

Post-Infectional Biochemical Changes in *Cymbopogon martinii* (Roxb.) Wats and *Cymbopogon citratus* (DC) Stapf. Due to Leaf Rust Disease

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ABSTRACT

Post-infectional changes in sugars, ascorbic acid, protein, phenols, chlorophyll and carotene of two *Cymbopogons* viz., *Cymbopogon martinii* and *Cymbopogon citratus* due to leaf rust caused by *Puccinia nakanishikii* (Diet) were investigated. All the biochemical constituents decreased in plants infected by the fungal pathogen.

Keywords: Ascorbic Acid; Biochemical Changes; Carotene; Chlorophyll; Cymbopogon Martini; Cymbopogon Citrates; Phenols; Protein; Sugars

1. Introduction

Cymbopogon martinii (Roxb) Wats (Palmarosa) and *Cymbopogon citratus* (DC) Stapf. (Lemongrass) are essential oil bearing aromatic plants belonging to the genus *Cymbopogon*. The oil *Cymbopogon martinii* is used as the base for fine perfumery and is valued because of its geraniol contents. Besides the perfumery value, the oil has a great wound healing effect. *Cymbopogon citratus* is one of the sources of citral, an important monoterpene aldehyde, large quantity of which are being utilized for production of ionones, vitamin A and geraniol besides the use in perfumery soaps and cosmetics.

Although *C. citratus* and *C. martinii* are two economically important essential oil yielding grasses, due to the infection by rust fungus, these two species of *Cymbopogons* show serious losses in terms of herb yield, oil content and its quality. Rust fungi are obligate parasites and are highly destructive. Boruah and Bordoli [1] reported the pathogen associated with rust disease of *Cymbopogons* as *Puccinia nakanishikii* (Diet).

The ultimate impact of host-pathogen interaction is manifested in alteration of composition of nutritional and structural metabolites in the host [2]. Metabolic changes due to fungal infection have been reported in various plants [3-6]. The present investigation was undertaken to study the changes in sugars, ascorbic acid, protein, phenols, chlorophyll, carotene, respiration and transpiration

during pathogenesis in *Cymbopogon martinii* and *Cymbopogon citratus*.

2. Materials and Methods

2.1. Sugar

Following the Indole reaction method [7] both reducing and non-reducing sugars were estimated taking glucose as standard.

2 g of fresh leaf samples from respective cases were boiled in 20 ml of distilled water for 20 minutes. The extract was purified by treating with 5% ZnSO₄ and 5% Ba(OH)₂ solution and then filtered and volume was adjusted to 250 ml and further diluted to give readings at 480 nm with 1% Indole reagent. The estimation of sugars was carried out in a Unicam SP 600 series 2 Spectrophotometer. Non-reducing sugar was calculated by subtracting the value of reducing sugar from total sugar. Results were expressed as g of sugar per 100 g of fresh leaf.

2.2. Ascorbic Acid

Fresh leaves (5 g) were extracted with 5% metaphosphoric acid solution and made up to 50 ml. Ascorbic acid content was estimated volumetrically using 2,6-dichlorophenol indophenol as indicator [8].

2.3. Protein

Protein content of plant samples was estimated by the method of Lawry *et al.* [9] and Wildman and Jagendorf [10] using bovine albumin as standard.

2.4. Phenols

Phenolic compounds were extracted by boiling 5 g plant material with 80% alcohol for 5 min [11]. Total phenols in the alcohol extract were estimated by employing Folin-Ciocalteu reagent [12] and Catechol was used as standard.

2.5. Chlorophyll and Carotene

Chlorophyll & carotene contents were estimated by the following method [13]. 100 mg of fresh leaves from each variety were collected and extracted separately in 15 ml of acetone in a test tube by standing tubes overnight in dark. The extract was centrifuged at 5000 rpm for 15 minutes. The supernatant was taken and absorbance measurements were recorded at 662, 664 and 440.5 nm in a Unicam SP 600 Series 2 Spectrophotometer. The chlorophyll and carotene contents were calculated by incorporating the absorbance values into the following equation.

$$\text{Chl}_a = 9.78 A_{662} - 0.99 A_{664}$$

$$\text{Chl}_b = 21.40 A_{664} - 4.65 A_{662}$$

$$\text{Car} = 4.69 A_{440.5} - \text{Chl}_a + \text{Chl}_b \times 0.26$$

3. Results and Discussion

3.1. Sugar Content

The reducing and non-reducing sugar contents decreased in infected plants of the species from healthy ones (**Table 1**). Percent losses of reducing sugars were 45.312 and 45.454 in *C. martinii* and *C. citratus* respectively. Similarly, non-reducing sugars were recorded as 22.845% and 28.264% in *C. martinii* and *C. citratus* respectively.

