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Assessment of *Casuarina equisetifolia* Forst. Clones against Blister Bark Disease Resistance

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Abstract

Blister bark disease, a serious disease that usually found in young plantations of *Casuarina equisetifolia* Forst. caused by *Trichosporium vesiculosum* Butl. In this study, 250 clones of *C. equisetifolia* have been screened to identify the blister bark disease resistance. The clones were vegetatively propagated and inoculated with the pathogen. The inoculated clones were assessed for disease resistance through a disease severity score under nursery conditions. Totally 37 clones are showed resistant and 55 clones showed moderately resistant. Rest of the other clones showed reaction to the disease. Analysis of total phenols for all the 250 clones was performed and found that total phenol content was directly related to disease resistance. The resistant clones of *C. equisetifolia* showed higher content of Phenols (20 to 26 ml g^{-1}), whereas the moderate resistant clones showed lower phenol content (11 to 18 ml g^{-1}) than resistant clones. The resistant clones showed higher phenol content that influenced the disease resistance against blister bark disease.

Key words: Blister bark, *Casuarina equisetifolia*, disease resistance, total phenols, *Trichosporium vesiculosum*

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Casuarina equisetifolia Frost. is an important multipurpose trees species mainly grown for scaffolding in building construction, paper pulp and fuel-wood. It plays a major role as a windbreak and shelterbelt along coasts in several tropical countries. Poles cut from the main stems are also extensively used for construction purposes. It is also a nitrogen fixing tree and grows up to 50m height. *C. equisetifolia* has been planted approximately 8,00,000 hectare in India and the annual production of pulp wood alone is 10 million tonnes worth of ₹ 200 crores and it was estimated that the yield is within 3.5 to 4 years (Nicodemus, 2009). At present the poles of *C. equisetifolia* costs ₹ 6000 per tonne in India. Due to its economical value farmers are interested in planting this tree as an agroforestry crop in Southern parts of India. However, blister bark disease is the most destructive disease of this tree caused by a hyphomycete fungus, *Trichosporium vesiculosum* Butl. and was first reported in India during 1905. This disease has been reported to cause large-scale mortalities in India as well as in China, Kenya, Thailand and Vietnam (Narayanan et al 2003). It is reported mostly from regions to which the species has been introduced. The pathogen attacks trees of different age causing large-scale mortalities, particularly in monoculture plantations. Mortality rates as high as 90 per cent have been reported from India and Vietnam (Sharma 1994). This fungus is able to multiply rapidly and spread over the main stem of the tree that causes death (Karthikeyan et al 2011).

Many plantations in Tamilnadu and Pondicherry (India) are affected by this dreadful disease because of the quick spread. Though much research has been carried out on the management of the disease by silvicultural practices, chemical and bio control agents, identification of blister bark resistant source is the permanent and long term solution to manage this disease. Hence, the study to screen 250 clones of *C. equisetifolia* to identify the resistant candidates against blister bark disease through artificial inoculation of *T. vesiculosum* was undertaken.

Materials and Methods

Collection of diseased sample. Diseased samples were collected from *C. equisetifolia* plantations in 3 different sites located at Panampally, Kerala (11° 700 N & 77° 713 E) Rameswaram, Tamilnadu (9.282° N & 79.301 E) and Tuticorin, Tamilnadu (8.811° N & 78.142°E).

Isolation of pathogen. Infected five bark samples were surface sterilized with 0.1 per cent of hydrogen peroxide placed in sterile potato dextrose agar (PDA) containing petri plates. Then they were incubated at 32 C in the Orbitol Shaker (Gyromax 703 R, USA) and rotated at 20 rpm for 7 days. After 7 days white fungal mycelia growth have been noticed in Petri plates and the mycelia were again inoculated in PDA Petri plates for pure culture. The fungal cultures showed blackish brown and berry shaped conidia which are the specific character of *T. vesiculosum*. Thus obtained pure cultures of *T. vesiculosum* were maintained in potato dextrose agar

broth in the laboratory for further inoculations in to *C. equisetifolia* clones.

