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Variation of Nitrate Reductase Activity in Rumex Maritimus Linn. During Infection With Ustilago Parletoreii F.A.Wald

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Abstract - The present study deals with the variation of nitrate reductase activity in Rumex maritmus Linn. during infection with smut fungus, Ustilago parletoreii F.A.Wald. It is an annual herb growing wild in different marshy places of Manipur, India. The leaves are cooked as vegetable curry items by the local people. The plant is often infected with smut fungus mostly at the midrib and soft portion of the stem (shoot). During the infection with smut fungus Ustilago parletoreii, different enzymatic activities changes were taken placed. There is a fluctuation of nitrate reductase activities amongst the degree of diseases response during infection of plant by this fungus. The maximum activity was found in the pre-sporulation stage of infected plants and chilling spores.

Keywords: Rumex maritimus, Nitrate reductase (NR), Ustilago parletoreii, Pre-sporulation (PS), Chilling spores.

1. INTRODUCTION

It has long been known that numerous biochemical changes are induced in host plants in response to attack by fungi. Some of the pathogenic fungi damages the plant tissue damaged, breakdown to the cell wall by secreting pathogenic enzymes. Rumex maritimus is an annual angiospermic herb belonging to the family Polygonaceae and normally attain a height of 1-2 feets, mesophytic distributed throughout the World. All parts of the plant were found useful and have a number of health benefits like antioxidant, anti-inflammatory, antifertility, antibacterial, antiviral and antipyretic. In young condition the shoot is filled with paranchymatus tissue and gradually become hollow at the pit portion with age. The leaves and young shoots are often infected with smut fungus, Ustilago parletoreii. The infected plant is also used as food by the local people of Manipur, India. The infected plant does not produce flowers and there are no chances of seed formation. A number of workers has been studying smut species for its nutritional value [5,9,17] and pharmaceutical activities on different Rumex species [12,13]. A limited information is available on the study of physiological and biochemical changes in control and infected tissue of Rumex maritimus [13,16,17,19]. Enzymes of nitrogen metabolism is related to nitrate reductase. biochemicalolved in assimilation of nitrogen from NO_3 - to NO_2 – is the nitrate reductase. Enzyme of nitrate metabolism have been excessively studied by a number of workers [2,3,4,5,8,10]. Much work has been reported on nitrate reductase in higher plants [6,11,14,15,17,18]. No information is available on the study of the infected fungus activity of *Rumax maritimus*. Therefore, the present study aims to investigate the variations of nitrate reductase activities in *R.* maritimus during infection with smut fungus.

2.MATERIALS AND METHODS

The Rumex maritimus plants were grown in the experimental field, both healthy and inoculated germinating seedling separately. The plants matured and start flowering after 90 days of sowing. The nitrate reductase activity in the leaves and young shoots were measured in vivo for each sample by using slightly modified method of Hageman and Hucklesbay[1]. On the basis of different stages of infection, the samples are divided into different terminologies i.e. pre- flowering (PF), flowering (F), pre-sporulation (PS), very young sporulation (YS) and mature sporulation (MS) and spores. The tissue was thoroughly washed with distilled water and then placed in layers of filter paper to remove surface moisture. These leaves and young shoots were cut separately into narrow pieces and weighed quickly for the enzyme assay. 500 mg of the sample strips were kept in a 15 ml capacity light proof serum vials. The weighted sample were incubated in the assay mixture, which is in a final volume, 5 ml containing 4 ml potassium phosphate buffer 100 µM, pH 7.4; 0.5 ml KNO3, 100 µM and 0.5 ml of n- propanol (5% v/v). Each sample were incubated at 30 ± 2oC in an incubator, and nitrite formed during the incubation period was measured by adding 1 ml of sulphanilamide (1% in HCl w/v) and 1 ml of N-(1-Napthyl) ethylene diamine dihydrochloride (0.02%) in distilled water (w/v) to 1 ml of the incubation mixture for each suitable interval. The absorbance of the pink colour develops was recorded at 540 nm using spectrophotometer (Spectronic-20). The nitrate reductase activities have been calculated in terms of µ mol NO_2 – produced h-g- tissue. Sodium nitrite was used as standard.

