Eurasscience Journals

Eurasian Journal of Forest Science (2015) 3(1): 14 - 21

CLONAL VARIATION IN EUCALYPTUS WITH REFERENCE TO BIOCHEMICAL PARAMETERS

Ranjani VIJAYAKUMAR, Chelliah BUVANESWARAN, Rekha R. WARRIER* AND Rathinam Saviour Caesar JAYARAJ

*Institute of Forest Genetics and Tree Breeding (Indian Council of Forestry Research and Education) P.B.No.1061, R.S.Puram, Coimbatore-641002 (Tamil Nadu). email: rekha@icfre.org, rekhawarrier@gmail.com

Abstract

The Institute of Forest Genetics and Tree Breeding, Coimbatore is involved in systematic tree improvement programme of *Eucalyptus* and in the process, has identified highly productive clones based on productivity. In the present study, selected clones were evaluated for biochemical parameters – carbonic anhydrase (CA) activity, chlorophyll and organic acids which play a major role in carbon accumulation and therefore may aid in future breeding programmes as markers for carbon sequestration. Results revealed highly significant variations in all the parameters studied among the selected *Eucalyptus* clones. The CV for the experiments was also low indicating low relative magnitudes in the standard deviation of means. Cluster analysis for grouping clones on the basis of the biochemical traits revealed that these clones could be segregated into three major groups. Among the parameters used, Fumaric acid, followed by Oxalic acid and total chlorophyll determined the clustering of selected eucalyptus clones. This suggests the suitability of using these parameters as novel markers for screening clones for carbon sequestration potential.

Keywords: Eucalyptus, Carbonic anhydrase, Fumaric acid, Oxalic acid, Coimbatore.

Özet

Coimbatore Ağaç Islâhı ve Genetiği Enstitüsü okaliptüs türü ile ilgili olarak sistematik bir ağaç iyileştirme programına dâhil olmuş ve verimlilik esasına göre yapılan değerlendirmelere göre çok üretken bir klonun varlığı tespit edilmiştir. Bu çalışmada seçilen klonlar; CA aktivitesi, klorofil ve organik asitler gibi karbon birikiminde önemli rol oynayan ve haddizatında bitkiler tarafından karbon tutulması ile ilgili çalışmalarda da önemli bir müşir vazifesi görebilecek parametreler incelenmiştir. Seçilen bütün klonlar arasında çalışılan parametreler bakımından anlamlı farklar bulunmuştur. Deneme desenindeki CV düşük standart sapma ortalamaları göstermiştir. Biyokimyasal özellikleri bakımından klonları gruplandırmak için yapılan küme analizi sonucu üç farklı büyük kümenin oluşturulabileceğini göstermiştir. Kullanılan parametreler arasında fumarik asit, bunu oksalik asit ve toplam klorofil izlemiştir. Elde edilen sonuçlar bu parametrelerin klonların karbon bağlama potansiyellerini belirlemek üzere izlenmesinde özgün müşirler olarak kullanılmasının uygun olduğunu göstermektedir.

Anahtar Kelimeler: Okaliptüs, Karbonic anhidraz, Fumarik asit, Oksalik asit, Coimbatore.

INTRODUCTION

Attempts are being made all over the world to increase the productivity of forest resources and plantations by planting of high yielding species or clones to meet the forest based growing needs. One such species introduced to meet demands of paper and pulp industries is Eucalyptus. The species primarily finds use in making pulp/paper and for charcoal in India (Kulkarni, 2006). It is also used as fuel wood, poles, stakes and fence posts, as a minor timber and in particleboards. Clonal forestry programmes operating on a large scale are mostly with several species in the genus Eucalyptus and a number of clones are being deployed to increase the productivity of this species in India (Lal, *et al.,* 2006).

