J. Phytol. Res. **21**(1) : 119-122, 2008

CHANGES IN α-ASCORBIC ACID, PIGMENTS AND PROTEIN IN *RUMEX MARITIMUS* LINN. DURING INFECTION WITH SMUT FUNGUS, *USTILAGO PARLETOREII* F.A. WAL.

O. NOYON SINGH and L. NONGDREN KHOMBA SINGH*
Department of Botany, Lilong Haoreibi College, Manipur, India.
*Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India.

Rumex maritimus Linn. is an annual herb, mesophyte, distributed prominiently in Manipur. The plant is normally infected with smut fungus, *Ustilago parletoreii*, F.A. Wal. Both the infected and fresh uninfected plant sometimes used as food by a few sections of the people of Manipur. There are fluctuations of α-ascorbic acid, pigments and protein during infection of the plant by this smut fungus.

Keywords : α-Ascorbic acid; β-Carotenes; Chlorophylls; Protein; *Rumex maritimus* Linn; *Ustilago parletoreii* F.A. Wal.

Introduction

Rumex maritimus Linn. is angiospermic herb belonging to the family Polygonaceae, and normally attains a height of 1-2 feets. In young condition the shoot is filled with parenchyma tissue and gradually becomes hollow at the pit portion with maturity. The young shoots and leaves are often infected with the smut fungus, *Ustilago parletoreii* F.A. Wal. The infected plant is used as food by the local people of Manipur. The black and brown teleutospores are also used as palatable food by these people. The infected plant does not produce flower and hence, there is no formation of seeds. Variation of α-ascorbic acid, pigments and protein during infection has been studied. The paper discusses the reason of decrease and increase of these metabolites during infection.

Materials and Methods

Fresh plant materials both healthy and infected were collected. These materials were cut into pieces and crushed thoroughly. For the estimation of α-ascorbic acid the procedure adopted by Roe[1] was used. For the assay of chlorophyll,a,b, and β-carotene the method used by Lichtenthaler *et al.*[2] was adopted and that of protein the procedure adopted by Lowny *et al.*[3] was used. In all the samples 5 stages were devided viz., preflowering stage (PF), Flowering stage (F), Initial Infection stage (IN), Medium Infection stage (ME), Severe Infection Stage (SEV) and Spore.

Results and Discussion

Table 1 shows the levels of α-ascorbic acid in the control (PF and F) and infected tissues (IN and SEV). In the controlled tissues the level of α-ascorbic acid was 0.86mg and 0.87mg in PF and F, respectively. With the start of infection the level of total α-ascorbic acid in the leaf tissues decreased from 0.83mg (IN_1) to final stage of infection, *i.e.*, 0.66mg (SEV). In the case of host shoot the same trends were recorded. In the shoot the amount of α-ascorbic acid in the PF and F was recorded to be 1.3 mg and 1.2 mg, respectively. There was gradual decrease of the same till the infection reaches the final stage, *i.e.*, (0.81 mg). The value obtained in the infected tissues was always lower than the control. Mahadevan and Sridhar[4] described that in the diseased plant ascorbic acid reduces quinones to phenols and this reaction has created the defence mechanism of the host plant. Gunasekaran and Weber[5] viewed that the low content of ascorbic acid in the infected tissues might be due to host-pathogen interaction in which the ascorbic acid from the host might serve as a carbon source for the fungus in the formation of other metabolites. The decline in the concentration of ascorbic acid in the infected host tissues might be due to the production of certain ascorbic dehydrogenase enzyme by the fungus itself and also possible that during host-pathogen interaction this very enzyme might have been synthesised[6-8]. Isherwood and Mapson[9] also reported that the biosynthesis of ascorbic acid is markedly influenced by the chloroplast content. The reduction of chlorophyll content in the leaf tissues (present findings) would have resulted the decrease in ascorbic content of the infected tissues. Parmar *et al.*[10] and Prasad[8] reported that the decrease of ascorbic acid might be due to transformation to dehydroxy α-ascorbic acid or by the enzymic activities

Table1. Variation in the α-ascorbic acid content expressed in mg/g fresh wt. in different stages of *Rumex maritimus* infected with *Ustilago parletoreii.*

Pigment	PF	F	IN_1	IN_2	ME_1	ME_2	SEV
Leaf	0.86	0.87	0.83	0.75	0.71	0.66	0.66
Shoot	1.3	1.2	1.2	1.17	1.13	1.01	0.81

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

Table 2. Changes in chlorophyll content expressed in mg per gram fresh tissue and β-carotene in μg per gram fresh tissue in different stages in the leaf tissues of *Rumex maritimus* infected with *Ustilago parletorell.*

