$)

In the case of stomatal conductance, the lowest stomatal conductance was recorded in the C-188 (0.0293) and check clone 7 registered the maximum of 0.0513 compared to the mean of 0.0386.

C. Transpiration ($mmolm^{-2}s^{-1}$)

From the established clonal trials, transpiration rate recorded the lowest value of 2.84 (C-188) to the highest value of 9.25 in check clone 10 compared to the mean of 5.32.

II. Intercellular CO_2 level and Intrinsic Carboxylation rate

Intercellular CO_2 level and Intrinsic Carboxylation rate data collected and analysed statistically and the results showed that, there is a significant difference among the clones.

A. Intercellular CO_2 level (Ci-μl l^{-1})

In the case of intercellular CO_2 level presence, higher the level of the CO_2 which helps in tree species for effective nutrient use efficiency and high photosynthetic activity which facilitate the growth over a period. Clone C-188 recorded the maximum intercellular CO_2 levels (276.07) and followed by C-186 (273.01). The lowest level of intercellular CO_2 was found in the clone in check clone 10 (138.17). Clones of C-188, C-186, C-19, C-111 and C-111 form one cluster and register the higher intercellular CO_2 levels of more than the mean of 222.28. The check clones 10, 9, 1, 2 3 and 8 are recorded the lower the rate of the intercellular CO_2 and form a group. The remaining clone recorded intermediate values and form the clusters of intermediate.

B. Intrinsic Carboxylation rate (μmol m^{-2} s^{-1}).

The intrinsic carboxylation rate was recorded higher in the C-188 (0.073) followed by C-10 (0.071). The lowest rate of intrinsic carboxylation rate was found in the check clone 4 (0.045) followed by check clone 5 (0.049), compared to the mean of 0.054. The clones of C-188, C-14, C-186 and C-123 forms a single cluster and registered the intrinsic carboxylation rate of more than 0.058. The check clones 4, 5, 1, 2 and 3 are recorded the carboxylation rate ranged between 0.034 and 0.045.

III. Water Use Efficiency

A. Intrinsic water use efficiency

The simplest form to define the Intrinsic water use efficiency (WUE_{intr}), the ratio of photosynthesis to stomatal conductance to water use. Higher the ratio between the photosynthesis and the stomatal conductance showed that better water use efficiency and the clone C-188 recorded the maximum value of 547.52 followed by C-123 (490.03). The lower the intrinsic water use efficiency value was found in check clone 4 (134.71) followed by and check clone 7 (135.48) with the mean of 301.05. The clones of C-188, C-123, C-10, C-14 and C-186 are recorded greater intrinsic water use efficiency value and form a single group having water use efficiency on a par. The lowest values are recorded in the check clones 4, 7, 6, 5, 3 and 8 and these clones were recorded the minimum value of intrinsic water use efficiency.

Table 1: Physiological parameters of Eucalyptus clones.

Clone no	Net photosynthetic rate (Pn- µ mol $m^{-2}s^{-1}$)	Stomatal conductance (gn- $molm^{-2}s^{-1}$)	Transpiration rate (E- $mmolm^{-2}s^{-1}$)
C 7	9.32^{a-b}	0.0330^{a-b}	4.43^{a}
C 9	10.16^{b-c}	0.0379^{b-c-d}	3.80^{a}
C 10	14.30^{d-e}	0.0292^{a}	3.73^{a}
C 14	15.32^{d-e}	0.0300^{a}	3.17^{a}
C 19	13.93^{d-e}	0.0336^{a-b}	3.21^{a}
C 63	14.99^{d-e}	0.0342^{a-b}	3.34^{a}
C 66	13.81^{d-e}	$0.0416^{c-d\ e}$	3.80^{a}
C 100	12.89^{c-d-e}	0.0420^{c-d-e}	4.37^{a}
C 111	14.86^{d-e}	0.0332^{a-b}	3.67^{a}
C 115	12.74^{c-d}	0.0416^{c-d-e}	4.05^{a}
C 123	15.85^{d-e}	0.0291^{a}	3.01^{a}
C 124	14.68^{d-e}	0.0388^{b-c-d}	3.55^{a}
C 186	15.97^{d-e}	0.0325^{a-b}	3.06^{a}
C 187	14.60^{d-e}	0.0340^{a-b}	3.50^{a}
C 188	16.71^{e}	0.0273^{a}	2.84^{a}
C 196	15.01^{d-e}	0.0441^{d-e}	3.42^{a}
Check 1	7.44^{a-b}	0.0330^{a-b}	8.10^{b-c}
Check 2	7.23^{a-b}	0.0393^{b-c-d}	7.82^{b-c}
Check 3	7.53^{a-b}	0.0379^{b-c-d}	7.73^{b-c}
Check 4	6.00^{a}	0.0441^{d-e}	7.56^{b-c}
Check 5	7.14^{a-b}	0.0393^{b-c-d}	6.75^{b}
Check 6	8.44^{a-b}	0.0423^{c-d-e}	7.40^{b-c}
Check 7	7.07^{a-b}	0.0483^{e}	6.90^{b}
Check 8	7.14^{a-b}	0.0292^{a}	7.90^{b-c}
Check 9	7.29^{a-b}	0.0300^{a}	8.80^{b-c}
Check 10	7.70^{a-b}	0.0336^{a-b}	9.12^{c}
Mean	**11.47**	**0.0402**	**5.1**

