EFFECT OF HYPERTHERMIA ON THE NITROGEN METABOLISM OF *PENNISETUM TYPHOIDES*

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(Communicated by S. M. Sircar, F.N.I.)

(*Received August 17, 1967*)

This investigation was undertaken to study the effects of hyperthermia on the nitrogen metabolism of *Pennisetum typhoides*. For this purpose alterations in the levels of various nitrogenous substances have been studied after exposing three-week-old plants to high temperature conditions (48°±1° C) for different durations up to 24 hours. Soil water stress was negligible during the experimental period.

High temperature conditions triggered off proteolysis leading to increased accumulation of soluble nitrogenous substances. Degradation of proteins brought about an increase in the level of amino acid nitrogen. Ammonia nitrogen was found in detectable quantities after 12 and 24 hours' treatment which caused injury to plants. It has been speculated that during the first six hours of heat treatment the level of ammonia was kept low by its increased neutralization with organic acids as a detoxicating measure. Although amide production was enhanced beyond this period, fast catabolic processes and possible limitations of organic acid turn-over caused an increase in the ammonia level. The results, therefore, suggest that increased organic acid production under hyperthermia may be a basis for heat hardiness in plants. Large increase in nitrate and nitrite nitrogen beyond six hours has been thought to be caused by progressive oxidation of ammonia. The mechanism of action of heat and moisture stress has been compared and differences in metabolic events have been outlined.

INTRODUCTION

Apart from drought, plants growing under arid conditions are often subjected to adverse supra-optimal temperatures. In dry habitat, heat stress is frequently associated with moisture stress, where plants try to evade overheating by increased transpiration. However, hyperthermia, as such, is known to bring about marked changes in the metabolism of plants. Results of various investigations (Henckel 1964) suggest that heat stress causes increased proteolysis. This leads to accumulation of soluble nitrogenous substances, particularly of ammonia, which being toxic inflict injury to plants. Other metabolic alterations like reduced photosynthesis (Lundegord 1937), disturbances of metabolic reactions (Belehradek 1935), adverse effects on respiration (Goodman and Wedding 1956) may be explained on the basis of changes in proteins and changes in configuration and activity of enzymes.

In this relation, temperature mediated increase in organic acids and associated decrease in respiratory quotient has been thought (Petinov and Molotkovsky 1962) to be a direct confirmation of Prianishnikov's (1945) classical postulation, where the existence of an efficient mechanism for increased amide formation has been envisaged, as a measure to render the ammonia (arising from protein degradation) harmless. Proteolysis was also thought (Mothes 1928, 1931, 1956; Petrie and Wood 1938) to be the primary effect of water stress. However, subsequent findings (Zholkerich and Koretskaya 1959; Lahiri and Singh 1968) indicated that in the initial stages of moisture deficit synthesis of proteins is primarily affected and hydrolysis, if any, may be associated only under extreme conditions of wilting. Possibilities of the existence of a similar action mechanism under heat stress still remains a matter of speculation.

In view of the above there seems to be need for detailed investigation on the impact of high temperature on the levels of various nitrogenous substances of plants which may furnish information on the role of amides and depict the trend of nitrogen metabolism under hyperthermia. Present study on *Pennisetum typhoides*, a major crop of the arid areas of Rajasthan, was undertaken to throw light on these problems.

Material and Methods

Seeds of *Pennisetum typhoides S* and *H* (var. T55) were sown on 1-3-66 in earthen pots of 30 cm diameter, containing a mixture of sand, soil and farmyard manure (*ca.* 1:2:1). The pots were kept in the open and were watered regularly to field capacity up to the third week when the plants were approximately 12–14 cm high. Each pot contained eight seedlings. Ten of these pots at this stage were randomly chosen for this experiment from a total of 80 pots. Eight of these pots were kept at $48°\pm1°$ C and in darkness in a 'Labmaster' forced-draft oven, where water was also kept in a separate container in order to maintain high humidity. Soil moisture and tissue moisture were determined at 0, 6, 12 and 24 hours from two of the treated pots. Two of the remaining treated pots were removed at 6, 12 and 24 hours respectively after the onset of temperature treatment and on each occasion, whole of the above ground shoot portions were harvested from both the pots for the purpose of analysis. Similarly, samples were also collected from two other untreated pots at the beginning of the experiment.

The nitrogen fractions estimated were total, soluble, amide, amino acid, nitrate and nitrite and ammonia nitrogen. The total and soluble nitrogen (from the filtrate after addition of tri-chloro acetic acid to the aqueous extract) were determined by Micro-Kjehldahl technique and the protein nitrogen content was assessed from the difference of the two. Analyses of other nitrogen fractions were carried out according to methods suggested by Sircar

and Datta (1957) with minor modifications as described earlier (Lahiri and Singh 1968). Mean of three determinations has been presented in all cases and quantities of nitrogen have been expressed as mg per 100 g dry tissue. Apart from these, soil moisture (as percentage of dry weight) and tissue moisture (as percentage of fresh weight) contents were also noted at the time of each sampling.

Results

From Fig. 1 it may be observed that the tissue moisture content and soil moisture showed only a minor decrease up to 12 hours of exposure to high temperature. During 0 to 12 hours soil moisture decreased from 12·90 to 11·07 per cent and the tissue moisture fell from 78·30 to 73·01 per cent. However, with 24 hours of exposure to high temperature tissue dehydration was aggravated (45·1 per cent) and soil moisture decreased further to 9·75 per cent. Nevertheless, soil moisture was not limiting at this stage since the permanent wilting percentage was around 3·5–4·0.