Pigment	PF	F	IN_1	IN_2	ME_1	ME_2	SEV
Total Chl.	1.47	1.40	1.28	1.19	1.19	1.16	1.11
Chl.a	0.69	0.68	0.58	0.56	0.51	0.45	0.43
Chl.b	0.78	0.72	0.7	0.63	0.68	0.70	0.68
β- carotene	0.30	0.28	0.25	0.25	0.22	0.19	0.15
Chl a/b	1.45:1	1.28:1	0.82:1	0.89:1	0.75:1	0.64:1	0.63:1

Table 3. Changes in chlorophyll content expressed in mg and β- carotene in μg per gram fresh tissue in different stages in the host shoot tissues of *Rumex maritimus* infected with *Ustilago parletoreii.*

Pigment	PF	F	IN_1	IN_2	ME_1	ME_2	SEV
Total Chl.	0.41	0.34	0.53	0.31	0.24	0.21	0.20
Chl.a	0.16	0.17	0.19	0.14	0.12	0.09	0.09
Chl.b	0.25	0.17	0.34	0.17	0.14	0.12	0.11
β- carotene	0.10	0.09	0.08	0.06	0.03	0.02	0.02
Chl a/b	0.06:1	1:1	0.56:1	0.82:1	0.86:1	0.75:1	0.82:1

Table 4. Variation of protein content (mg/g) in the dried material of the host plant *Rumex maritimus* infected with *Ustilago parletereii.*

Pigment	PF	F	IN_1	IN_2	ME_1	ME_2	SEV	Spore
Leaf	14.28	13.18	16.78	18.68	19.50	16.36	15.51	21.67
Shoot	9.90	10.06	14.12	14.16	13.20	11.20	10.46	

of the pathogen which was strengthened by the increase in the contents of reducing sugar in the infected part. Hence, it may be concluded that the decrease in the level of ascorbic acid during infection of *Rumex maritimus* might be due to the metabolic activity or host-parasite interaction of *Rumex maritimus* and *Ustilago parletoreii*.

Findings shown in Tables 2 and 3 clearly indicated that there was a gradual decrease in different types of plant pigments, viz., chlorophyll a & b, β-carotene, during infection. The decrease in pigments in the infected plants was observed by different workers[11-15]. The decrease in the amount of pigment contents in the diseased plant might be due to the reduction in the number and size of the chloroplast caused by the pathogen[16]. It has also been reported that the low pigment content in the diseased tissues might be due to inhibitation of its production by the fungus[11]. It was reported that the increased chlorophyllase activity also helps in the lowering of the chlorophyll in the defence of host pathogen combination[17-18]. It may also be explained that during the infection certain inhabitors might be secreted by the pathogen which affect the synthesis of chlorophyll pigments[15-16]. Hence, it may be concluded that the gradual decrease of plant pigments in the plant tissues during infection may be explained due to the influence of the invading fungus on the normal metabolism of the host plant.

The findings of protein in the healthy and infected tissues of the host plant *Rumex maritimus* are shown in Table 4. The level of protein in the control leaf, pre-flowering (PF) and Flowering (F) stage was indicated to be 14.23 mg and 13.18 mg, respectively. With the start of the infection the level of protein in the leaves decreased from 16.78 mg (IN1) to 15.51 mg (SEV) with the advancement of infection, except in Medium Infection (ME1) where the value was recorded to be 19.50 mg. In the case of control host shoot sample (PF and F) the protein value was 9.9 mg and 10.06 mg, respectively. There was slight increase till infection reached upto (IN2) 14.16 mg and after reaching the maximum value at this stage it declined till it reached the final stage i.e. SEV (10.16 mg). In the case of leaf the minimum value obtained in the tissues was always higher over the control. However, the situation was also same in the case of host shoot during the infection period. But the amount of protein value was found highest in the case of fungal spores (21.67 mg). From the above result it is clear that the maximum amount of protein value was found in the leaves over the shoot. It was also clearly seen that the accumulation of protein concentration gradually increases from early stage of infection and decreases with the severity of infection. The decrease in the amount of protein in the diseased tissues was brought about due to sporulation of the infected fungus. Yamamoto *et al*[19], Tani and Yamamoto[20] reported that the increase during infection was due to increase in protein synthesis accelerated by the biosynthetic enzyme and other proteins involved in plant defence. Staples and Ledbter[21] observed the increase in protein in the infected plant was mainly incorporated into the fungal mycelia and spores. Many other researchers also gave their views on the increase in both protein and amino acids in the infected tissues[22-23]. They further viewed that the increase might be due to translocation of the same from other part of the host to the infected tissues or increased synthesis of both protein and amino acids. Albersheim and Valent[24] viewed that fungal pathogen produces or secretes protein which inhibits enzyme of the host capable of attacking the pathogen. Rudolph[25] also reported that the increase of additional protein in the tissues was due to the increased metabolic activity in stress. Hence, it may be concluded that the increase in the level of protein during infection might be due the metabolic activities or the host parasite interection.