India is the largest planter in the world having about 3.943 million ha (Eucalyptus Global Map, 2010) covering 22 per cent area. Systematic *Eucalyptus* tree improvement programmes have been taken up with an aim to identify trait based requirements of the end users. This has been done in India with large scale introductions of different provenances from its native range, mostly Australia. Provenance and family level selection through multi location trials have led to the selection of highly productive clones based on biometrical parameters. Considerable variations have been reported in clones of *Eucalyptus camaldulensis* Dehnh. selected by Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu (IFGTB) subject to biometric and physiological studies (Warrier and Venkataramanan, 2010).

Physiological characters like photosynthetic efficiency or chlorophyll fluorescence have been used to select high-quality seedlings or clones for a particular environment. Biochemical components (chlorophyll, nitrogen, etc.) are among essential parameters that control physiological processes. Foliar biochemicals can also be used as indicators of nutrient availability, ecosystem functioning and plant stress especially in aquatic and wetland plants and this property has been deployed in remote sensing for hyperspectral measurements of plant nutrients (Siciliano et al, 2008). The activity of carbonic anhydrase (CA) has been reported to be crucial in photosynthetic acclimation. CA activities have been predicted to enhance the rate of photosynthesis by catalysing rapid equilibration of inorganic carbon and thus increasing the supply of CO2 across the stroma in the chloroplast (Woodrow, 2009). Enhanced CA activities were noticed in *Arabidopsis* and *Zea mays* (maize or corn), grown at elevated CO2, indicating difficulties in the interpretation of the role of CA in photosynthetic acclimation (Raines et al., 1992). Changes in the enzyme activities and photosynthetic machinery can have a direct bearing on the metabolic activities within the plant which would in turn lead to variations in traits related to growth, productivity, tolerance to different biotic and abiotic stresses. Therefore, information on general growth, physiological and biochemical traits enables characterizing the germplasm and would support designing further tree improvement and breeding strategies. Hence, these clones selected for productivity and gas exchange characteristics are evaluated for biochemical traits related to the already mentioned traits which would impart higher weightage over the existing available data. This would aid in future breeding programme and also act as markers for selection especially for traits like pest resistance, salt and drought tolerance etc. Hence, the objective of the study was to screen selected clones of Eucalyptus for carbonic anhydrase (CA) activities and also to estimate variation in levels of chlorophylls and organic acids with a focus to categorise clones for different traits, especially carbon sequestration.

MATERIALS AND METHODS

The Institute of Forest Genetics and Tree Breeding, Coimbatore is working towards improvement of Eucalyptus species from the past two decades. First generation provenance trials were established in ten different locations and about 100 clones of *E. camaldulensis* and *E. tereticornis* were selected, based on individual tree superiority for height, diameter at breast height and straightness of

stem through index selection method. The clonal trials were established in three different locations, viz., Coimbatore (11° 00' N, 76° 58' E, 400 m altitude, 900 mm rainfall), Sathyavedu (13° 25' N, 79° 57' E, 215 m altitude, 1150 mm rainfall) and Kulathupuzha (8° 50' N, 77° 15' E, 230 m altitude, 2800 mm rainfall). In the present study, twenty five clones from two species *viz. Eucalyptus tereticornis* and *E. camaldulensis* identified for improved growth were selected from the Vegetative Multiplication Garden, Institute of Forest Genetics and Tree Breeding, Coimbatore, India (11° 00' N, 76° 58' E, 400 m altitude, 900 mm rainfall). The experiment was conducted under greenhouse conditions. Ten replicates of each clone were used in each of the treatments and n=5 for all measured parameters.

Preparation of Plant Extract: Fully expanded leaves were collected at random from five ramets per clone and after cleaning, the leaves were cut into small pieces. Extraction was carried out with different types of buffer for the experiment (0.1 M phosphate buffer (pH 8.3), 80% ethanol, 25mM phosphate buffer (pH 7.0) and cold acetone depending on the experiments), centrifuged and then the supernatant was taken for the estimations.

