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Biochemical Indicators for Rooting in *Casuarina equisetifolia* Clones

[1]K. Satyavani, [2]Kannan C.S. Warrier, [1]T. Ramanathan and [1]S. Gurudeeban
[1]Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai 608502, India
[2]Institute of Forest Genetics and Tree Breeding, Coimbatore 641002, India

Abstract: *Casuarina equisetifolia* is the wide spread exotic trees in peninsular Indian and well known member of the family Casuarinacea. The present study was carried out biochemical indicators of ten good rooting and poor rooting clones of *Casuarina equisetifolia*. The DNS and anthrone method of reducing sugar and carbohydrate was estimated respectively. The result of the analysis revealed significant difference between the good rooting and poor rooting clones with higher the content reducing sugar (6.99-12.68) in better rooting, lesser the content of carbohydrates (20.09-58.59) present in better rooting. The present investigation reveals reducing sugar and carbohydrates plays a major role in rooting of *Casuarina equisetifolia*.

Key words: Australian pine, cladode, clones, root indicators, DNS, t-test

INTRODUCTION

Casuarinas equisetifolia is naturally found along the sea coast of Malaysia to subtropical Australia, Melanesia, Micronesia, the Philippines, Polynesia, Southern Myanmar, Southern Myanmar, South Thailand and Andaman Nicobar Islands (Pinyopusarerk and House, 1993). Australian pine is a deciduous tree with soft, wispy, pine like appearance with 100 feet or more in height. The fruit is a nut let about one and half inch in diameter. It produces dense shade and thick blankets of leaves (Swearingen, 2005). Casuarinas are deep rooted and roots are found to have been arranged in clusters around the taproot. The lateral roots are spread at all angles with 150 to 240 cm length and 3 to 18 cm girth near the branching point and ends as fine roots, all the lateral roots have fine roots of their ends (George *et al*., 1996). Rooting generally occurs in 15 to 20 days of time. Clonal variation can also be observed with respect to rooting (Whistler and Elevitch, 2006). *Casuarina equisetifolia* can be propagated by seed, stem cuttings and air layering. Basal portion from the cutting was cut and used for the determination of peroxidase activity at pH 5.5 and 7.0. A close relationship between enzymes, specific protein and the process of fruit development could be observed. The genetic variation of seeds from 20 trees of *Aroeira* the lipid content analyzed varied between 200 and 334 mg g^{-1} seed, the total sugars also varied (26.5-46.3 mg g^{-1} of seed) and the starch content varied from 0.35-1.58 mg g^{-1} of seed (Tani and Sasakawa, 2006). The content of individual sugars and the some protein bound amino acids showed seasonal changes in mature leaves but not in young developing leaves. Na^+ concentration in the shoots gradually increased with increasing the NaCl concentration in the culture solution (Hedge and Hofreiter, 1962). GA_3 reduced NaCl inhibition of shoot growth, but not of root growth but the sugars (sucrose, fructose and glucose) were able to reduce NaCl-induced growth inhibition of shoots and roots (Lin and Kao, 1995). The present investigations carry out the biochemical indicators for rooting between the good and poor rooting clones of *Casuarina equisetifolia*.

MATERIALS AND METHODS

Plant material: The study was conducted during December 2008 and the cladode tissues of 20 different clones were collected and authenticated from the plant nursery of Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu and India.

Preparation of plant extract: Extraction was usually carried out with different type of buffer for experiment (phosphate buffer, hydrolyzed by 2.5 N HCl and 80% ethanol) centrifuged and then supernatant was taken for the estimation.

Estimation of protein: Different concentration of BSA (0.2, 0.4, 0.6, 0.8 and 1 mL), 0.1 and 0.2 mL of the extract was taken in the various test tubes. All the tubes were

Corresponding Author: K. Satyavani, Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai, Tamil Nadu, India Tel: (0) 9786445483

made up to 1 mL with distilled water. Added alkaline $CusO_4$ 5 and 0.5 mL folin to all the tubes, b lank was prepared simultaneously. The intensity of blue colored was measured at 660 nm after 30 min of incubation (Lowry *et al*., 1951).

Estimation of reducing sugar: Pipetted out 0.5 to 3 mL of the extract in test tubes and equalized the volume to 3 mL with water in all the test tubes. Added 3 mL of DNS reagent and boiling in water bath for 5 m. Added 1 mL of 40% Rochelle salt solution. After cooling the intensity of dark red color was measured at 510 nm (Miller, 1959).