Table 2: Intercellular CO_2 level and Intrinsic Carboxylation rates of *Eucalyptus* clones.

Clone no	Intercellular CO_2 (Ci-µl l^{-1})	Intrinsic Carboxylation (µmol m^{-2} s^{-1})
C 7	225.43 $^{c-d-e}$	0.043$^{a-b-c-d}$
C 9	252.91 $^{e-f}$	0.047$^{a-b-c-d}$
C 10	263.35 $^{e-f}$	0.067^{d-e}
C 14	232.44 $^{d-e-f}$	0.069^{d-e}
C 19	271.26 $^{e-f}$	0.051$^{a-b-c-d-e}$
C 63	264.93^{e-f}	0.057^{c-d-e}
C 66	248.53 $^{e-f}$	0.057^{c-d-e}
C 100	235.13^{d-e-f}	0.057^{c-d-e}
C 111	273.33 $^{e-f}$	0.055$^{b-c-d-e}$
C 115	242.229 $^{d-e-f}$	0.054$^{b-c-d-e}$
C 123	271.26 $^{e-f}$	0.058^{c-d-e}
C 124	245.43 $^{d-e-f}$	0.051$^{a-b-c-d-e}$
C 186	274.14^{e-f}	0.058^{c-d-e}
C 187	261.84^{e-f}	0.056^{c-d-e}
C 188	277.20 ^{f}	0.072^{e}
C 196	271.25 $^{e-f}$	0.055$^{b-c-d-e}$
Check 1	179.85^{a-b}	0.042$^{a-b-c-d}$
Check 2	165.64 $^{a-b}$	0.044$^{a-b-c-d}$
Check 3	169.09 $^{a-b}$	0.045$^{a-b-c-d}$
Check 4	176.49 $^{a-b}$	0.034^{a}
Check 5	176.41 $^{a-b}$	0.040 $^{a-b-c}$
Check 6	185.43 $^{a-b-c}$	0.046$^{a-b-c-d}$
Check 7	199.46 $^{b-c-d}$	0.036 $^{a-b}$
Check 8	160.0 $^{a-b}$	0.045$^{a-b-c-d}$
Check 9	146.31 ^{a}	0.050$^{a-b-c-d-e}$
Check 10	139.30 ^{a}	0.056^{c-d-e}
Mean	**223.41**	**0.052**

Table 3: Water Use Efficiency in *Eucalyptus* clones.

