FREE PROLINE ACCUMULATION IN RESPONSE TO INFECTION BY *COLLETOTRICHUM FALCATUM* IN SUGARCANE

O. K. SINHA, R. RAJ BHANSALI and KISHAN SINGH
Division of Plant Pathology, Indian Institute of Sugarcane Research, Lucknow 226 002, India.

THE phenomenon of free proline accumulation in plants exposed to diverse environmental and biological stresses has considerable physiological significance. In response to water stress conditions, large amount of free proline accumulates in plants[1–4]. Similarly, accumulation of free proline in plants takes place under salinity stress[5,6], low temperature conditions[7–9] and when plants get exposed to air pollutants[10–12]. The reports indicate that free proline synthesised from glutamate serves as energy donor during such environmental stresses[1,13]. Recently proline accumulation in plants has been demonstrated by biological stresses; the level of proline increased in tomato and tobacco plants parasitised by *Meloidogyne javanica* or *Agrobacterium tumefaciens*[14,15]. Sugarcane plant is subjected to biological stress by the attack of various diseases. One of the most important and common diseases is red rot which mainly affects the stalk of sugarcane. The present study was performed to observe proline accumulation in different parts of sugarcane plant as a result of stress by *Colletotrichum falcatum* Went, the causal agent of red rot disease.

Five-month-old healthy sugarcane plants (cv. CoJ 64) were inoculated with spores and mycelium of *C. falcatum* grown on oat meal agar medium. The inoculation was done in the third internode from ground level. The control plants were inoculated without fungus. After two months, tissue samples were collected from the inoculated plants. The stalk was split open and the tissue samples were taken from three places: (a) unaffected internodal tissue adjacent to soil level, (b) red rot affected portion near inoculation point, and (c) unaffected internodal tissue just above to rotted portion. Besides stalk, samples were taken from nodal buds (located near the inoculation point) and leaves (6th from top). Similarly, after 5 months of inoculation such samples were taken again for analysis.

The samples were frozen overnight, thawed and extracted with 90% ethyl alcohol (1:20, w/v) in a chilled mortar. The extract was centrifuged to remove activated charcoal. Further analysis was performed by the methods of Troll and Lindsley[16] and Meon *et al*[15] with minor modifications by adding 2 ml glacial acetic acid and 2 ml of freshly prepared acidic ninhydrin reagent (125 mg ninhydrin: 3 ml acetic acid: 2 ml of 6 M phosphoric acid). The transmittance was recorded at 520 nm. Free proline concentration was determined from the standard curve prepared from pure proline. The data were statistically analysed.

The results presented in table 1 show that there was pronounced rise in proline content of nodal buds, leaves and internodal tissue of the plant inoculated with *Colletotrichum falcatum* than uninoculated one (control). Earlier, similar findings were reported in plants infected with fungi[17], bacteria[14,15], viruses[18] and those parasitised by nematodes[15]. In 2-month-inoculated plants, proline content increased three folds (75.4 μg) in the nodal buds, while in leaves and internodal tissues a substantial increase was noticed. Out of three portions of internode maximum proline accumulation (140.2 μg) was found in rotted tissue followed by its lower (95 μg) and upper (87.2 μg) portions.

After 3 months, in 5-month-inoculated plants, significant increase in free proline was recorded in leaves (99.6 μg), and upper and bottom internodal portions (186.8 and 150.6 μg, respectively). Conversely, there was marginal decrease in the nodal buds (70.2 μg) and the rotted portion of the internode (137 μg).

These results indicate that consequent upon injury in the internode by the fungal pathogen, proline accumulation was induced in the adjoining plant parts to meet the enhanced energy requirement. Various workers[1,13] consider that during stress conditions

Table 1 *Changes in free proline content in sugarcane (cv. CoJ 64) plant parts after infection by Colletotrichum falcatum*

	Mean proline content (μg/g fresh wt*)				
	2 months after inoculation		5 months after inoculation		
Plant part	Diseased	Healthy	Diseased	Healthy	C. D. (5%)
Internode (I_1)	95.0	87.4	150.6	92.8	
Internode (I_2)	140.2	93.8	137.0	99.8	
Internode (I_3)	87.2	81.8	186.8	86.0	3.26
Leaf	94.8	60.2	99.6	59.8	
Nodal bud	75.4	25.0	70.2	24 8	

* Mean of 5 replicates; I_1-bottom portion adjacent to soil level; I_2-red rot affected portion; I_3-just above to affected part.

proline is synthesised from glutamate and, thus, serves as energy reserve. After 5 months of inoculation when considerable rotting occurred in the internode, proline content reduced in the rotted portion and adjoining nodal buds. This trend was suggestive of proline oxidation to fulfil the energy demand at the place of high metabolic activity.