Estimation of total carbohydrates: Different concentration of glucose (0.2, 0.4, 0.6, 0.8 and 1 mL), 0.5 and 1 mL of the aliquots was taken in various test tubes. All the tubes were made up to 1 mL with distilled water. Four milliliter of anthrone reagent was added to all the test tubes. Blank was prepared simultaneously. Cool rapidly and the intensity of dark green color was measured at 630 nm (Yemm and Fokes, 1954).

RESULTS

Estimation of protein: Protein content was found to be higher in good rooting clones when compared to poor rooting clones. The t-test perform could not reveal any significant differences between the good rooting and poor rooting clones. In good rooting clones the quantity of protein varied from 20.24 to 64.88 mg g^{-1} of cladode tissue whereas in poor rooting clone it ranged between 37.48 to 53.59 mg g^{-1} of cladode tissue. The results revealed that protein content could not be used as a biochemical indicator for rooting. The results on protein estimation by Lowry's method are presented in Table 1.

Estimation of reducing sugar: In good rooting clones the values ranged from 6.99 to 12.68 mg g^{-1} of cladode tissue. In poor rooting clones the amount of reducing sugar varied from 6.86 to 10.19 mg g^{-1} of cladode tissues. Significance difference between the values of reducing sugar was noticed when the data were subjected to t-test. The results revealed that reducing sugar could be used as a biochemical indicator for rooting. The results are presented in Table 2.

Estimation of carbohydrates: The data on estimation of carbohydrates was carried out by Anthrone Method are given in the Table 3. The T-test performed revealed significant difference between both the groups of clones. For the good rooting clones the quantity of carbohydrates ranged from 20.09 to 58.59 mg g^{-1} of cladode tissue. The values varied from 32.52 to 73.36 mg g^{-1} of cladode tissue in poor rooting clones. The results revealed that carbohydrates content can be used as biochemical indicators for rooting. Lower carbohydrate content was found to be beneficial for rooting.

Table 1: Protein estimation by Lowry's method

Results	Amount of protein (Mg g^{-1}) in cladode tissue^{NS}
Clones which showed good rooting ability	
G1	31.83±0
G2	22.50±1.47
G3	30.98±0
G4	20.24±0.98
G5	46.24±0
G6	64.88±0
G7	49.91±2.45
G8	38.33±1.96
G9	46.80±0.09
G10	41.71±0.99
Clones which showed poor rooting ability	
P1	47.94±0
P2	53.59±3.92
P3	50.76±0.98
P4	38.89±1.95
P5	42.00±0
P6	34.65±0.49
P7	40.31±1.47
P8	49.37±0.44
P9	47.83±0
P10	37.48±2.45

^{NS}: Not significantly different from the corresponding observation as per t-test ($p \leq 0.05$)

Table 2: Estimation of reducing sugar by dinitrosalicylic acid method

Results	Amount of reducing sugar (mg g^{-1}) in cladode tissue*
Clones which showed good rooting ability	
G1	10.81±0.34
G2	9.84±0.34
G3	12.68±0.78
G4	9.06±0
G5	10.03±0
G6	6.99±0.11
G7	9.58±0.11
G8	10.74±0.11
G9	10.29±0.11
G10	11.58±0
Clones which showed poor rooting ability	
P1	7.51±0
P2	9.45±0
P3	7.12±0
P4	9.84±0
P5	7.12±0
P6	9.26±0
P7	6.99±0.11
P8	9.64±0
P9	6.87±0.11
P10	10.19±0.08

*Significantly different from the corresponding observation as per t-test ($p \leq 0.05$)

Table 3: Estimation of carbohydrates by anthrone method

Results	Amount of carbohydrates in (mg g^{-1} Good rooting ability) in cladode tissue*
Clones which showed good rooting ability	
G1	26.31±2.15
G2	41.83±3.23
G3	58.59±0
G4	48.66±1.07
G5	28.17±2.15
G6	31.27±1.08
G7	20.09±2.15
G8	44.31±2.15
G9	41.83±3.23
G10	43.69±0
Clones which showed poor rooting ability	
P1	32.52±3.23
P2	43.07±8.60
P3	56.78±0
P4	73.36±0
P5	69.77±0
P6	56.73±0
P7	41.83±0
P8	60.45±0
P9	54.87±0
P10	54.87±0

