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Bio-chemical screening of the clones of *Nothapodytes nimmoniana* (an anti-cancerous drug yielding tree), for leaf spot disease resistance

Shwetha VR, Gurudatt M Hegde and R Vasudeva

Abstract
The clonal variation of reducing sugars and phenols in 44 different clones of *Cylindrosporium* leaf spot affected *Nothapodytes nimmoniana* (anti-cancerous drug yielding tree) have been investigated. Based on the per cent disease index the clones were classified into highly resistant (0%), resistant (1-15%), moderately resistant (21-35%), susceptible (36-50%) and highly susceptible (>50%). The results indicated the strong association between biochemical content and disease resistance. In moderately resistant clones (clone-P_x and clone-O) reducing sugar content was 78.23 per cent and 72.33 per cent respectively. Whereas, reducing sugar content in check (healthy) plant was as high as 90 per cent. Similarly in phenol estimation moderately resistant reaction two clones *viz.* P_x and O have shown highest per cent of phenol content, 51.80 and 53.73 per cent respectively. However, the phenol content in check (healthy) plant was recorded to be 71.40 per cent.

Keywords: bio-chemical screening, clones of *Nothapodytes nimmoniana*, leaf spot disease resistance

Introduction
Medicinal plants are widely used to treat diseases but in recent decades medicinal trees are threatened by many stress factors. One of the major threats to such reserves is diseases by plant pathogens. The most important medicinal tree facing severe disease is *Nothapodytes nimmoniana*. This plant has anti- cancer properties which has made it an endangered species. It is being exploited clandestinely in the domestic market and is also shipped abroad. The profit potential is enormous as the alkaloid camptothecin is extracted from the plant. Alkaloid content (CPT) is maximum in various parts, but this species is suffering from *cylindrosporium* leaf spot disease which is damaging the economic part of the tree. The information on leaf spot disease of this species is very limited and there is no systematic work done on various aspects of this disease. Hence, the biochemical analysis of the available clones was undertaken.
Plants need bochemical compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. Plants synthesize a greater array of secondary compounds than animals because they cannot rely on physical mobility to escape their predators and have therefore evolved a chemical defence against such predators (Kolb *et al.*, 2001) [4]. In most plants which are affected by fungal pathogens, a high level of reducing sugars and phenols in tissues enhances resistance. Several hypotheses have been proposed to explain the mechanisms of high biochemical resistance. Sugars and phenols constitute the primary substrate providing energy and structural material for defence responses in plants, while they may also act as signal molecules interacting with the hormonal signalling network regulating the plant immune system (Varonica *et al.*, 2006) [9].

Material and Methods
Forty four clones of *N. nimmoniana* maintained in the clonal bank of department of Forest Biology and Tree Improvement at College of forestry, Sirsi were selected for screening against leaf spot disease. The screening for disease was conducted for two consecutive years (2015-16 and 2016-17) during high disease pressure period (October-December). The severity or

percent disease index of various clones was recorded by using 0-5 scale developed by Sharma and Mohanan, 1984 [7]. Based on the per cent disease index the clones were classified into highly resistant (0%), resistant (1-15%), moderately resistant (21-35%), susceptible (36-50%) and highly susceptible (>50%) (Table 1).

Table 1: Grouping of *N. nimmoniana* clones into HR, R, MR, S and HS against leaf spot disease

Sl. No.	Reaction	Clones Responded	Number of Clones
1	Highly resistant (HR)	0	0
2	Resistant (R)	0	0
3	Moderately resistant (MR)	P_x and O	2
4	Susceptible (S)	D, Z, Y, B, N_0, EBR_2, and A	7
5	Highly susceptible (HS)	R, E, C, A_1, V, U, Q, K, B_1, EBR_4,N_X, T, I, P_0, M, X, S, L, F,W, N_R,G, H, N_2, N_{11}R, J, PN_{15}, EBR_1, EBR_3, P_4, NR_1, P_2, N_9, N_{15}, N_8	35

Recording of observations

Per cent disease index (PDI) for all the clones were calculated, with the help of formula given by Wheeler (1969).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum grade used}} \times 100$$

Estimation of reducing sugars in diseased clones by Nelson- Somogyi's method (Nelson and Somogyi, 1945) [6]

