

Effect of Biochemical Indicators for Rooting in Horsetail Trees (*Casuarina equisetifolia* L.)

¹K. Satyavani, ²Kannan C.S. Warriar, ¹S. Gurudeeban and ¹T. Ramanathan

¹Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, India

²Institute of Forest Genetics and Tree Breeding, Coimbatore 641002, Tamil Nadu, India

Abstract: *Casuarina equisetifolia* Linn. is native from Australia Eastward into Melanesia, extotic trees in peninsular India and coastal Southeast Asia. The present investigation was carried out ten good rooting and poor rooting clones of *Casuarina equisetifolia* were subjected to various biochemical analysis including starch, amylase, peroxidase and IAA oxidase. The result of the analysis revealed significant difference ($P < 0.05$) between the good rooting and poor rooting clones. Starch was found to be lower in (10.16 to 39.97 mg g⁻¹) better rooting clones, but greater in poor rooting clones (36.86 to 82.19 mg g⁻¹). Similarly lesser content of amylase (0.003 to 0.065 mg g⁻¹) were also present in good rooting as compared with poor rooting clones (0.033 to 0.080 mg g⁻¹ of cladode tissue). Results also revealed that peroxidase and IAA oxidase did not exhibit any significant difference between the good rooting and poor rooting clones. Therefore, it can be concluded that starch and amylase plays a major role in rooting of *Casuarina equisetifolia*.

Key words: Amylase • Australian pine • Cladode tissues • Anthrone method

INTRODUCTION

Casuarina equisetifolia L. is the wide spread exotic trees in peninsular Indian and well known member of the family Casuarinaceae. Nearly 86 species have been recognized so far [1]. Casuarinas are deep rooted and roots are found to have been arranged in clusters around the tap root. 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. It is extensively cultivated for fuel consumption (calorific value of 4950 k.cal/kg), erosion control and manufacturing charcoal, source of pulp for production of papers and rayon. Its bark is widely used in traditional medicines for treating digestive track problems. Clonal variation can also be observed with respect to rooting [2]. *Casuarina equisetifolia* can be propagated by seed, stem cuttings and air layering. Rooting generally occurs in 15 to 20 days of time. Research revealed that basal portion from the cutting can cut and could be 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 seed, the total sugars also varied (26.5 -46.3 mg/g of seed) and the starch content varied from 0.35-1.58 mg g⁻¹ of seed [3]. 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 [4]. GA₃ 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 [5]. The present investigations are therefore carried out about the biochemical indicators for rooting between the good and poor rooting clones of *Casuarina equisetifolia*.

MATERIALS AND METHODS

Plant Material: The cladode tissues of 20 different clones were collected and authenticated from the plant nursery of Institute of Forest Genetics and Tree Breeding (ICFRandE), Coimbatore, Tamil Nadu and India.

Preparation of Plant Extract: Extraction was usually carried out with different type of buffer for experiment (0.1 M phosphate buffer, 80% ethanol, 25mM phosphate buffer and cold acetone) centrifuged and then supernatant was taken for the estimation.

Estimation of Starch: 0.8 ml of glucose with 0.2 ml of supernatant was taken in test tubes. Then added 4ml of anthrone reagent and heated for 8 mints. After cooling, the intensity of dark green color was measured at 630nm. It was followed by the method of King [6].

Estimation of Amylase: 1ml of starch solution diluted with 1ml of enzyme in a test tubes. Incubated at 27°C for 15m. The reaction was stopped by addition of 2ml of DNS. 1ml potassium sodium tartrate solution added after heated for 5mints. After cooling the volume made up to 10 ml by addition of 6ml of water. Read the absorbance at 560nm following the procedure described by [7].

Estimation of Peroxidase: Peroxidase estimation was followed by the method of Satisha *et al.*, [8]. Simply, 3ml of buffer solution, 0.05ml of guaiacol solution, 0.1 ml of enzyme extract and 0.03 ml hydrogen peroxide was pipetted in a cuvette. Bring the buffer solution to 25°C before assay. Mixed well and placed the cuvette in spectrophotometer until the absorbance has increased by 0.05.