Biochemical analyses: To determine the chlorophyll contents, pigments were extracted in 80 per cent acetone, measured with a UV-VIS spectrophotometer (Labtronics, India) at 645, 654 and 663 nm. Chlorophyll (a, b a:b ratio and total) contents on fresh weight basis were calculated using the method of Yoshida et. al.(1976). Electrometric method was used for analyzing CA activity, in which the time required for a saturated CO_2 solution to lower the pH of 0.02 M Tris HCL buffer from 8.3 to 6.3 at 0° C was determined (Wilbur and Anderson, 1948). The organic acids were estimated by titrimetric method.

Statistical analysis: Each experiment was replicated five times. The values for various parameters of the plants were subjected to statistical analysis following the standard procedure described by Gomez and Gomez (1984). The means were compared by the least significant difference (LSD) test to study the significance at a 5% level of probability. The data on various biochemical parameters were subjected to clustering analysis using 'Statistica' Version 3. The cases-wise analysis was done by using Un-weighted Pair Group Mean Average (UPGMA) and the horizontal tree was generated. Euclidean distance matrix was used for clustering clones.

RESULTS

Carbonic Anhydrase activity

In the present study, the activity of Carbonic Anhydrase was assessed in 25 selected clones of eucalyptus and it is observed that the highest activity for carbonic anhydrase was in clone- C116 (3054.5 Units) and least was recorded in clone- C115 (1442.9 units). The average of te clones was 2275.7 ± 48.49 Units / g tissue. Statistical analysis of Carbonic anhydrase activity revealed that highly significant variation ($P<0.001$) exists in CA activity among the selected *Eucalyptus* clones (Fig 1 and Table 1).

Chlorophylls

Table 2 presents estimates on chlorophyll contents in the clones studied. The minimum chlorophyll-a was observed in clone - C196 (0.819 mg g^{-1}) and the maximum value for chlorophyll-a was noted in clone - C198 (9.299 mg g^{-1}). The overall mean value for chlorophyll a was 3.293 ± 0.447 mg g^{-1}. As observed for chlorophyll-a, the minimum value for chlorophyll-b was also observed in clone - C196 (0.565 mg g^{-1}) and the maximum chlorophyll-b was noted in clone - C198 (7.251 mg g^{-1}). The overall mean value for chlorophyll-b was 2.493 ± 0.346 mg g^{-1}. The minimum total chlorophyll was observed in clone- C196 (0.577 mg g^{-1}) and the maximum total chlorophyll was noted in clone - C31 (7.013 mg g^{-1}). The mean total chlorophyll content was 2.517 ± 0.344 mg g^{-1}. Chlorophyll a:b ratio, which indicates the functioning of the photosynthetic machinery, ranged from 0.878 to 2.058 with a mean and SD of 1.357 and 0.31.

Organic acids

Fumaric acid was minimum in clone - C100 (101.57 mg g^{-1}) and maximum in clone - c123 (273.76 mg g^{-1}). The mean value registered was 154.81 ± 8.66 mg g^{-1} (Table 3). The minimum Malic acid was

estimated in clone - C88 and maximum in clone-C17 (2.91 and 15.87 mg g^{-1} of leaves respectively). The mean value for Malic acid was 7.83 ± 0.67 mg g^{-1} (Table 3).