Leaf samples were cut into small bits and grounded with alcohol in pezzele and mortar. Leaf sample was grounded to fine paste and homogenised with 80% alcohol. Obtained filtrate was pooled and made clear solution by adding lead acetate. Supernatant was taken and used as sample. In a series of labelled test tubes, aliquots of working standard (10-100 µg) were pipetted out and made up to 1 ml with distilled water. A reagent blank was maintained with 1 ml of distilled water. One milliliter of freshly prepared alkaline copper reagent was added to all the tubes including reagent blank, mixed well and placed in boiling water bath for exactly 20 mins. Then test tubes were cooled under tap without shaking and 1 ml of arsenomolybdate reagent was added to all the test tubes and mixed immediately till the effervescence stops. Then the volume was made up to 15 ml with distilled water and read at 510 nm against reagent blank set to 100% transmittance. The amount of reduced sugar presented in the sample was calculated from the standard graph of D-glucose and the values were expressed in percentage.

Estimation of phenols in diseased clones by Folin-Ciocalteau method (Bray and Thorpe, 1954) [2]

In a series of labelled test tubes, working standard solution containing 10-50 µg standard catechol was pipetted out and the volume was made up to 1 ml in all the test tubes. A blank with 1 ml distilled water was maintained. One ml of 1N FCR was added to all the test tubes, after shaking thoroughly 2 ml of sodium carbonate solution was added to all the test tubes and mixed well. Then the tubes were placed in boiling water bath for exactly 1 min, cooled and made up to 15 ml. the per cent transmittance of the standard and samples were read against reagent blank which was adjusted to 100% Transmittance at 650 nm. The total phenol content was calculated from standard graph and expressed in per cent.

Result and Discussion

Reducing sugar

Table 2: Biochemical content (Reducing sugar) in leaf spot affected clones of *N. nimmoniana*

Sl. No.	Clone ID	Reducing Sugars (%)	Sl. No.	Clone ID	Reducing Sugars (%)
1	P_X	78.33 (62.26)*	23	P_0	23.47 (28.98)*
2	O	72.33 (58.26)	24	M	19.97 (26.54)
3	D	55.33 (48.06)	25	X	19.80 (26.42)
4	Z	54.47 (47.56)	26	S	19.13 (25.94)
5	Y	54.80 (47.75)	27	L	18.83 (25.72)
6	B	46.17 (42.80)	28	F	18.17 (25.23)
7	N_0	46.77 (43.15)	29	W	18.50 (25.47)
8	EBR_2	46.53 (43.01)	30	N_R	18.83 (25.72)
9	A	44.80 (42.02)	31	G	16.97 (24.33)
10	R	36.70 (37.29)	32	H	16.53 (23.99)
11	E	34.20 (35.79)	33	N_2	16.67 (24.10)
12	C	34.53 (35.99)	34	$N11_R$	16.07 (23.63)
13	A_1	32.77 (34.92)	35	J	15.40 (23.11)
14	V	34.33 (35.87)	36	PN_{15}	15.17 (22.92)
15	U	31.20 (33.96)	37	EBR_1	15.17 (22.92)
16	Q	33.93 (35.63)	38	EBR_3	14.63 (22.49)
17	K	36.27 (37.03)	39	P_4	14.73 (22.57)
18	B_1	33.87 (35.59)	40	NR_1	13.37 (21.45)
19	EBR_4	29.27 (32.75)	41	P_2	13.57 (21.62)
20	N_X	25.93 (30.61)	42	N_9	12.30 (20.53)
21	T	24.70 (29.80)	43	N_{15}	10.50 (18.91)
22	I	25.87 (30.57)	44	N_8	6.67 (14.97)
Healthy plant		90.00			
SEm±		1.09			
CD @ 1%		3.08			

*Figures in the parentheses are arcsine values

Reducing sugar content of all the 44 clones were analysed by following the standard procedures and the results are presented in Table-2. Significant difference was observed in reducing sugar content of leaves of different clones. In moderately resistant reaction two clones *viz.* P_x and O have shown highest per cent of reducing sugar, 78.32 and 72.33 per cent respectively. However, the sugar content in check (healthy) plant was recorded to be 90 per cent. In susceptible reaction the clones *viz.* D, Z, Y, B, N_0, EBR_2, and A have recorded the reducing sugar content of 55.33, 54.47, 54.80, 46.17, 46.53, and 44.80 respectively. In highly susceptible reaction (R, E, C, A_1, V, U, Q, K, B_1, EBR_4,N_X,T, I, P_0, M, X, S, L, F,W, N_R,G, H, N_2, N_{11}R, J, PN_{15}, EBR_1, EBR_3, P_4, NR_1, P_2, N_9, N_{15}, and N_8) per cent sugar content was ranged from 6.67 to 36.70.

