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CHANGES IN PHYTOSTEROL CONTENT IN THE LEAVES OF *RUMEX MARITIMUS* (L) INFECTED WITH *USTILAGO PARLETOREI* F.A. WALD.

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The phosphate buffer soluble membrane containing fraction isolated from *Rumex maritimus* leaves contains free sterol, sterol-glycosides and sterol esters. The three sterol forms decreased on fresh weight basis with the severity of disease intensity over healthy. The results supported the suggestion that plant cells may have altered the physiological function associated with membrane due to host-pathogen interactions.

Keywords : Phytosterol, *Rumex maritimus, Ustilago consimilis.*

Introduction

It has long been known that numerous biochemical changes are induced in host plant in response to attack by fungi. Some of the pathogenic fungi make the plant tissues damaged, break down the cell wall. It causes the accumulation or decrease of organic compounds and brings about directly and indirectly a more or less complete disorganization of the host tissues. Results of studies with polyene antibiotics have pointed out to an interaction with membrane[1]. These interactions were reported to occur specifically with sterol[2]. It has also been noted that certain phytotoxins produced by plant pathogen interact with plant cell membranes affecting their permeability and occasionally causing them to rupture[3]. Although the biosynthesis and identification of sterols in plants have received much attention in recent years, their function in higher plant is unknown[4]. It has been shown that sterol in plant may stabilize membrane in the same way sterols function in animal cell membrane and might be involved in controlling the permeability of membrane[5]. Since permeability may be involved in some mechanisms of disease resistance, it appears logical to study a particular host pathogen combination and to determine what differences if any, existed in sterol content between healthy and infected host. So the present objective of this study was to examine the sterol distribution in healthy and infected leaves of *Rumex maritimus* plant due to a smut fungus *Ustilago parletorei.*

Materials and Methods

The *Rumex maritimus* plants were grown in the field, both healthy and inoculated germinating seedling separately for 90 days. The plants were in flower at this time. Hundred grams of fresh leaves of different disease intensity were homogenized in a crusher in 100ml of 0.1 M phosphate buffer at pH 7.5 containing 0.5M sucrose, 0.01 M NaCl, 0.04 M disodium EDTA. The homogenate was centrifuged 5000 rpm for 20 min. The pellets thus obtained were dried at 80°C. The total free, glycoside esterified sterols were isolated by following the methods of Gurnwald[4]. The pellets were extracted with 15ml of acetone and alcohol (1:1, v/v) and divided into two equal parts. One part was evaporated to dryness and 10ml of 95% ethanol containing 0.13ml H_2SO_4 was added and refluxed for 12 hrs to cleave the sterol glycosides. Fifteen ml of 10% KOH in 95% ethanol was added and refluxed for 30 mins to hydrolyze the esterified sterol. This sample gave total sterol since both the glycosides and ester have been hydrolyzed. The sterols were extracted from the mixture with 30 ml of n-hexane and enough water to obtain two layers. The n-haxane fraction was back extracted with 90% methanol and taken to dryness.

The second part was also evaporated to dryness and extracted with hot ethanol. This sample gave free sterol and extracted further n-hexane. After extracting n-hexane, the alcohol treated aliquote is again treated with 0.13 ml H_2SO_4. After refluxing for 12 hrs the sterol glycosides were extracted as free sterol by n-hexane. The n-hexane fractions were back extracted with 90% methanol and taken to dryness. The dry residue was dissolved in 10ml chloroform and treated with Acetic anhydride and concentrated H_2SO_4 mixture, (30:1 v/v). The color intensity was measured in a colorimeter. The standard was used a ß-sitosterol. Thus values for free, glycosides and total sterols were obtained directly and values for esterified sterols were calculated.

Results and Discussion

The leaves extract in aqueous medium consist of sterol. The amount of sterol present per gram fresh weight of tissue decreases with intensity of disease. The extract when compared to the leaf tissue contains only a small amount of sterol. The free sterol decreased with severity of infection, lower values of glycosides and ester remain more or less same. The level of sterol in the control leaf (pre flowering /PF and flowering /F) was indicated to be 37.32 µg/g and 36.65 µg/g respectively. With the advancement of infection the level of sterol in the leaves decreases from 30.321µg/g (PS) to 24.65 µg/g (MS). The same trends were recorded in the case of free and glycoside sterol except flowering (F) where the free and glycoside value recorded to be 4.35 µg and 7.24 µg/g respectively. However, the percentage value of ester in disease tissues show gradual increase in the level from 70.3% (PS) to 74.5% (MS) respectively over the control 69.4% (PF) and 68.8 (F).

On comparing the value of phytosterol extracted from healthy and different stages of infected plants, it was found that the infection did not cause increase in phytosterol content. The reduction and alteration of sterol content in host plant is due to pathogen interaction[6,7]. The reduction in the sterol content runs parallel with the findings of Jennings[6]. The differences in sterol content in healthy and infected tissue might be the results of the degree of penetration during the disease development. According to Richardson[7] there are slight differences in sterol contents in normal and tobacco mosaic virus infected plants and explained the decreased in sterol content in diseased tissue might be due to toxin

Table 1. Phytosterol contents of healthy and infected *Rumex maritimus* leaves infected with *Ustilago parletoreii* (express in µg/g fresh weight tissue).

Sample	Total sterol	Free sterol	%	Sterol Glycoside	%	Sterol Esterified	%
PF	37.32	4.22	11.3	7.00	18.7	26.10	69.9
F	36.65	4.35	11.6	7.20	19.6	25.10	68.8
PS	30.32	2.28	7.5	6.90	22.2	21.14	70.3
YS	26.98	1.45	5.4	6.20	22.3	19.33	72.3
MS	25.65	1.08	4.3	5.30	21.5	18.27	75.5

All the values are the mean of 5 replicates.

Healthy - PF - Pre flowering
F - Flowering

Disease - PS - Pre sporulation
YS - Very young sporulation
MS - Mature sporulation

produced by the pathogen host interaction. The present findings with decrease in sterol and related compounds indicate the decline of the contents with the advacement of infection and sporulation. The interaction of the host and pathogen during infection might have suppressed the synthesis of phytosterol and brought about changes in the host cell permeability and ultimately leading to loose the sterol during catabolic action of the disease.

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