Clone no	Intrinsic Water Use Efficiency	Instantaneous Water Use Efficiency	WUE by Isotope Mass Spectrophotometer
C 7	282.42$^{a-b-c-d}$	0.007 $^{a-b-c}$	26.82 $^{f-g}$
C 9	268.07$^{a-b-c-d}$	0.010 $^{b-c-d}$	26.73 $^{f-g}$
C 10	489.73^{c-d}	0.010 $^{b-c-d}$	26.75 $^{f-g}$
C 14	510.67^{c-d}	0.011^{c-d}	27.15 ^{g}
C 19	414.58^{c-d}	0.012^{d}	27.03 ^{g}
C 63	438.30$^{a-b-c-d}$	0.010 $^{b-c-d}$	26.57 $^{e-f-g}$
C 66	331.97$^{a-b-c-d}$	0.011 $^{c-d}$	25.62 $^{a-b-c-d-e}$
C 100	306.90$^{a-b-c-d}$	0.010 $^{b-c-d}$	25.01 $^{a-b}$
C 111	447.59$^{a-b-c-d}$	0.009 $^{b-c}$	26.54$^{d-e-f-g}$
C 115	306.25$^{a-b-c-d}$	0.006^{a-b}	24.79 ^{a}
C 123	544.67^{d}	0.012^{d}	26.78 $^{f-g}$
C 124	378.35$^{a-b-c-d}$	0.006^{a-b}	25.38^{a-b-c}
C 186	491.38^{b-c-d}	0.011 $^{c-d}$	27.12 ^{g}
C 187	429.41$^{a-b-c-d}$	0.010 $^{c-d}$	25.60 $^{a-b-c-d-e}$
C 188	612.09^{d}	0.013 ^{d}	27.21 ^{g}
C 196	340.36$^{a-b-c-d}$	0.011 $^{c-d}$	26.33 $^{c-d-e-f-g}$
Check 1	225.45^{a-b-c}	0.004 ^{a}	25.51 $^{a-b-c}$
Check 2	183.97^{a-b}	0.005 ^{a}	25.97 $^{b-c-d-e-f}$
Check 3	198.68^{a}	0.005 ^{a}	25.26 $^{a-b}$
Check 4	136.05^{a}	0.006 ^{a}	25.75 $^{a-b-c-d-e}$
Check 5	181.68^{a-b}	0.006 $^{a-b}$	24.85 ^{a}
Check 6	199.53^{a-b}	0.006 $^{a-b}$	25.21^{a-b}
Check 7	146.38^{a-b-c}	0.007 $^{a-b}$	25.62$^{a-b-c-d-e}$
Check 8	244. ^{a}	0.004 ^{a}	25.28 $^{a-b}$
Check 9	243.00^{a--b-c}	0.003 ^{a}	25.56 $^{a-b-c-d}$
Check 10	229.17 $^{a-b-c}$	0.004 ^{a}	25.21 $^{a-b}$
Mean	**330.05**	**0.008**	**26.28**

B. Instantaneous water use efficiency

Instantaneous water use efficiency is defined as the ratio of CO_2 assimilation into the photosynthetic biochemistry (*A*) to water lost, via transpiration through the stomata (T) or the ratio between the photosynthesis and transpiration. Increasing the external concentration of CO_2 will increase instantaneous WUE, as the driving force for water loss will remain unchanged, while that for CO_2 uptake will increase. Higher the ratio between the photosynthesis and the transpiration showed that better the instantaneous water use efficiency. The clone C-188 recorded the maximum value of 0.013 followed by C-123 (0.012). The lower the instantaneous water use efficiency value was found in the check clones 9 (0.004) followed by check clone 10, 1 and 8 (0.004, 0.004 and 0.004) with the mean of 0.009. The clones of C-188, C-123, C-10, C-14 and C-186 are recorded the more the intrinsic water use efficiency value and form a single group in the case of high instantaneous water use efficiency. The lowest values are recorded in the check clones of 9, 10, 1 and 8 and these clones were recorded the minimum value of intrinsic water use efficiency.

C. Water Use Efficiency discrimination by Stable Isotope Mass Spectrophotometer

Carbon isotope discrimination has been used to assess the genetic variability in the driving force for CO_2 uptake. Stable isotope discrimination delta (Δ) has been used to assess genotype variation in WUE and physiological responses to environmental factors. Higher the value of the Δ which indicates the better water use efficiency in the clones. The highest Δ value of 27.21 was recorded in the C-188 clone followed by 27.15 in C-14, 27.12 in C-186 and 27.03 in C-19. The clone of C-115 registered the Δ lowest value of 24.75 followed by 24.85 (in check clone 5) and 25.01 in C-100. The water use efficiency ranged between 24.75 and 27.15 among the clones. The grouping was done by using the DMRT and from the analysis, the clones of C-188, C-186, C-14, C-10, C-123 and C-19 are falls in one cluster and the water use efficiency for the Δ values are higher when compared to other clones and these clones are ranked first for high water use efficiency. The check clones of 5, 4, 10, 9, 6 and C-100 are formed one cluster and registered the lower Δ value and having lower water use efficiency.