These results indicate that the strong association exists between the per cent disease index and per cent sugar content in the clones (Fig-1). Higher the sugar content, higher will be the conversion of sugar to phenolics through shikimic acid pathway. Phosphoenol pyruvate formed in glycolysis reacts with Erythrose phosphate formed in Pentose pathway and results in formation of dehydroquinic acid which is highly toxic to pathogens (Naveenkumar and Naik, 2012) [5]. Higher sugar content in resistant clones might have imparted higher degree of resistance. And it may reduce the toxin production by pathogen which is essential for successful pathogenesis and also proved to inhibit the activity of pectolytic and cellulolytic enzymes secreted by pathogen. These results supported from the finding of Horsfall and Diamond (1957) and Bell (1981) [3, 1].

Phenols

Phenol content of all the 44 clones were analysed and the results are presented in Table- 3. Significant difference was observed in phenol content of leaves of different clones. In moderately resistant reaction two clones *viz.* P_x and O have shown highest per cent of phenol content, 51.80 and 53.73 per cent respectively. However, the phenol content in check (healthy) plant was recorded to be 71.40 per cent. In susceptible reaction the clones *viz.* D, Z, Y, B, N_0, EBR_2, and A have recorded 22.13, 25.23, 33.87, 23.40, 43.40, 26.77 and 23.00 per cent respectively. In highly susceptible clones (R, E, C, A_1, V, U, Q, K, B_1, EBR_4,N_X, T, I, P_0, M, X, S, L, F,W, N_R,G, H, N_2, N_{11}R, J, PN_{15}, EBR_1, EBR_3, P_4, NR_1, P_2, N_9, N_{15}, and N_8) per cent phenol content was ranged from3.87 to 25.53.These results indicate that the strong association exists between the per cent disease index and per cent phenol content in the clones (Fig-2). Varying quantity of phenol content in different clones may be due to constitutive makeup of different clones, which decides varying levels of resistance. In shikimic acid pathway, phenolics may be produced in higher quantity from different metabolisms after infection which indicates that higher constitutive phenol and in plants may offer greater resistane (Umesh Kumar, 1990) [8].

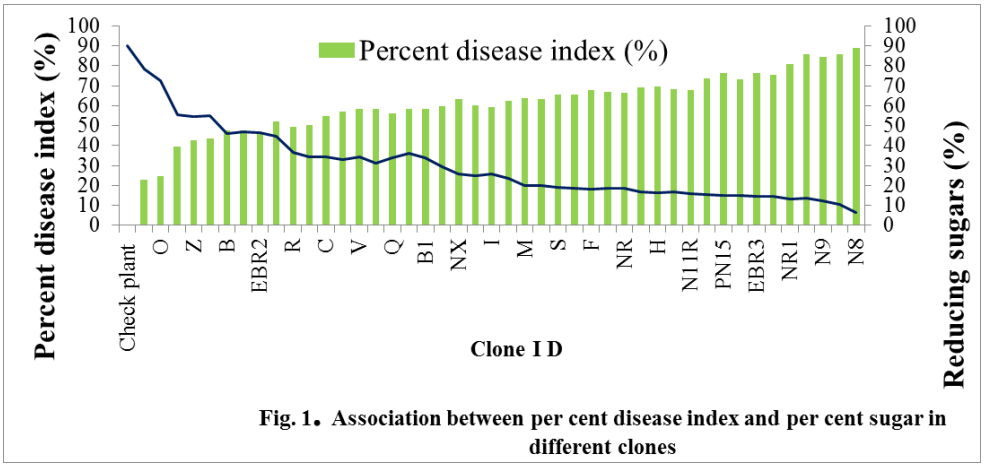

Fig. 1. Association between per cent disease index and per cent sugar in different clones