Vegetative multiplication of *C. equisetifolia* clones. Institute of Forest Genetics and Tree Breeding (IFGTB) Coimbatore has a plant germ plasm bank where 250 clones of *C. equisetifolia* housed. These clones have been used in this study. The stem cuttings of each clone were collected from the mother clone and the basal end of the stem cuttings have been cut crossed. Stem cuttings (5 cm length (± 0.43); 0.3 cm girth (±0.052)) of these clones were treated with 0.1 per cent carbendiazim fungicide for 3 min. After that the stem cuttings were treated with 2000 ppm of Indole Butyric Acid (IBA) (40mg IBA + 20 g of talcum powder) at the basal end of the cuttings for 0.5 min. Thus treated stem cuttings have been placed in 100cc root trainers filled with vermiculite and maintained in poly tunnels for 15 days.

Inoculation of *T. vesiculosum*. IBA treated clones of *C. equisetifolia* grown in 100 cc root trainers were inoculated with 5 ml *T. vesiculosum* culture (10^5 ml^{-1} contains 1.2µg protein ml^{-1}) by making an injury at the collar region. The injured portion was sealed with thin parafilm to avoid contamination. Totally 250 clones were inoculated with *T. vesiculosum* and each clone was replicated at 15 times. All the clones were maintained in a chamber with a dimension of 90 x 60 x 45 cm made with aluminum angles and covered with a polythene sheet on all sides except the base. This chamber was maintained to create a desired humidity (75 %) for the inoculated clones for 30 days. After 30 days, the pathogen inoculated clones were assessed for disease severity index scale (score 0 = No symptoms; 1= Needle leaves showing shrunken, stem showing healthy, Moderately resistant; 2=Needle leaves showing partially drying and brittle, Susceptible; 3= Needle leaves completely dead and necrotic roots, Highly susceptible;)

Assessment of total phenol. After the assessment of disease severity of *C .equisetifolia* clones, the resistant clones were collected and analyzed for the phenol estimation test using Folin and Ciocalteu (1927). Standard Error SE (±) was performed on the data of total phenol content of all clones.

Results and Discussion

Symptoms of blister bark disease developed after 30 days on the bark and needles of the clones inoculated with *T. vesiculosum*. The bark starts peeling out and showed black spores of *T. vesiculosum*. These symptoms were exactly matched with the infections of diseased trees found grown in field (Fig.1 A and B). The needles showed brownish in the infected clones and starts drying whereas the resistant clones showed healthy and greenish needles.

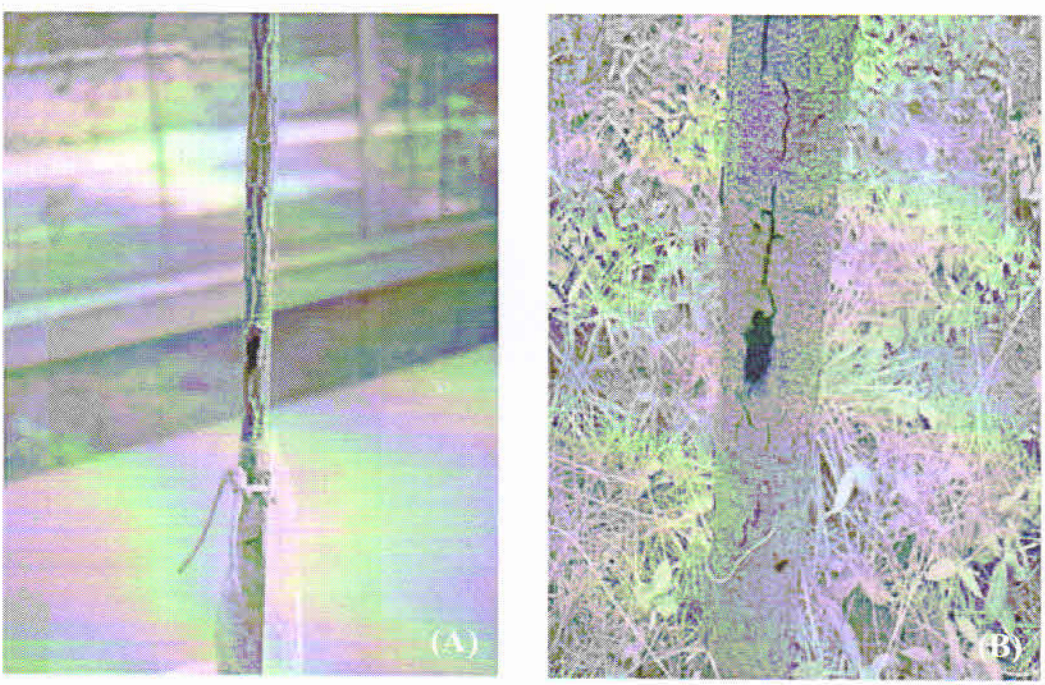

Figure 1. (A) Development of Blister bark disease symptoms in a clone of *C. equisetifolia* after 30 days of artificial inoculation of *T. vesiculosum*; (B) Blister bark diseased *C. equisetifolia* tree at the age of 3 years