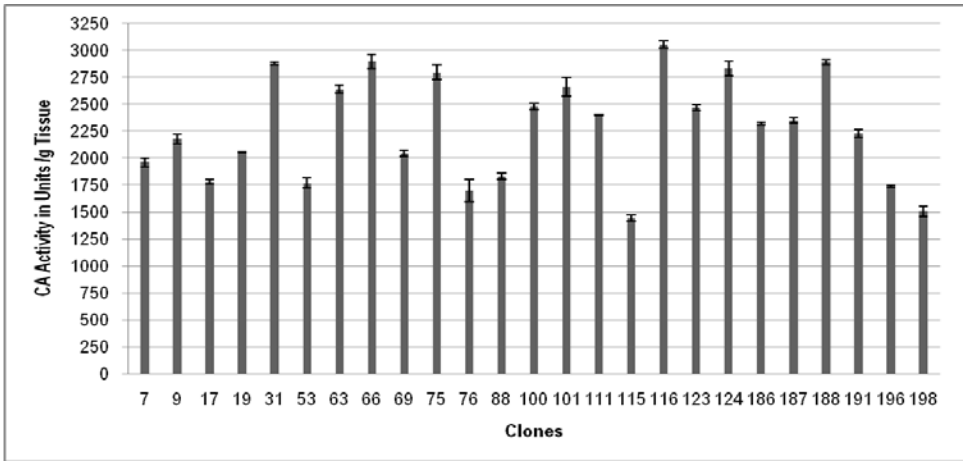

Figure 1: Carbonic anhydrase activity (Units / g tissue) in selected clones of *Eucalyptus*

Table 1: ANOVA for Carbonic anhydrase assay in clones of Eucalyptus

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between clones	116.3854	24	4.849392	3.4355	0.000115	1.73708
Within clones	70.57767	50	1.411553			
Total	186.9631	74				

Table 2: Levels of Chlorophylls (mg g^{-1}) in selected Eucalyptus clones

Clones	**Chlorophyll a**	**Chlorophyll b**	**Chlorophyll a/b ratio**	**Total Chlorophyll**
C7	1.158	0.850	1.362	0.862
C 9	1.121	0.760	1.475	0.778
C 17	3.047	2.154	1.415	2.194
C 19	5.680	3.819	1.487	3.917
C 31	6.364	7.251	0.878	7.013
C 53	3.923	4.296	0.913	4.170
C 63	1.030	0.923	1.116	0.914
C 66	3.354	3.058	1.097	3.022
C 69	1.498	1.089	1.376	1.105
C 75	1.825	1.375	1.327	1.389
C 76	2.116	1.456	1.453	1.488
C 88	1.638	1.084	1.511	1.114
C 100	5.648	3.340	1.691	3.490
C 101	4.581	4.501	1.018	4.414
C 111	1.334	1.004	1.329	1.014
C 115	2.103	1.433	1.468	1.467
C 116	5.705	3.841	1.485	3.939
C 123	3.931	2.533	1.552	2.614
C 124	7.005	3.404	2.058	3.677
C 186	3.353	3.081	1.088	3.042
C 187	1.862	1.442	1.291	1.452
C 188	2.394	1.514	1.581	1.566
C 191	1.529	1.638	0.933	1.593
C 196	0.819	0.565	1.450	0.577

C 198	9.299	5.906	1.575	6.106
Mean	**3.293**	**2.493**	**1.357**	**2.517**
SD	**2.237**	**1.729**	**0.271**	**1.720**
SE	**0.447**	**0.346**	**0.089**	**0.344**

Values are means of five replications

Table 3: Photosynthetic intermediates (mg g^{-1}) in selected Eucalyptus clones

Clone No.	Fumaric Acid (mg g^{-1})	Malic Acid (mg g^{-1})	Citric Acid (mg g^{-1})	Oxalic Acid (mg g^{-1})
C7	150.90	10.06	9.22	0.0081
C 9	198.30	3.13	9.90	0.0026
C 17	104.47	15.87	10.93	0.0060
C 19	110.28	5.36	8.54	0.0107
C 31	183.79	10.73	12.30	0.0024
C 53	125.75	5.81	12.98	0.0035
C 63	148.97	7.60	10.58	0.0029
C 66	105.44	7.37	11.95	0.0044
C 69	198.30	3.58	10.93	0.0033
C 75	138.33	11.62	8.54	0.0036
C 76	145.10	3.80	20.83	0.0038
C 88	142.20	2.91	10.59	0.0032
C 100	101.57	8.04	14.00	0.0047
C 101	207.98	11.40	7.17	0.0030
C 111	165.41	4.25	12.98	0.0041
C 115	222.49	7.15	18.79	0.0060
C 116	182.83	5.81	9.90	0.0056
C 123	273.76	10.50	11.27	0.0032
C 124	155.74	8.27	6.49	0.0041
C 186	115.11	14.97	10.25	0.0125
C 187	121.88	7.15	8.88	0.0044
C 188	107.37	8.49	10.93	0.0059
C 191	188.63	7.82	10.25	0.0038
C 196	129.62	6.93	8.54	0.0045
C 198	146.07	7.37	11.61	0.0027
Mean	154.812	154.81	7.84	11.13
SD	43.326	43.33	3.38	3.17
SE	8.665	8.67	0.68	0.63
Mean	5.60	8.62	5.70	10.63