DISCUSSION

Reports stated that photosynthetic rate varies among the plants belonging to different taxa and also among the varieties within the same species (Arora and Gupta, 1996). Olbrich *et al*. (1993) stated that, drought resistant clones may be selected for improving silvicultural practices for higher productivity and such clones need to be assessed physiologically under different conditions. Rekha Warrier *et al*. (2013) reported that, Clone EC 52 ranked top with reference to the net photosynthesis rate followed by EC 70. Clones EC 9, 10, 19 and 111 exhibited poor photosynthetic rates. The Pn values varied from 0.204 to 7.94μmol m^{-2} s^{-1} with a mean of 3.01μmol m^{-2} s^{-1} in different Eucalyptus clones. Net photosynthesis rate (Pn), is the important factor that determine the biomass production and Water Use Efficiency of a species. Variation in Pn, has been reported as determinant of plant productivity in rubber (Nataraja and Jacob, 1999). Significant differences in Pn and stomatal conductance (gs) have been reported to exist in different tree species (Zipperlen and Press, 1996), viz., *Eucalyptus camaldulensis* (Farrel *et al.,* 1996) *Populus* (Kalina and Ceulemans, 1997), *Azadirachta indica* (Kundu and Tigerstedt, 1998) and *Hevea brasiliensis* (Nataraja and Jacob, 1999). Considerable variation has been reported in clones of *Eucalyptus camaldulensis* for important physiological characteristics including high photosynthesis, carboxylation efficiency and water use efficiency (Warrier *et al.,* 2010). Kannan and Venkatramannan (2010) studied the net photosynthetic rate in Eucalyptus clones and stated that, The Pn values varied from 10.05 to 37.80 μmol m^{-2} s^{-1} with a mean of 18.45 ± 6.70 μmol m^{-2} s^{-1}. Kuyah Shem *et al*. (2009) reported that A, E and gs varied between species, being highest in *Eucalyptus* hybrid GC 15 (24.6 μmol m^{-2} s^{-1}) compared to *Eucalyptus* hybrid GC 584 (21.0 μmol m^{-2} s^{-1}), *E. grandis* (19.2 μmol m^{-2} s^{-1}), *C. africana* (17.7 μmol m^{-2} s^{-1}) and *G. robusta* (11.1 μmol m^{-2} s^{-1}). The mean maximum net photosynthetic value for GC 15 (24.6 μmol m^{-2} s^{-1}), GC 584 (21.0 μmol m^{-2} s^{-1}) and *E. grandis* (19.2 μmol m^{-2} s^{-1}) showed significant differences ($P<0.001$) in eucalyptus species. Balasubramanian *et al*. (2009) studied the photosynthetic rate in different Eucalyptus clones and revealed that, there is a significant difference among the clones under water logged conditions. Marrichi (2009) observed values from 25.7 to 31.6 μmol m^{-2} s^{-1} in eucalyptus plants at 16 months of age. White Head and Beadle (2004) found values of around 13-32 μmol m^{-2} s^{-1} for 11 species of *Eucalyptus*. Photosynthetic rate of any species is a direct indicator of plant growth and metabolism. Therefore, selection of a variety or species for a given geographical location could also be on the basis of its photosynthetic activities. A reduction in photosynthesis affected the accumulation of biomass in the plant.

The growth and development of trees on sites experiencing occasional periods of drought stress depends on the ability of stomata to control water loss while maintaining growth. Stomata also respond to CO_2

as stomatal conductance decreases as CO_2 concentration increases (Medlyn *et al.,* 2001). The stomatal conductance decline in response to increase in CO_2 concentration will, to some extent, compensate for the effect of increased CO_2 on photosynthesis and may also reduce the transpiration rate and the integrated result of these effects is that an increase in atmospheric CO_2 concentration generally increases water use efficiency (WUE) (Centritto *et al.,* 1999). Stomatal conductance is of utmost importance when photosynthesis is concerned. Stomata play a pivotal role in controlling the balance between assimilation and transpiration. Rekha warrier *et al.* (2013) studied the clonal differences in stomatal conductance and the results showed that stomatal conductance varied between 0.008 to 0.264 mol^{-2} s^{-1} with a mean of 0.08 mol^{-2} s^{-1}. The role of stomata in determining the water use efficiency is also well understood (Li, 2000). Kallarackal and Somen (1998) observed significant variations among different species of *Eucalyptus* viz. *E. tereticornis, E. camaldulensis, E. urophylla, E.brassiana, E. pellita* and *E. deglupta* in stomatal conductance. It was lowest in *E. urophylla* and highest in *E. camaldulensis*. As such, leaves must adjust their stomatal aperture to maximize photosynthesis while minimizing consequences of excessive water loss.

Intrinsic carboxylation efficiency was derived as the ratio of net photosynthetic rate to intercellular CO_2 concentration (Pn/Ci). This result is in tune with Kannan and Venkatramannan (2010) who studied in Eucalyptus clones and stated that, among the 59 clones, EC 130 ranked first (197.30 µl l^{-1}) for intercellular CO_2 concentration. Clone EC 148 recorded the lowest value (92.58 µl l^{-1}). The mean and standard deviation were 125.34 and 26.45 µl l^{-1} respectively. Among the eucalypt clones, EC 17-1, EC 1-7, EC 71, EC 72 and EC 130 recorded higher values for intrinsic carboxylation efficiency coupled with superior growth when compared to others. This ratio varied from 0.100 (EC 12-11) to 0.198 µmol m^{-2} s^{-1} (EC 17-1) with a coefficient of variation of 20 per cent (Kannan and Venkatramannan, 2010).