Estimation of Indole Acetic Acid Oxidase: Pipette out 2ml of phosphate buffer (pH 6.2), 1ml of paracoumeric acid, 1ml of manganese chloride and 2ml of enzyme extract. Started the reaction by addition of 4ml IAA solution and incubated in dark after shaking at 30°C. Withdrawn 2ml of the mixture after 0 and 50m of incubation and added 5.2 ml perchloric acid and 0.5ml ferric nitrate solution. After diluted with 10ml distilled water and read the absorbance at 535nm. Significance of the treatment effects was determined using analysis of variance (ANOVA, $P \leq 0.05$) and comparison between mean values of treatments were made by Tukey's test

RESULTS

Starch Content in 20 Clones of C. Equisetifolia: The results on estimation of starch by Anthrone method is given in Table 1. Starch content was found to be lower in good rooting clones when compared to poor rooting clones. Significant difference could be observed between the two groups of clones when the data were subjected to

Table 1: Estimation of Starch in different Clones of *Casuarina equisetifolia*

S.No	Clones which showed good rooting ability	Amount of starch (mg/g) in cladode tissue
1.	G1	12.08 ±0
2.	G2	39.97 ±0
3.	G3	22.58 ±1.076
4.	G4	39.97 ±0
5.	G5	21.34 ±0
6.	G6	21.34 ±0
7.	G7	10.16 ±0
8.	G8	27.58 ±10.76
9.	G9	23.20 ±0
10.	G10	29.41 ±4.30
	Clones which showed poor rooting ability	Amount of starch (mg/g) in cladode tissue *
11.	P1	54.87 ±0
12.	P2	66.04 ±0
13.	P3	54.86 ±0
14.	P4	51.14 ±0
15.	P5	66.04 ±0
16.	P6	46.79 ±1.08
17.	P7	36.86 ±1.07
18.	P8	82.18 ±1.07
19.	P9	45.55 ±0
20.	P10	59.83 ±8.60

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

Table 2: Estimation of Starch in different Clones of *Casuarina equisetifolia*

S.No	Clones which showed good rooting ability	Amount of amylase (mg/g) in cladode tissue *
1.	G1	0.039 ±0
2.	G2	0.065 ±0
3.	G3	0.029 ±0
4.	G4	0.003 ±0
5.	G5	0.039 ±0
6.	G6	0.023 ±0
7.	G7	0.019 ±0
8.	G8	0.042 ±0
9.	G9	0.014 ±0
10.	G10	0.049 ±0
	Clones which showed poor rooting ability	Amount of amylase (mg/g) in cladode tissue *
11.	P1	0.043 ±0
12.	P2	0.069 ±0
13.	P3	0.059 ±0.01
14.	P4	0.049 ±0
15.	P5	0.056 ±0
16.	P6	0.070 ±0.
17.	P7	0.080 ±0
18.	P8	0.035 ±0.02
19.	P9	0.050 ±0.
20.	P10	0.033 ±0.0

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

Table 3: Estimation of Peroxidase in different Clones of *Casuarina equisetifolia*

S.No	Clones which showed good rooting ability	Amount of peroxidase in unit / litre ^{NS}
1.	G1	8.34
2.	G2	16.66
3.	G3	5.55
4.	G4	16.66
5.	G5	8.34
6.	G6	8.34
7.	G7	16.66
8.	G8	8.34
9.	G9	16.66
10.	G10	5.55
	Clones which showed poor rooting ability	Amount of peroxidase in unit / litre ^{NS}
11.	P1	16.66
12.	P2	4.17
13.	P3	5.55
14.	P4	16.66
15.	P5	16.66
16.	P6	8.34
17.	P7	16.66
18.	P8	8.34
19.	P9	16.66
20.	P10	8.34

NS: Not significantly different from the corresponding observation as per T- test ($P \leq 0.05$)

Table 4: Estimation of IAA Oxidase in different Clones of *Casuarina equisetifolia*

S.No	Clones which showed good rooting ability	Amount of IAA oxidase ($\text{min}^{-1} \text{mg}^{-1}$) in cladode tissue ^{NS}
1.	G1	0.092 \pm 0
2.	G2	0.120 \pm 0
3.	G3	0.106 \pm 0
4.	G4	0.101 \pm 0.02
5.	G5	0.092 \pm 0
6.	G6	0.069 \pm 0.01
7.	G7	0.149 \pm 0
8.	G8	0.092 \pm 0
9.	G9	0.078 \pm 0
10.	G10	0.120 \pm 0
	Clones which showed poor rooting ability	Amount of IAA oxidase ($\text{min}^{-1} \text{mg}^{-1}$) in cladode tissue ^{NS}
11.	P1	0.163 \pm 0
12.	P2	0.144 \pm 0.01
13.	P3	0.106 \pm 0
14.	P4	0.135 \pm 0
15.	P5	0.106 \pm 0
16.	P6	0.135 \pm 0
17.	P7	0.097 \pm 0.01
18.	P8	0.064 \pm 0
19.	P9	0.064 \pm 0
20.	P10	0.078 \pm 0

NS: Not significantly different from the corresponding observation as per T- test ($P \leq 0.05$).

