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VARIATION OF NITROGENOUS COMPOUNDS IN *RUMEX MARITIMUS* LINN. DURING INFECTION WITH *USTILAGO PARLETOREII*, F.A. WAL.

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Rumex maritimus is an annual herb growing wild in different marshy places of Manipur. The plant is also used as food vegetables by the local people of Manipur. The plant is often infected with the smut fungus mostly at the midribs and branch ribs of the leaves and soft portion of the stem. During the infection with the smut fungus *Ustilago parletoreii* different metabolic changes take place. There are variation of nitrogenous compounds-total nitrogen, soluble and insoluble nitrogen fractions, nitrates, nitrites and amino acids in the leaves and stems during the infection.

Keywords : Amino acids; Nitrates; Nitrites, *Rumex maritimus*; Soluble and insoluble nitrogen; *Ustilago parletoreii*.

Introduction

Rumex maritimus Linn. was an angiospermic plant belonging to the family Polygonaceae and normally attains a height of 1-2 ft. It was an annual herb, mesophyte, distributed through out the world. It grows in the wet lands, ditches even in the water logging areas and floating mat (phoomdi). In Manipur the plant was growing wild and serves as the food vegetables. The plant was normally infected with smut fungus *Ustilago parletoreii* F.A. Wal. The black and brown spores are also used as palatable food by the local people of Manipur. With the infection of this fungus, there occurs abnormal swelling of young shoot and midribs of the lamina. Cavities are formed by degelatinization of the parenchyma cells filled with mass of fungal spores. The changes in nitrogenous compounds, like, soluble and insoluble nitrogen fractions, nitrite and nitrate nitrogen, amino acids etc. are seen in the leaves and stems during infection.

Materials and Methods

The procedure for the determination of soluble and insoluble nitrogen fractions was performed following the procedures adopted by Lang[1]. The estimation of nitrites and nitrates was done by the procedure adopted by Peach and Tracey[2]. The estimation of amino acids was done following the procedure adopted by Yemm and Cocking[3]. On the the basi, of the different stags of infection the samples are divided into different terminologies, Preflowering (PF), Flowering (F), Initial infection (IN), Medium infection (ME) and Severe infection (SEV).

Results and Discussion

Table 1 and 2 show the levels of total nitrogen, insoluble and soluble nitrogen fractions in the leaves and stems, respectively. The level of total nitrogen in the control leaves (PF) and (F) was indicated to be 2.9 mg and 2.52 mg, respectively. With the start of the infection the level of the nitrogen in the leaves varied from 3.19 mg (IN_1) to 2.58 mg (SEV) with the advancement of infection except in the cases of IN_2 where the nitrogen value was recorded to be 3.23 mg. However, the amount of the nitrogen value was found highest in the case of fungal spores (4.54 mg). In the case of host shoot there was little variation in nitrogen content in control sample (PF and F) having the value of 1.94 mg and 1.97 mg, respectively. However, with the start of infection the amount decreased from 2.2 mg to 1.72 mg, except in IN_2 (2.25 mg). There was gradual increase in insoluble nitrogen fraction during the infection of leaf and shoot tissue of the host. The insoluble nitrogen fraction in the case of control leaves PF and F was recorded as 2.06 mg and 1.8 mg, respectively. There was gradual increase in this fraction till the infection reached the stage IN_2 (2.69 mg) and after reaching the maximum value there was decline in this fraction till it reached the final stage, i.e. SEV (2.31 mg). However, the minimum value obtained in the infected tissues was always higher over the control ones. The situation was little bit different in the case of host plant during and after infection periods. Though, there was gradual increase in the insoluble nitrogen fraction over the control ones upto the IN_2 stages (1.74 mg) and the decrease of the same upto severe stage (SEV) 1.07 mg, the amount of this nitrogen fraction in the control samples was found to be higher than the minimum value recorded in the final stage of infection (SEV) 1.07 mg unlike

Table1. Variation in Nitrogen content, expressed in mg/g, in the dried material at different stages of infection in the leaf tissues of the host plant *Rumex maritimus* infected with *Ustilago parletoreii.*

Leaves	PF	F	IN_1	IN_2	ME_1	ME_2	SEV	Spore
Total Nitrogen	2.90	2.52	3.19	3.23	2.96	2.79	2.58	4.54
Insoluble Nitrogen fraction	2.06	1.81	2.64	2.69	2.52	2.41	2.31	3.28
Soluble Nitrogen fraction	0.82	0.72	0.55	0.54	0.44	0.38	0.28	1.26