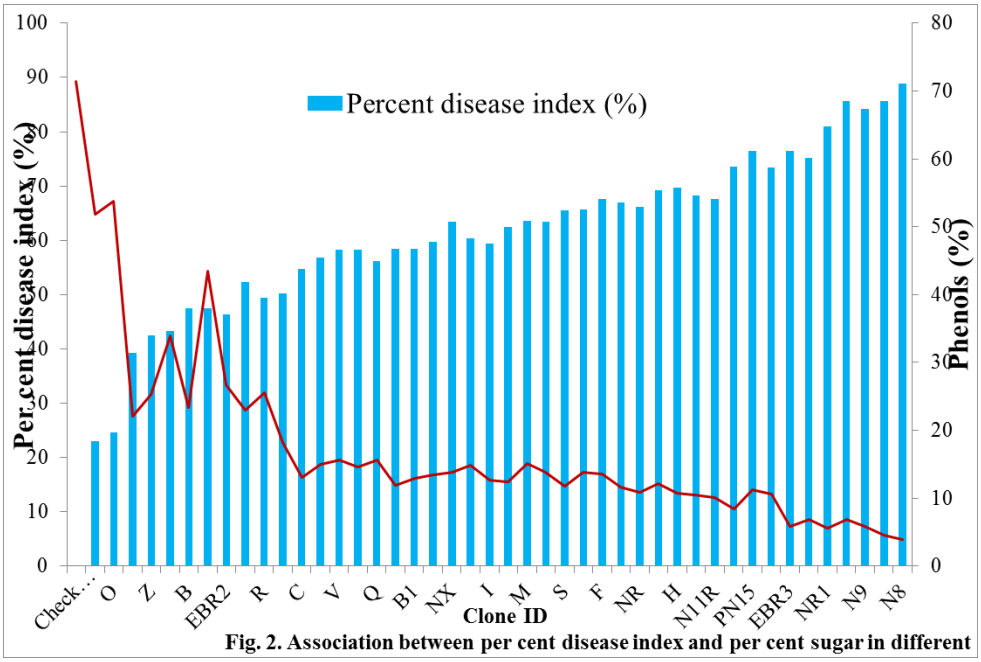

Fig. 2. Association between per cent disease index and per cent sugar in different

Table 3: Biochemical content (Phenol) in leaf spot affected clones of *N. nimmoniana*

Sl. No.	Clones	Phenol (%)	Sl. No.	Clones	Phenol (%)
1	P_X	51.80 (46.03)*	23	P_0	12.47 (20.68)*
2	O	53.73 (47.14)	24	M	15.07 (22.84)
3	D	22.13 (28.06)	25	X	13.87 (21.87)
4	Z	25.23 (30.15)	26	S	11.73 (20.03)
5	Y	33.87 (35.59)	27	L	13.80 (21.81)
6	B	23.40 (28.93)	28	F	13.60 (21.64)
7	N_0	43.40 (41.21)	29	W	11.67 (19.98)
8	EBR_2	26.77 (31.16)	30	N_R	10.87 (19.25)
9	A	23.00 (28.66)	31	G	12.20 (20.44)
10	R	25.53 (30.35)	32	H	10.73 (19.12)
11	E	18.27 (25.30)	33	N_2	10.53 (18.94)
12	C	13.07 (21.19)	34	$N11_R$	10.07 (18.50)
13	A_1	14.93 (22.73)	35	J	8.40 (16.85)
14	V	15.67 (23.32)	36	PN_{15}	11.27 (19.62)
15	U	14.60 (22.46)	37	EBR_1	10.67 (19.07)
16	Q	15.67 (23.32)	38	EBR_3	5.87 (14.02)
17	K	11.93 (20.21)	39	P_4	6.87 (15.20)
18	B_1	12.93 (21.07)	40	NR_1	5.53 (13.60)
19	EBR_4	13.40 (21.47)	41	P_2	6.87 (15.20)
20	N_X	13.87 (21.87)	42	N_9	5.80 (13.94)
21	T	14.80 (22.63)	43	N_{15}	4.60 (12.38)
22	I	12.73 (20.90)	44	N_8	3.87 (11.35)
Healthy plant		71.40			
SEm±		1.09			
CD @ 1%		3.08			

*Figures in the parentheses are arcsine values

Conclusion

In general where ever the disease index was low the per cent phenol and reducing sugar content were found higher. Per cent disease index and biochemical content in both infected and healthy leaves of different clones were strongly associated.

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