Fig. 1: Clones and Cladode tissues of *Casuarina equisetifolia*

T- test. In good rooting clones the quantity of starch varied from 10.16 to 39.97 mg/g of cladode tissue whereas in poor rooting clone it ranged between 36.86 to 82.19 mg/g of cladode tissue. The results revealed that starch content can be used as a biochemical indicator for rooting.

Enzymatic Amylase Level in 20 Clones of *C. Equisetifolia*:

The quantity of amylase in good rooting clones varied between 0.003 to 0.070 mg/g of cladode tissue. In poor rooting clones the values ranged from 0.033 to 0.080 mg/g of cladode tissue. The amount of amylase was found to be lower in good rooting clone when compared to poor rooting clones in general. The T-test performed revealed significant difference between the two groups of clones. The results indicate that amylase can be used as a biochemical indicator for rooting. The results are presented in Table 2.

Enzymatic Peroxidase Level in 20 Clones of *C. Equisetifolia*:

The data on estimation of peroxidase are given in Table 3. The T-test could not revealed significant difference between the 2 groups of clones. The peroxidase activity ranged from 5.55 to 16.66 unit/liter of cladode tissue in good rooters whereas it varied from 4.17 to 16.66 unit/liter of cladode tissue in poor rooting clones. Among the poor rooting clones five out of ten clones recorded the maximum values of 16.66 unit / liter for peroxidase activity.

Enzymatic Iaa Oxidase in 20 Clones of *C. Equisetifolia*:

Table 4 represents the results on IAA oxidase activity in clones of *Casuarina equisetifolia*. The data were subjected to T-test could not reveal any significant differences between the good rooting and poor rooting

clones. Therefore, the IAA oxidase activity cannot be used as a biochemical indicator for rooting. In good rooting clones the values varied from 0.078 min⁻¹/mg to 0.149 min⁻¹/mg cladode tissue. In poor rooting clones the value different from 0.064 min⁻¹/mg to 0.163 min⁻¹/mg cladode tissue. The results for the present study indicate that the IAA oxidase cannot be used as a biochemical indicator for rooting.

DISCUSSION

The current study is focused to understand the biochemical aspects behind rooting of cuttings. Biochemical indicators of *Casuarina equisetifolia* revealed significant difference between the good and poor rooting clones with respective carbohydrate and reducing sugar [9]. Cladode cuttings of *Casuarinas* treated with 2000 ppm IBA. All candidate plus trees were rooted within 15 days. The rooting percentage of the clones varied from 6 to 95.33%. The average rooting potential of the clones was 57.06% and the average survival potential of clone was 70.52% [10]. The two genotypes of *Ebenus cretica* L. showed different rooting response when grown in green house after the application of 0.5g l⁻¹ IBA. The peroxidase activity increased during rooting process; as a result it could be used as an analytical criterion for predicting rooting ability of cutting in various clones of *Ebenus cretica* L. Peroxidase is involved in both root initiation and elongation process of *Jatropha curcas* [11], *Roystonea regia*, a big tryptophan pool (1555.1° g/g fresh mass) was found in the root nodules, which might serve as a source of IAA production. The presence of IAA-metabolizing enzymes, IAA oxidase and peroxidase indicated metabolism of IAA in the root nodules [12]. Also examined the presence of phenolic compounds, peroxidase, polyphenols oxidase and IAA oxidase activities in the corm and apical bud of *Crocus sativus* L. Moreover the content of phenolics and IAA in the corm tissue during flower formation and growth were higher than at the lower developmental stage [13]. The quantitative analysis of total phenolic contents also revealed that old leaves contained high level of phenolic con as compared to old stem, while young leaves and stem showed mix trend towards the total phenolic contents [14]. The leaves of haploid tobacco plants recorded lower free IAA level (by 40%, higher peroxidase level (by 160%) and IAA oxidase (by 70%) and produce less ethylene (by 25%) than leaves of corresponding

diploid plants [15]. The increase in the peroxidase activity in haploid was due to increase in the activity of the cathodic isozyme which is known to have high IAA oxidase activity. 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 an increase in TSP content for leaves and buds was noticed during fruit ripening.

CONCLUSIONS

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 Starch, Amylase, Peroxidase and IAA Oxidase. 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 their respective starch and amylase. Therefore, concluded that starch and amylase can be act as biochemical indicators for rooting of *C. equisetifolia*.

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