Table 2. Variation in Nitrogen content, expressed in mg/g dry weight, at different stages of infection in young shoot tissues of the host plant *Rumex maritimus* infected with *Ustilago parletoreii.*

Shoot	PF	F	IN_1	IN_2	ME_1	ME_2	SEV
Total Nitrogen	1.94	1.97	2.20	2.25	2.26	2.00	1.72
Insoluble Nitrogen fraction	1.22	1.28	1.66	1.74	1.41	1.25	1.07
Soluble Nitrogen fraction	0.69	0.69	0.54	0.78	0.85	0.75	0.65

Table 3 (a) and (b). Variation in Nitrogen content, expressed in mg/g, in the dried material at different stages of infection in the leaf tissues of the host plant *Rumex maritimus* infected with *Ustilago parletoreii.*

Table (a)

Leaf	PF	F	IN_1	IN_2	ME_1	ME_2	SEV	Spore
Nitrate	1.72	1.65	1.62	1.30	1.42	1.36	1.24	0.95
Nitrite	0.91	0.93	0.75	0.70	0.96	0.88	0.84	0.72

Table (b)

Shoot	PF	F	IN_1	IN_2	ME_1	ME_2	SEV
Nitrate	2.13	2.11	2.20	2.25	2.30	2.36	2.60
Nitrite	0.54	0.54	0.72	0.75	0.81	0.82	0.80

PF - Preflowering; F - Flowering; IN - Initial infection;
ME - Medium infection; SEV - Severe.

Table 4. Changes in amino acid content, expressed in mg/g, in the dried materials of *Rumex maritimus* infected with *Ustilago parletoreii.*

	PF	F	IN_1	IN_2	ME_1	ME_2	SEV	Spore
Leaf	3.23	3.36	3.60	3.62	3.67	3.70	3.45	5.85
Shoot	2.71	2.52	2.10	2.15	2.19	2.80	2.75	

PF - Preflowering; F - Flowering; IN - Initial infection;
ME - Medium infection; SEV - Severe.

that of leaf.

The condition was quite reverse in the case of the soluble nitrogen fraction in the leaves with this smut fungus. The amount of this nitrogen faction was found to be maximum in the control samples (0.82 mg and 0.71 mg) and minimum in the SEV (0.28 mg). However, unlike the leaves the shoots showed gradual increase in this nitrogen fraction upto IN_2 stage (0.69 mg to 0.85 mg) but it decreased there after upto the final stage (ME_2) from 0.85 mg to 0.65 mg. Changes in the nitrogenous constituent of the host plant in response to pathogen attack have been observed and discussed by many workers[4-7].

Table 3 and 4 show the level of nitrite and nitrate fractions in the leaves and shoots of *Rumex maritimus,* respectively. The level of nitrate nitrogen fraction in the control leaves (PF) and (F) was indicated to be 1.72 mg and 1.65 mg, respectively. There was gradual decrease in nitrate during the infection in leaves from 1.62 mg (IN_1) to 1.24 mg (SEV). There was little bit changes in the nitrite fraction upto ME1 (0.96 mg) and after reaching the maximum value there was decline in the fraction till it reached the final stage i.e SEV (0.84 mg). The condition was quite reverse in the case of infected shoot. The amount of nitrite fraction was found to be minimum in PF and F (0.54 mg and 0.54 mg) and maximum in MF_2 infection stage (0.82 mg). Unlike the leaves the shoot showed gradual increase in nitrite fraction upto ME_2 (0.72 mg and0.82 mg). Changes in the concentration of nitrogen in the infected tissues in response to pathogen attack have been observed by different researchers. Lilly and Barnett[8] reported that nitrate were excellent source of nitrogen for many fungi. Evans and Nason[9] reported the reduction of nitrate to nitrite was responsible to the activity of enzyme nitrate reductase. This view was also supported by the findings of Johnson *et al.*[10] and Harandez *et al.*[11]. Hence, it may be concluded that the changes that took place in the concentration of nitrate and nitrite fractions in the leaves and shoots during the infection might be due to the activities of nitrite and nitrate reductase.