A similar trend of decreased sugar levels in diseased plants was observed by Prasad *et al.* [5] and Nema [6]. The depletion of sugars during host-parasite interaction might be due to increased respiration or utilization of sugars by the fungi which depends on the capability of fungi to secrete carbohydrate degrading enzyme [5]. Nema [6] suggested that reduction in sugars during disease development might be due to utilization of sugars probably for energy and synthetic reactions involved in multiplication of the pathogen.

3.2. Ascorbic Acid Content

In infected plants ascorbic acid content was decreased than healthy ones in both the cases (**Table 1**). In *C. mar-*

tinii percent loss was recorded as 28.260 while in the case of *C. citratus* it was 27.272.

The decreased level of ascorbic acid in infected plants might be due to ascorbic acid degenerating enzymes either by the fungus alone or by the activity of the host-pathogen complex. Reddy *et al.* [3] observed a gradual loss in ascorbic acid content in infected fruits of acid lime (*Citrus aurantifolia*).

3.3. Protein Content

It was observed that higher amount of protein content was available during healthy stage which gradually decreased in diseased plants of both the species (**Table 1**). Result indicated that 32.197% of protein was lost due to infection in *C. martinii* while it was 32.207% in the case of *C. citratus*.

Decreasing in protein content might be due to degradation of the host proteins by the proteolytic enzymes secreted by the pathogens. Similar results were also reported by Prasad *et al.* [5] in muskmelon fruits infected with fruit-rot fungi.

3.4. Phenol Content

A decreasing manner in phenol content was observed in rust-infected plants in both the *Cymbopogons* (**Table 1**). Percent loss in phenol content was recorded as 25.093 and 29.859 in *C. martinii* and *C. citratus* respectively.

Khatri *et al.* [4] have observed that the amount of phenols reduced in rice leaves due to infection by *Entyloma oryzae*. Working on betelvine leaves infected with *Colletotrichum gloeosporioides*, Naik *et al.* [14] had reported a rapid decline in phenolic compounds. Nema [6] was of the opinion that the growth of the pathogen is inhibited by the phenolic compounds but when the pathogen is successful in causing disease the ratio of phenolic compound changes, and in highly susceptible cultivar, the phenolic compounds mostly deplete.

3.5. Chlorophyll and Carotene

In the present investigation, it was noticed that chlorophyll-a, Chlorophyll-b and Carotene metabolism gradually declined in the diseased condition of the *Cymbopogon* (**Table 1**).

Chlorophylls played a major and positive role in the synthesis of carbohydrates. The scenario was very much different in a diseased plant which leads to degradation of chlorophyll. Carbohydrate synthesis in leaves had a positive correlation with chlorophyll content. The initial stage of disease development led to the breakdown of chlorophyll which probably facilitated subsequent establishment of the parasite. This content was supported by

Table 1. Changes of host metabolites in relation to disease.

Metabolic constituents	<i>C. martinii</i>			<i>C. citratus</i>		
	Healthy (%)	Diseased (%)	Percent loss/gain	Healthy (%)	Diseased (%)	Percent loss/gain
Reducing sugar	1.792	0.980	45.312	1.639	0.894	45.454
Non-reducing sugar	15.942	12.300	22.845	14.053	10.081	28.264
Total sugar	17.734	13.050	26.412	15.692	10.895	30.369
Chlorophyll-a	6.042	4.620	23.535	5.292	3.084	41.723
Chlorophyll-b	5.352	3.829	28.456	4.930	3.092	37.281
Carotene	0.734	0.629	14.305	0.496	0.331	33.266
Protein	4.842	3.283	32.197	5.210	3.532	32.207
Ascorbic acid	0.184	0.132	28.260	0.132	0.96	27.272
Phenol	130.032	97.428	25.093	135.020	94.704	29.859

the findings of Allen [15]. During infection, the chlorophyll molecules were destroyed and further synthesis of chlorophyll was checked which in turn disturbed the synthesis of sugar.

Along with the decrease of Chl_a and Chl_b, the carotene synthesis was also decreased. After attaining a particular level, the chlorophyll content didn't increase because it was protected from photooxidation by the carotenoids of leaf [16]. This decrease in the carotene content in palmarosa and lemongrass might be due to environmental factors [17].

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