Values are means of five replications

Among the 25 *Eucalyptus* clones the minimum Citric acid was observed in clone-C124 leaves (6.49 mg g^{-1}) and maximum was observed in clone C76 (20.83 mg g^{-1}). The mean value registered was 11.13 ± 0.63 mg g^{-1}. The minimum Oxalic acid was observed in clone-C31 and minimum in clone-C186 (0.002 and 0.012 mg g^{-1} respectively). The mean value registered was 0.0047 ± 0.0004 mg g^{-1}. ANOVA on organic acids revealed that there exist highly significant variations among the clones of eucalyptus. Among the organic acids studied, Oxalic acid registered the greater variation (CV of 10.63%) and Fumaric acid registered least variation (CV of 5.60).

Cluster Analysis

Clustering analysis was carried out for grouping the related clones with reference to biochemical traits. The results revealed that these 25 selected clones formed three major groups. The first

group consisted of two sub-groups. The first sub-group consisted of clones: C7, C63, C88, C75, C124, C198, C76 AND C111. The second sub-group consisted of the clones: C17, C19, C66, C188, C100, C186, C53, C187 and C196. The second group consisted of C9, C69, C101, C31, C116, C191 and C115. The clone C123 formed the third group. Among the biochemical parameters used for clustering of clones, Fumaric acid, followed by Oxalic acid and total chlorophyll mainly determined the clustering of selected eucalyptus clones (Fig 2).