Table 4 indicates the changes in amino acids during the infection on *Rumex maritimus* with *Ustilago parletoreii.* The level of total free amino acids in the control leaf (PF and F) was indicated to be 3.23 mg and 3.36 mg, respectively. With the start of infection the level of total amino acid in the leaf increased from 3.60 mg (IN_1) to 3.70 mg (ME_2) except in case of SEV where the amino acid value was reported to be 3.40 mg. The highest value of amino acid was found in the fungal spores (5.58 mg). In the case of host shoot the same trends were recorded. In the shoot the amount of amino acid in the PF and F was recorded to be 2.71 mg and 2.52 mg, respectively. There was gradual increase in this nitrogen fraction till the infection reached the stage ME_2 (2.80 mg) and after reaching the maximum value it declined till it reached the final stage i.e SEV (2.75 mg). However, the minimum value obtained in the infected tissues was always higher over the control. The changes in amino acids concentration in diseased tissues due to the fungal infection were reported by a number of earlier workers[12-14]. The amino acid concentration in the host tissues was found greatly increased after fungal infection. Van Andel[15] reported that synthesis of amino acid in the growing fungus also causes an increase in the amino acid content in the invaded cell. Webster[16] reported that the increase in the synthesis of new amino acids might be due to the disturbed metabolism of the host, by the pathogen or host-parasite interection. On the other hand the decrease or depletion of amino acids in the infected tissues was also reported by various workers[17-19] who viewed that the accumulation of amino acids in the infected tissues might be due to the blockage of protein synthesis or enhanced protease activity in the diseased tissue. The accumulation of amino acids in the spores might be due to translocation from the host tissues or due to the synthesis of the same by the mycelia of the fungus during host-pathogen interection.

References

1. Lang C A 1958, Simple micro-determination of Kjeldahl in biological material. *Anal. Chem.* **30** 1692-1994.
2. Paech K and Tracey M V 1956, Modern Methods of plant analysis, Vol. 1 Sgringer-Verlag, Berlin.

3. Yemm E W and Cocking E C 1955, The determination of amino acids with ninhydrin. *Analysist.* **80** 209-213.
4. Tripathy R K and Chiranjeevi V 1976, Biochemical changes in sorghum leaves infected with Zonate leaf spot. *Ind. J. Mycol. and Plant Pathol.* **6** 121-125.
5. Singh R, Singh H C and Ganulee R 1979, Metabolic changes due to water melon mosaic virus infection in bottle guard fruits. *J. Mycol. and Plant Pathol.* **9(1)** 89-90.
6. Prasad S M and Sahambi H S 1983, Biochemical changes brought about by *Sesamum phyllody. Ind. Phytopath. Chem.* **33** 617-618.
7. Shaw M and Colotelo N 1961, The physiology of plant host parasite reaction. VII. The effect of stem rust on the nitrogen and amino acids in wheat leaves. *Can. J. Bot.* **39** 1351-2137.
8. Lilly YC and Barnett H L 1951, Essential metabolic elements in physiology of fungi. McGraw Hill Book Company.
9. Evan H J and Nason A 1953, Pyridinenucleotide-nitrate reductase from extracts of higher plants. *Plant Physiol.* **28** 233.
10. Johnson V A, Schmidt J W and Mattern P T 1968, *Eco. Bot.* **22** 16.
11. Harandez H H, Walsh D and Baur A 1974, *Cereal Chem.* **51**(3) 330-336.
12. Farkas G L and Kiraly Z 1961, *Plany Physiol.* **14** 344-353.
13. Aulakh K S, Grover K and Malhotra S 1970, Utilization of free amino acids *in vivo* and *in vitro* by isolates of *Phoma destructiva. Plowr-Phytopath. Medit.* **9** 8-12.
14. Tandon R N, Tandon M P and Jamaliddin 1974, Studies on the post-harvest dwaseases of fruits and vegetables. In : Current Trends in Plant Pathology. (S.N. Das Gupta ed.) pp-209-220.
15. Van Andel O M 1966, Amino acids and plant disease. *An. Rev. Pytopath.* **4** 349-368.
16. Webster J 1956, Succession of fungi on decaying cocksfoot culms. *Ind. J. Eco.* **44** 517-544.
17. Mc Combs C L and Winstead M N 1964, Changes in sugars and amino acids of cucumber fruits infected with *Pythium aphanidermatum. Phytopath.* **54** 233-234.
18. Touz'e P 1964, Formation of free amino acids and amides during infection of melon by *Collectotichum lagenarium* Pass. *Phytochem.* **3** 143-147.
19. Goodman R W, Kiraly Z and Zaitlin M 1967, The biochemistry of infectious plant diseases. Von Nostrand Co. Princeton, New Jersy.