Water use efficiency is the ratio of CO_2 assimilation into the photosynthetic biochemistry (Pn) to water lost, via transpiration, through the stomata (E). Also defined as Water use efficiency is the ratio of A to transpiration (E), and is a measure of the amount of water used per carbon gain. $\Delta^{13}C$ is related to the ratio of A/gs and termed intrinsic water use efficiency (Wi). Chunying Yin *et al*. (2005) found that there were significant interspecific differences in early growth, dry matter allocation, and WUE between two sympatric *Populus* species under well-watered and water-stressed treatments. Li (2000) reported that measurement of intrinsic WUE may be a useful trait for selecting genotypes with improved drought adaptation and biomass productivity under different environmental conditions. Higher intrinsic WUE was associated with productivity in *Prosopis glandulosa* and *Acacia smallii* (Polley *et al*.1996). It is reported that long-term structural and growth adjustments as well as changes in intrinsic WUE are important mechanisms of *Acacia koa* to withstand water limitation. Instantaneous WUE is estimated as the ratio of net photosynthetic rate to transpiration. Higher the value, better the efficiency of the plant to divert water for photosynthesis than transpiration. This result is in tune with Kannan *et al*. (2007) and revealed that, productive clones of Casuarina exhibited superior values of instantaneous WUE and the values ranged from 0.169 in CP 2401 to 0.477 µmol $mmol^{-1}$ in CH-3004 clone. Similar findings have been reported for *U. americana* (Reich *et al*. 1989), *Eucalyptus* spp. (Sheriff, 1992), maritime pine (Guehl *et al*. 1995), *Pinus radiata* D. Don (Sheriff and Mattay, 1995), poplar clones (Liu and Dickmann, 1996), white spruce (Livingston *et al*. 1999) and *Quercus robur* L. (Welander and Ottosson, 2000). In these studies, the increase in intrinsic and instantaneous WUE was related to higher net photosynthetic rate coupled with low stomatal conductance and transpiration rate and resulted higher productivity.

Stable carbon isotope ratios ($\delta^{13}C$) in tree rings are the result of discrimination against the heavier $^{13}CO_2$ during carboxylation and diffusion through the stomata, which are linearly related to the ratio of intercellular and atmospheric CO_2 (ci/ca). Therefore, time-integrated, intrinsic water-use efficiency can be inferred using stable carbon isotope ratios ($\delta^{13}C$) of plant tissues given its inverse linear relationship with Ci/Ca, whereby high water use efficiency is indicated by less negative $\delta^{13}C$ and low Ci/Ca and vice versa. Plant drought stress can be reflected to the degree by which plants discriminate against the heavier isotope carbon $\delta^{13}C$ during photosynthesis. Debbie Le Roux *et al*. (1996) reported that water use efficiencies differed significantly between clones and clonal variation in $\delta^{13}C$ is associated with variation in WUE. that water use efficiencies differed significantly between clones and clonal variation in $\delta^{13}C$ is associated with variation in WUE. Significant correlations between carbon isotope discrimination, instantaneous and growing season water use efficiencies were also found in western larch seedlings (Zhang and Marshall, 1993).

CONCLUSUSION

In the present study, the highest WUE value of 27.21 was recorded in the C-188 clone followed by 27.15 in C-14,-27.12 in C-186 and-27.03 in C-19. The clone of C-115 registered the lowest value of 24.75 followed by 24.85 (check clone 5) and 25.01 in C-100. The water use efficiency ranged between 24.75 and 27.15 among the clones. The grouping was done by using the DMRT and from the analysis, the clones of C-188, C-186, C-14, C-10, C-123 and C-19 are falls in one cluster and the water use efficiency values are lower when compared to other clones and these clones are ranked first for high water use efficient clones for better productivity. The above said clones exhibited superior growth coupled with favourable physiological characteristics including high photosynthesis, carboxylation efficiency and water use efficiency. Also, these clones are registered better intrinsic and instantaneous WUE and recorded the higher productivity. Further, these clones were tested for WUE by using the stable isotope mass spectrophotometer and the above said clones recorded higher values for better WUE positively correlated with higher productivity.

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