*Significantly different from the corresponding observation as per t-test ($p \leq 0.05$)

DISCUSSION

The current study is focused to understand the biochemical aspects behind rooting of cuttings. Biochemical indicators of *Casuarina equisetifolia* revealed significant differences between the good rooting and poor rooting clones with respective Starch, Amylase, reducing sugar and carbohydrates (Satyavani, 2009). The results revealed that protein content could not be used as a biochemical indicator for rooting. The Biochemical studies were conducted to analyze the characteristics of juveline and adult tissue of *Casuarina equisetifolia*. Changes in the protein profile of Total Soluble Protein (TSP), peroxidase (PO) and polyphenoloxidase (PPO) activities in leaves and buds of olive trees (cv. Zard) from the Gilvan region were studied by Motamed *et al*. (2007), as a result an increase in TSP content for leaves and buds was noticed during fruit ripening. A close relationship between enzymes, specific protein and the process of fruit development could be observed. In all cases, protein content and both enzyme activities in buds were higher than leaves during the growth and development if olive trees. Abdala *et al*. (2002) analyzed the genetic variation of seeds from 20 trees of Aroeira (*Myracrodruon urundeuva*). Reflectance spectrometry methods were used for estimating the foliar biochemical concentration of ground tree leaves. The concentration of 12 foliar biochemical such as chlorophyll a, chlorophyll b, total chlorophyll, lignin, nitrogen, water, cellulose, phosphorous, protein, amino acids, sugar and starch was estimated. As a result the 12 foliar biochemical concentrations were found to be high (Paul *et al*. 1999). Fifty three clones of *Populs deltoides* were collected and quantitatively estimated the moisture, polyphenol, protein, nitrogen, potassium and sodium. Significant variation ($p<0.01$) was observed in protein content among selected clones. Maximum amount of protein was found in clone LL88/89 (11.68%) followed by clone 6402 (11.44%) while minimum protein content was recorded as (1.0%) (Jain and Smita, 2002). A wide range in rooting capability of *Persea americana* Mill was resulted the carbohydrate content in leaves and cutting bases at the beginning was not associated with rooting capability. Starch accumulation at the cutting bases occurred while cuttings were correlated with the rooting capability (Reuveni and Raviv, 1980). The present results revealed that reducing sugar could be used as a biochemical indicator for rooting. An increasing in the level of reducing sugar was reported in the pre-girdled tissues of *Heritiera littoralis* at initiation was well as at subsequent stages of root development, which was further enhanced by the use of auxins, a decrease in the total sugar, carbohydrates and polyphenols and an increase in the total nitrogen and a high C/N ratio were noted at the root initiation stage in both species. Interaction of IBA and NAA promoted starch hydrolysis during root development and subsequently reduced the C/N ratio and increase the protein-nitrogen activity during the development of root primordial (Das *et al*., 1997). The results revealed that carbohydrates content of our study can be used as biochemical indicators for rooting. Carbohydrate composition of nodules of soybean plants (*Glycine max* L. Merr.) roots and leaf blades was determined and resulted nitrate inhibits nodule growth and activity by reducing the accumulation of carbohydrate in nodules (Streeter, 1981). The biochemical composition of Mother Vines plays an important role in propagation growth and development. Each root stock has it own inherent capacity to synthesis the biochemical constituents which influence scion physiology either directly or indirectly after grafting (Satisha *et al*., 2007). Marmit and Sharma (2008) observed significantly higher contents of total sugar, starch, alpha- amylase and invertase enzymes activity and lower content of reducing sugar in *Magnifera indica* subsequent to gall induction. Further more, it would be much more valuable if biochemical indicators are specified for individual species rather than generalized for all species.

CONCLUSION

The present investigation was conducted at Institute of Forest Genetics and Tree Breeding (Indian Council of

Forestry Research and Education) Coimbatore to find out biochemical indicators if any for rooting. Ten good rooting and ten poor rooting clones were selected for the study. Cladode tissue collected from ten good rooting and ten poor rooting clones were subject to various biochemical analysis including protein, reducing sugar and carbohydrates. The replicated (3) data were subjected to T-test. The results of the analyses revealed significantly differences between the good rooting and poor rooting clones with respective reducing sugar and carbohydrates. Therefore reducing sugar (higher the content better rooting) and carbohydrates (lesser the content better rooting) can be used as a biochemical indicators for rooting in *Casuarina equisetifolia.*

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