Table -1: NR activities of leaves of R. maritimus and spores

Time	Control		Infected tissue of leaves			Spores
	PF	F	PS	YS	MS	4.32 before chilling
30 mins	2.13 ± 0.04	3.37 ± 0.06	3.58 ± 0.06	2.14 ± 0.04	3.12 ± 0.06	
45 mins	4.30 ± 0.08	6.70 ± 0.11	7.50 ± 0.13	4.16 ± 0.06	3.92 ± 0.06	
60mins	8.52 ± 0.14	9.32 ± 0.19	11.81 ± 0.20	7.55 ± 0.11	6.92 ± 0.09	

Data represents are means ± SD of 5 replications

PF=Pre-flowering, F= Flowering, PS= Pre-sporulation, YS= Young sporulation, MS= Mature sporulation

Table -2: NR activities of young shoot of R. martimus and spores

Time	Control		Infected tissue of leaves			Spores
	PF	F	PS	YS	MS	14.71 after chilling (10±2oC) for 12 hours
30 mins	2.87 ± 0.05	2.92 ± 0.05	2.95 ± 0.06	2.86 ± 0.06	2.95 ± 0.05	
45 mins	3.02 ± 0.04	3.05 ± 0.05	3.22 ± 0.05	3.13 ± 0.05	3.06 ± 0.05	
60mins	4.65 ± 0.06	4.7 ± 0.07	4.78 ± 0.07	5.1 ± 0.07	4.77 ± 0.09	

Data represents are means ± SD of 5 replications.

PF=Pre-flowering, F= Flowering, PS= Pre-sporulation, YS= Young sporulation, MS= Mature sporulation

3.RESULTS AND DISCUSSION

Changes in the rate of Nitrate reductase activities in healthy and infected Rumex maritimus leaves and shoots are express in µmol NO2 – /g- tissue. The highest value of NR was observed in PS (3.58 µ mol) and lowest at MS (2.124 µmol) / g tissue respectively at 30 minutes (Table 1). The value of NR in healthy leaves was found PF (2.13 µmol) and F (3.37 µmol)/ g tissue respectively at 30 mins. The activities of PF, (8.52); F, (9.32); PS, (11.81); YS, (7.55 and MS (6.92) µ mol/ g tissue were observed at 60 mins respectively. The highest value was found in PS, (11.81 µ mol) and lowest in MS, (6.92 µ mol)/ g tissue respectively amongst the disease infected plants.

The activities of NR at shoot was found lesser as compared to corresponding lamina. The activities from 30 to 60 minutes of PF, (2.87 to 4.65); F, (2.92 to 4.7); PS, (2.05 to 4.78); YS, (2.86 to 5.10); MS, (2.95 to 4.77) µ mol NO_2- /g tissue were observed (Table 2). The maximum activity was found at YS, (5.1 µ mol) NO_2- at shoot. However, the NR activities was also observed at pre-chilling (4.32) and Chilling spore (14.71) µ m NO2- /g spore at 60 minutes respectively.

From the above discussion it was found that the highest activity was found at PS, (11.81 µ mol NO_2- /g tissue) in case of leaves and YS (5.1 µ mol NO_2- /g tissue) in case of young shoot. The activity NR of shoots are found usually less in compared to leaves. From the above observation it is clearly shown that the rate of enzyme activity was found highest before sporulation (PS) and then gradually decrease with the advancement of spore formation. The decrease in the rate of activities among the disease is brought about due to the sporulation of the infected fungi. However, the maximum rate of NR activity was found in the chilling spore (14.075 µ mol NO_2- /g spore) over pre chilling spore (4.37 µ mol NO_2- /g spore)/per hour respectively. Nitrogen plays an important role in plant nutrition. Nitrate reductase can increase the activity and can rapidly reduce the accumulation of nitrate in plant tissues and indirectly prevent the occurrence of disease and insect pests. However, the excess soluble nitrogen compounds in plants will induce plant disease and insect pest [20]. Assimilation of nitrate reductase of higher plants is a most interesting enzyme both from its central function in plant primary metabolism and from the complex regulation of its expression. Nitrate reductase catalyzes the reduction of nitrate to nitrite and is a good example of the enzymes inducible by its substrate[4]. Nitrate is the major nitrogen source for plant in addition it is an important signaling molecule that influences plant growth and differentiation. Nitrate itself is expressed to be a signal that can directly affect the expression of genes related to nutrient uptake. Indirect nitrate may also affect plant pathogen interaction via the production of nitric oxide (NO) which is catalyzed by nitrate reductase under certain conditions.

4.CONCLUSION

From the above study it can be concluded that the maximum activities of nitrate reductase are found maximum at the mycelial or pre-sporulation stage (leaves) and young sporulation (shoot) then spore formation of the infected plant. The increase activity nitrate reductase during infection of the plant might be due to addition secretion by fungus.

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