The authors thank Dr Y. R. Saxena, for help and guidance.

13 June 1983

1. Barnett, N. M. and Naylor, A. W., *Plant Physiol.*, 1966, **41**, 1222.
2. Singh, T. N., Paleg, L. G. and Aspinall, D., *Aust. J. Biol. Sci.*, 1973, **26**, 45.
3. Rao, C. K. and Asokan, S., *Sugar J.*, 1978, **40**, 23.
4. Fukutoku, Y. and Yamada, Y., *Plant Cell Physiol.*, 1981, **22**, 1397.
5. Stewart, G. R. and Lee, J. A., *Planta*, 1974, **120**, 279.
6. Chu, T. M., Aspinall, D. and Paleg, L. G., *Aust. J. Plant Physiol.*, 1976, **3**, 219.
7. Draper, S. R., *Phytochemistry*, 1972, **11**, 639.
8. Chu, T. M., Jusaitis, M., Aspinall, D. and Paleg, L. G., *Physiol. Plant*, 1978, **43**, 254.
9. Withers, L. A. and King, P. J., *Plant Physiol.*, 1979, **64**, 675.
10. Mumford, R. A., Lipke, H. and Lanfer, D. A., *Environ. Sci. Tech.*, 1972, **6**, 427.
11. Godzik, S. and Linslens, H. F., *Environ. Pollut.*, 1974, **7**, 25.
12. Soldatini, G. F., Ziegler, I. and Ziegler, H., *Planta*, 1978, **143**, 225.
13. Dashek, W. V. and Erickson, S. S., *Bot. Rev.*, 1981, **47**, 349.
14. Seitz, E. W. and Hochster, R. M., *Life Sci.*, 1964, **3**, 1033.
15. Meon, S., Fisher, J. M. and Wallace, H. R., *Physiol. Plant Pathol.*, 1978, **12**, 251.
16. Troll, W. and Lindsley, J., *J. Biol. Chem.*, 1955, **215**, 655.
17. Bhansali, R. R., Sinha, O. K. and Singh, K., *Indian Phytopathol.*, 1983, **36**, 367.
18. Bozarth, R. F. and Diener, T. O., *Virology*, 1963, **21**, 188.

GENETICS OF MALE STERILITY IN A GENOTYPE OF CASSAVA

J. S. JOS and R. B. NAIR
Central Tuber Crops Research Institute, Trivandrum 695017, India.

THE germplasm of cassava (*Manihot esculenta* Crantz) maintained at this Institute comprises 1,320 genetic stocks assembled from different cassava growing countries of the world, and among them 60 clones are found to be male sterile. Cassava is heterozygous, cross-pollinated and generally propagated vegetatively. Male sterilities resulting from pachytene abnormalities[1], desynapsis[2], aberrant behaviour of tapetum[3], non-separation of microspores from tetrad[4] and functional male sterility[5] have already been reported. Among these about 40 clones exhibit male sterility caused by the non-separation of microspores and, due to its apparent importance an attempt has been made to understand the inheritance of male sterility in cassava.

The male sterile (MS) clone Ce-539 showing the characteristic non-separation of microspores, but having high female fertility, was selected and crossed with the male fertile (MF) clone Ce-613. Crosses were made between two other MF clones Ce-584 and Ci-326 and open-pollinated (OP) seeds were also collected from the MS 539, MF 584 and OP-4 clones. The resulting seedling progenies were screened for male sterility. Microsporogenesis and the sequence of pollen development in MS and MF clones were also compared.

Male fertile: The microsporogenesis was normal and tetrads lasted only for a short time (figure 1). The microspores separated and developed their own intine and exine. The tapetum showed signs of shrinking as the microspores separated and completely disappeared later (figure 2). The anthers were filled with pollen and the pollen fertility was above 90% (figure 3).

Male sterile: In the MS clone 539, the anthers were somewhat shrivelled and empty at the time of anthesis. The meiosis and early microsporogenesis was normal as in the case of MF clones. However, the microspores failed to separate from the tetrads and gradually degenerated (figure 4). The empty shells of the tetrads were discernible for a longer period (figure 5) but they also disappeared later. The tapetum was healthy and comparable with that of MF even at the time of early degeneration in the tetrads, but, later the tapetum also degenerated and disappeared leaving empty anthers (figure 6). In cassava, when male sterility was caused by the non-separation and degeneration of microspores,