References

1. Roe J H 1954, Chemical determination of ascorbic acid, dehydroascorbis and diketogluconic acids. *Meth. Biochem. Anal.* **1** 115-139.
2. Listenthaler H K, Thomas J Base and Alan R and Wellburn 1982, Cytoplasmic and plastidic isoprenoid compound of oat seedling and their distinct lebelling from 14 C-Mevalonate. In: Biochemistry and metabolism of plant lipid. (J .F. G. M. Wintermabs and P. J. C. kuipers eds.) pp 489-500. Elsevier Bimedical press Amsterdam, New York, Oxford.
3. Lowry O H, Rosebrough N J , Far A L and Randall R J 1951, Protein measuerment with Folin-phenol reagent. *J. Biol. Chem.* **193** 265-275.
4. Mahadevan A and Sridhar R 1982, Methods in physiological plant pathology (2nd Ed.) , Sivakami Publication, Madras.
5. Gunasekaran M and Weber D J 1972, Changes in ascorbic acid and phenols in apricot in response to *Rhizopus arrhizus*. *Can.J.Plant Sci.* **52** 662-664.
6. Ghost AK, Tandon R N, Bhargava S N and Srivastava M P 1964, *Naturwissenchaftou* **16** 478.
7. Tandon R N 1970, Observation on storage diseases of certain fruits. *Ind. Phytopath.* **20** 1-20.
8. Prasad M M 1980, Vitamin 'C' contents of Indian plum fruits after fungal infection. *Ind. Phytopath.* **33** (1) 117-118.
9. Isherwood F A and Mapson L W 1962, Ascorbic acid

metabolism in plants Part II. Biosynthesis: *Ann. Rev. Plant Physiol.* **13** 328-350.

10. Parmar S M S, Taneja S, Jain B L and Williamson R D 1983, Biochemical changes in *Cichorium intybus* infected by *Alternaria cichorei*. *Ind. Phytopath.* **36** (4) 680-682.
11. Pero R and Main C 1970, Chlorosis of tobacco induced by Alterbarid monomethyl ether produced by *Alternaria tenius*. *Phytopath.* **60** 1570-1573.
12. Thompson D P 1970, Phenols, carotene and ascorbic acid of Sweet potato root infected with *Rhizopus stolonifer*. *Can. J. Plant Sci.* **59** 1177-1179.
13. Billore S K, Mehta S C and Mall I P 1977, Changes in chlorophyll and carotenoid in summer leaves of a tropical deceduous tree *Buchanania lazan* Spreg. *Sci. and Cult.* **1977** 324-325.
14. Agarwal M L, Kumar S, Goel A K and Tayal M S 1982, Biochemical analysis in leaf spot disease of turmeric; some hydrolysing and oxidative enzymes and related metabolites. *Ind. Phytopath.* **35** (3) 438-441.
15. Singh L J and Singh P K 1992, Nutritive values of smut infected *Zizania latifolia* culm. 4th Manipur *Sci. Cong. Abstr.* **68**.
16. Tu T C, Ford R E and Krass C J 1965, Comparisions of chloroplasts and photosynthetic rate of plant infected and not infected by Maize Dwarf mossaic virus. *Phytopath.* **58** 285-288.
17. Manjeet K and Despande K B 1980, Phytosynthetic activities of cowpea plants infected with *Erysiphe polygony*. *Ind. Phytopath.* **33** 339-345.
18. Bedbrook J R and Mathews R E F 1977, Changes in the flow of products of photosynthetic carbon fixation associated with the replication of TYM I. *Virology* **53** 84-91.
19. Yamamoto H, Tani T and Hokin H 1976, *Ann. Phytopath, Jpn.* **42** 583-590.
20. Tani T and Yamamoto H 1979, Recognition and specificity in plant Host-Parasite interaction. *Jpn. Sci. Sco.* 273-282. Tokyo and University Park Press, Baltimore.
21. Staples R C and Stahmen M A 1964, Changes in protein and several enzymes in susceptible bean leaves after infection by bean rust fungus. *Phytopath.* **54** 760-764.
22. Shaw M and Colotelo N 1961, The physiology of host parasite reaction. VII. The effect of stem rust on the nitrogen and amino acids in wheat leaves. *Can. J. Bot.* **39** 1351-2137.
23. Hrushouetz S B 1954, The effect in infection by *Helminthosporium sativum* on the amino acid content of wheat roots. *Can. J. Bot.* **32** 571-575.
24. Albersheim P and Valent B S 1974, Host pathogen interactions VII. Plant pathogens secrete proteins which inhibit enzymes of the host capable of attacking the pathogen. *Plant Physiol.* **53** 684-687.
25. Rudolph K 1963, *Phytopath. Z.* **46** 276-290.