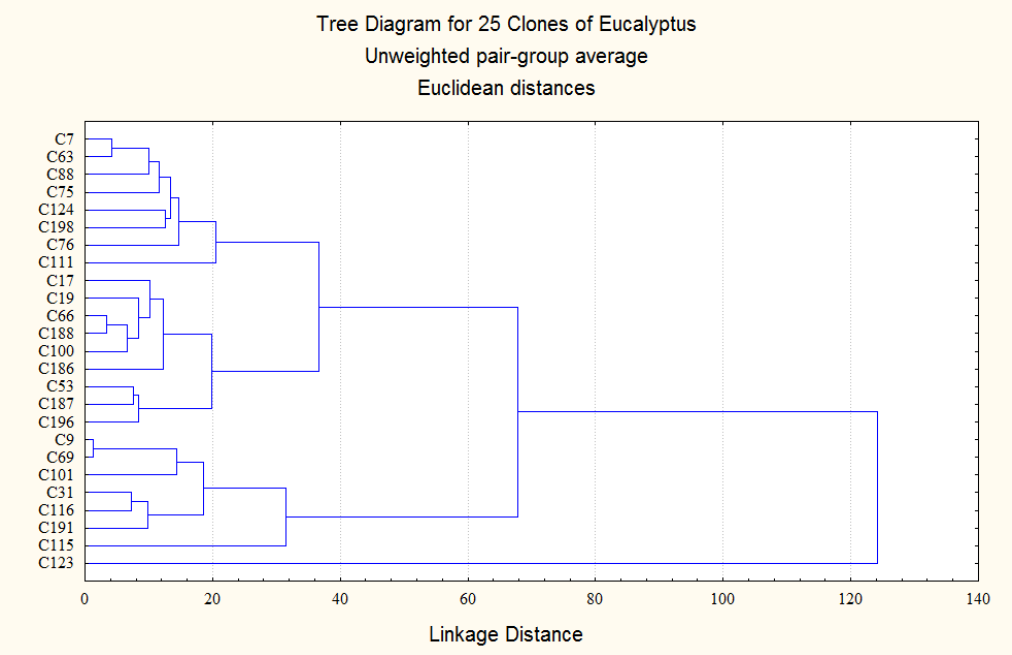

Fig 2: Clustering analysis of eucalyptus clones using biochemical traits

DISCUSSION

With respect to carbonic anhydrase activity, highly significant variation was observed among the selected *Eucalyptus* clones (Table1). The photosynthetic system is the backbone of plant system; therefore any changes in these attributes occur due to stress. Baseline information on these values in the clones would enable studies on their response to stress. CA activity is largely regulated by CO_2 concentration (Tiwari et al., 2005). CA is the enzyme that catalyzes the reversible hydration of CO_2 and maintains its constant supply to Rubisco (Majeau and Coleman, 1994). The reported decrease in the activity of CA by salinity is in agreement with other studies (Hayat et al., 2011).

Similar reports have been observed in teak (Anita *et al.*,2006) where significant variation ($P<0.005$) was observed in CA activity in teak across ten 1-year-old half-sib families and twenty one 5-year-old half-sib families. Greater diversity was observed in CA activity than in photosynthetic characteristics, suggesting use of CA as a biochemical marker for photosynthetic capacity in teak genotypes.

Rowland *et al.,* (2004) found significant stunting effects of high salinity on mean leaf size, plant height, total plant mass, root mass, and shoot mass, with no effects on chlorophyll content For all populations of *Eucalyptus* sp. This could suggest that baseline information on the levels of chlorophylls in the clones of Eucalyptus, as obtained in this study, would give an indication of the ability of the clones to overcome abiotic stresses like salinity. Clones like 31, 124, 196 and 198 would be adaptable to stress sites and can form part of the trials in problem soils. Nicolas *et al.* (2007) reported that there was no link of chlorophyll with tree growth irrespective of site and family in hybrids of poplar. Ashok Kumar and Paramathama (2005) reported that chlorophyll content was strongly associated with volume index in clones of *Casuarina equisetifolia* assembled from different places of South India. Reddy *et al.* (2003) reported that chlorophyll a, b a:b ratio and total

chlorophyll showed significant positive correlation with leaf area and yield in different genotypes of Mulberry.

Silva *et al.* (2004) reported increase in malic acid concentration in the root tips of all eucalypt species in response to Al treatment. A small increase in citric acid concentration was also observed in all species of eucalyptus suggesting their role in stress physiology of the plants.

This suggests that characterization of Eucalyptus clones can provide baseline information on the eucalyptus clones. In addition, they can also serve as indicators of the stress tolerance / adaptability of the clones when subjected to abiotic stresses like salinity, drought, metal tolerance, etc.

CONCLUSION

It is evident that clonal variations in eucalyptus could be detected using biochemical parameters. Intermediates of the process of photosynthesis and the main machinery involved, namely the chlorophylls could be used to discriminate the clones. Carbonic anhydrase activity was another parameter which could differentiate the clones. These parameters would be of significance in identifying the carbon sequestration potential of the clones as all the identified parameters contribute to carbon accumulation in the plant.

Acknowledgement

The authors are grateful to the Director, Institute of Forest Genetics and Tree Breeding and Director General, Indian Council of Forestry Research and Education, Dehradun for the direction and financial support.

References

Anita, T., Pramod Kumar, Chawhaan, P. H., Sanjay Singh, Ansari, S. A. (2006). Carbonic anhydrase in *Tectona grandis*: kinetics, stability, isozyme analysis and relationship with photosynthesis. *Tree Physiology*, 26(8): 1067-73.