Totally 159 clones out of 250 showed black pustules and brownish needles after 30 days of inoculation and they were scored as 2 or 3. These clones were notified as non resistant clones whereas 55 clones showed only wilting and withering of needles and scored as 1. These clones were considered as moderately resistant clones (Table 1). Thirty seven clones showed zero symptoms and they have been considered as resistant clones after 60 days of inoculation of *T. vesiculosum* (Table 2). This screening method against blister bark disease is found on par with the method of Harsh et al (2006) in *Dalbergia sissoo* against rust disease. Sun et al (2014) also suggested to screen the disease resistant clones by artificial inoculation method in nursery. Huberli et al (2002) found disease resistant clones of *Eucalyptus marginata* against *Phytophthora cinnamomi* causing die back disease. Ke et al (1994) screened 201 progeny clones of *C. equisetifolia* and found disease resistance against bacterial wilt. These studies were supported present study on identification of resistance clones against blister bark disease. The resistant clones of *C. equisetifolia* showed higher content of phenols (20 to 26 ml g^{-1}) whereas the moderate resistant clones showed lower phenol content (11 to 18 ml g^{-1}) than resistant clones (Table 1 and 2). Phenolic compounds are synthesized as secondary metabolites during normal development in response to stress conditions, such as wounding and UV radiation among others, Singer et al, 2003) thus protects the plants from pathogens. The presence of phenolic compounds in injured plants may also have an important effect on the oxidative stability and microbial safety. Because of these reasons 36 clones found resistance against blister bark disease. Venalaninan et al (2003) found disease resistance clones in *Painus sylvestris* against heart rot disease caused by *Coniophora puteana* as the clones had higher content phenolic compounds of Taxifolin and Taxifolin glucoside. *Salix myrsinifolia* clones were screened against *Melampsora* leaf spot disease and found that the resistant clones had higher content of phenolic compounds of Luteolin 7 glucoside (Hakinlinen and Julkanen Tittoo, 2000). Pusler et al (1995) found disease resistance clones of *Populus deltoides* against stem gall disease caused by *Phellinus tremulae* as the reason the clones had higher content of phenolic compounds of benzoic acid. Sun et al (2014) found seven *C. equisetifolia* clones resistant to wilt disease caused by *Ralstonia solanacearum* in similar lines.

High incidences of blister bark disease and heavy mortalities in *C. equisetifolia* plantations have been reported from several locations in India, Vietnam and Thailand (Jamaluddin 1998). Heavy mortality of six year old Casuarinas hybrid (*C. equisetifolia* x *C. junghuniana*) trees caused by blister bark disease was

Table 1. Total phenol content analyzed in moderately resistant clones (mean of 15 replicates) ± SE of mean