Ashok Kumar. and Paramathama, M. (2005). Correlation and path coefficient studies in *Casuarina equisetifolia* L. Johnson *Indian-Forester*. 131(1): 47-55.

GIT (2010). Consulting, Eucalyptus Universalis—Cultivated Eucalypt Forests Global Map.

Gomez, K. A., and A. A. Gomez, (1984). Statistical Procedures for Agricultural Research, 2nd Ed. John Wiley and Sons, NY.

Hayat S, Mir BA, Wani SA, Hasan SA, Irfan M, Ahmad A. (2011). Screening of salt-tolerant genotypes of *Brassica juncea* based on photosynthetic attributes. *J. Plant Interact*. 6:53-60.

Kulkarni, H.D. (2006). Current status of wood supply to industries based on agro-forestry related to pulp and paper industry. Paper presented in the Regional Conference in 'Scope of production forestry for enhancing carbon mitigation in India', held on 14-15, November 2005 at ATREE, Bangalore.

Lal, Piare., Dogra, AS., Sharma, S.C. and Chahal, G.B.S. (2006). Evaluation of different clones of *Eucalyptus* in Punjab, *Indian Forester* 132(11): 1383-1390.

Majeau N, Coleman J.R. (1994). Correlation of carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase expression in pea. Plant Physiol. 104:1393-1399.

Nicolas, M., Sophie, Y. D. and Reinhart C. (2007). Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. Environmental and Experimental Botany, 61: 103–116.

Raines, C. A., Horsnell, P. R., Holder, C. and Lioyd, J. C., (1992). *Arabidopsis thaliana* carbonic anhydrase: cDNA sequence and effect of CO2 on mRNA levels. *Plant Mol. Biol.*, 20, 1143–1148.

Reddy, B.K., Rao, D.M.R., Reddy, M.P., Jayaram, R.H., Suryanarayana, N. (2003). Variation of chlorophyll content and its relationship with leaf area and leaf yield in mulberry genotypes (*Morus* spp.). *Advances in Plant Sciences* 16(1): 277-280.

Rowland, D.L., Sher, A.A. and Marshall, D.L. (2004). Inter and intra population variation in seedling performance of Rio Grande cottonwood under low and high salinity. *Canadian Journal of Forest Research* 34(7): 1458-1466.

Siciliano, D., Wasson, K.**,** Potts, D.C., Olsen, R.C. (2008). Evaluating hyperspectral imaging of wetland vegetation as a tool for detecting estuarine nutrient enrichments. *Remote Sensing of Environment*. 112:420-433

Silva, I.R., Novais, R.F., Jham, G.N., Barros, N.F., Gebrim, F.O., Nunes, F.N., Neves, J.C.L. and Leite, F.P. (2004). Responses of eucalypt species to aluminum: the possible involvement of low molecular weight organic acids in the Al tolerance mechanism. *Tree-Physiology* 24(11): 1267-1277.

Tiwari A, Kumar P, Singh S, Ansari S.A. (2005). Carbonic anhydrase in relation to higher plants. Photosynthetica 43:1-9.

Warrier, K.C.S. and Venkataramanan, K.S. (2010). Gas exchange characteristics in eucalyptus clones. Indian Journal of Plant physiology. 15(3): 226-233

Wilbur, K. and Anderson, N. (1948). Electrical and colorimetric determination of CA. *J. Biochem*. 176: 147.

Woodrow, I. E., (2009). Flux control analysis of the rate of photosynthetic CO2 assimilation. In *Photosynthesis in silico* (eds Laiska, A., Nedbal, L. and Govindjee), Springer, The Netherlands, pp. 349–360.

Yoshida S, Forno DA, Cock JK, Gomez KA. (1976). Laboratory manual for physiological studies of rice. Manila, Philippines: International Rice Research Institute.

Submitted: 18.05.2013

Accepted: 09.04.2015