S N	Clone Number	Phenol content mg g^{-1}
1	TNRM-1	11.9066 ± 1.484
2	TNRM-3	17.533 ± 2.2368
3	TNRM-5	16.8333 ± 1.875
4	TNRM-6	12.25 ± 0.433
5	TNCN-1	16.8333 ± 1.875
6	TNAM-1	15.5343 ± 1.322
7	TNAM-2	17.5 ± 1.181
8	TNVM-2	16.8333 ± 1.2829
9	TNIPT-3	12.25 ± 0.433
10	TNIPT-4	17.533 ± 2.2368
11	TNIPT-5	16.5 ± 1.5
12	TNIPT-6	18.5 ± 1.089
13	TNIPT-8	18.916 ± 0.8779
14	TNIPT-9	17.75 ± 1.639
15	TNIPT-15	16.5833 ± 0.520
16	TNIPT-17	17.75 ± 0.6614
17	TNIPT-21	17.533 ± 2.236
18	PYP 2	16.833 ± 1.5
19	PYF 2	17.1666 ± 0.2886
20	PYX 2	16.75 ± 1.639
21	PYO 2	17.4166 ± 1.233
22	PYN	17.532 ± 1.125
23	PY 20	15.3166 ± 1.058
24	PY 130	16.2316 ± 1.116
25	PY 131	14.2312 ± 1223
26	TNIPT-18	15.689 ± 1.324
27	TNIPT-19	14.7532 ± 1.453
28	TN 501	14.6235 ± 1.13
29	TN 503	13.5678 ± 1.233
30	TN 504	15.5527 ± 1.253
31	TNBS-6	16.756 ± 1.321
32	TNBS-7	15.653 ± 1.263
33	TNBS-8	16.6325 ± 1.256
34	APKKD-1	14.365 ± 1.123
35	APKKD-3	12.356 ± 1.235
36	APVSP-14	11.3372 ± 1166
37	APVSP-15	14.667 ± 1.222
38	APVSP- 16	13268 ± 1.007
39	APSKLM-25	15.666 ± 1.221
40	APSKLM-26	15.367 ± 1.144
41	APSKLM-27	14.332 ± 1.121
42	CE 2002/2	14.645 ± 1.212
43	CE 2003/3	16.643 ± 1.256
44	CE 2003/4	13.652 ± 1.420

Contd.....

Table 1. Continued

S N	Clone Number	Phenol content mg g^{-1}
45	TCR 120102	17.633 ± 1.52
46	TCR 030202	17.236 ± 1.563
47	TCR 120203	16.4632 ± 1.510
48	TCR 060101	17.336 ± 1.369
49	TMPK- 4-15	16.4563 ± 1.265
50	APKKD-4	14.523 ± 1.652
51	APKKD-5	14.6321 ± 1.23
52	APKKD-6	17.124 ± 1.2673
53	APVSP-14	16.1453 ± 1.2741
54	APVSP-22	16.8754 ± 1.268
55	APSKLM 32	13..763 ± 1.117

Table 2. Total phenol content analyzed in resistant clones (mean of 15 replicates) ± SE of mean

S N	Clone Number	Phenol content mg g^{-1}
1	TNRM-4	23.5833 ± 1.376
2	TNRM-8	25.1666 ± 0.6291
3	TNIPT-7	26 ± 0.542
4	TNIPT-10	25.5 ± 1.322
5	TNIPT-11	21.5833 ± 1.233
6	TNIPT-12	20.933 ± 0.9878
7	TNIPT-13	26.416 ± 1.258
8	PY 157	21.5833 ± 3.8837
9	PY 75	26.75 ± 0.6614
10	PY 170	23.0833 ± 2.126
11	PY 171	25.1666 ± 0.6291
12	TN 502	23.5833 ± 1.376
13	TN 506	26.75 ± 0.6614
14	TNBS-1	23.5833 ± 1.376
15	TNBS-2	21 ± 3.3634
16	APKKD-4	20.7 ± 0.2408
17	APKKD-5	20.321 ± 1.554
18	APKKD-6	21.666 ± 1.7736
19	APKKD-7	20.933 ± 0.987
20	CE 100	26.5833 ± 0.6291
21	APVJM-32	21.75 ± 0.6614
22	CE-73	23.583 ± 1.233
23	CE-71	25.5146 ± 1.004
24	CE 79	21.916 ± 1.0103
25	TCR 020501	25.5 ± 0.661
26	TCR 050203	21.9166 ± 0.8779
27	TMPP-4-13	21.58333 ± 1.2332
28	TMMT-2-15	21.916 ± 0.8779
29	PY 119-14	25.416 ± 1.181
30	PY 116-15	21.9166 ± 1.01036
31	TMIPT-7-15	19.9333 ± 2.5740
32	TMIPT-5-15	22.583 ± 0.5204
33	TNIPT-12-10	21.2666 ± 1.061
34	TMIPT-8-15	21.25 ± 1.089
35	APKKD-9	20.666 ± 0.629
36	APVSP 23	21.333 ± 0.381
37	APSKLM-30	20.232 ± 0.1231

Contd.,...

also reported (Narayanan et al 1998). It was observed that *C. equisetifolia* plantations of different age were found affected and the disease has been also reported in 17 year old trees (Sharma, 1994). Hence, the disease resistant clones are the permanent solution to overcome the dreadful diseases like blister bark for better productivity.

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