During these 24 hours of heat treatment, proportion of protein nitrogen content progressively decreased and this was associated with a concomitant

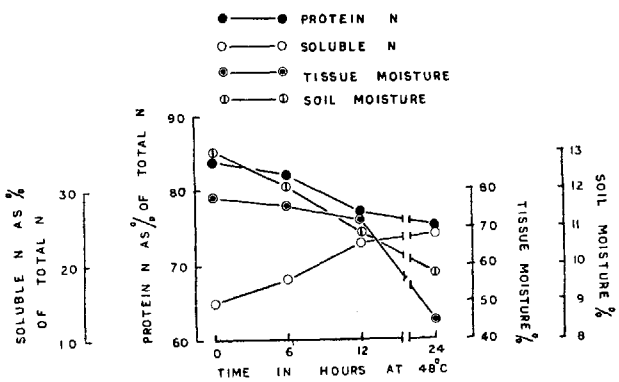

FIG. 1. The course of changes in soil moisture, tissue moisture, and in the relative proportions of protein and soluble nitrogen during the period of high temperature treatment.

increase in the proportion of soluble nitrogen content (Fig. 1). This suggested increased proteolysis with increasing duration of heat treatment. However, a situation was encountered in an earlier study (Lahiri and Singh 1968) where increase in the proportion of soluble nitrogen was brought about not by proteolysis but due to blocking in the pathway of protein synthesis.

Therefore actual changes in the quantities of total, soluble and protein nitrogen were examined (Fig. 2). It was found that the total nitrogen content of shoot tissue remained more or less unchanged up to 12 hours of heat treatment. This suggested that the transport of nitrogen from roots, if any, was negligible. Samples collected after 24 hours of heat treatment showed

rather a high nitrogen level which could be only due to biological variations. The protein nitrogen content decreased progressively from 0 to 12 hours. Relatively higher protein nitrogen content after 24 hours of heat treatment could be only due to higher total nitrogen value. Nevertheless, from the course of the curve for soluble nitrogen it becomes apparent that longer durations of heat treatment caused increased proteolysis.

In this respect the relative changes in the levels of different components of soluble nitrogen are of special interest. In Fig. 3(*a*), the alterations in the levels of amino acid nitrogen, amide nitrogen and ammonia nitrogen have been illustrated. The amino acid nitrogen tended to increase from 6 hours of treatment and the level progressively increased with longer duration. However, ammonia nitrogen increased to become detectable only after 12 hours of temperature treatment. Slower increase in amide nitrogen content observed during the first six hours was followed by faster rise in level during the next six hours. Thereafter concentration increases but the rate declines.

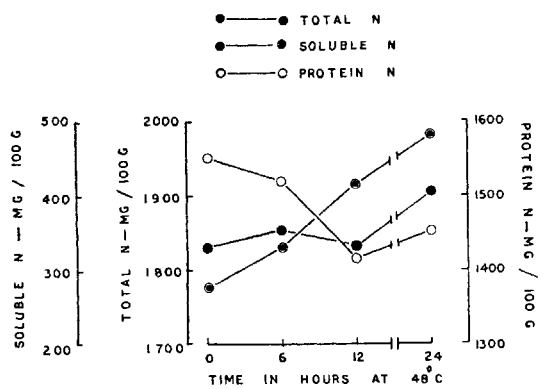

FIG. 2. The course of changes in total, soluble and protein nitrogen content during the period of high temperature treatment.

Changes in levels of total amino nitrogen and nitrate and nitrite nitrogen have been illustrated in Fig. 3(*b*). Total amino nitrogen increased slightly during the first six hours. But its level increased more after 12 hours. The rise in concentration between 12 and 24 hours was faster in comparison with the preceding six hours. The level of nitrate and nitrite nitrogen hardly showed any increase between 0 and 6 hours but the level tended to increase sharply thereafter up to 24 hours.

It may be mentioned here that high temperature treatment for six hours did not produce any visible adverse effect on plants. However, after 12 hours of treatment leaves showed slight yellowish discontouration of leaves and drying of leaf apices. These symptoms were much aggravated after 24

hours' treatment and plants had a 'scalded' appearance. Plants treated for 12 and 24 hours did not revive at room temperature.

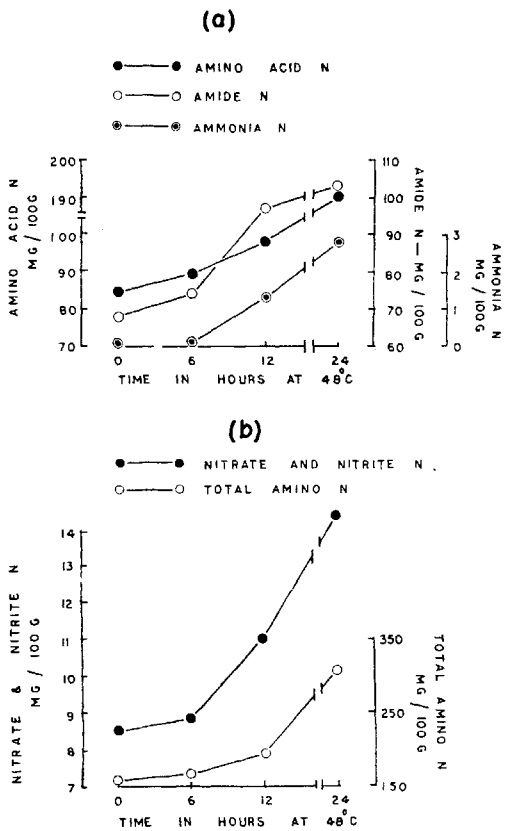

FIG. 3. (*a*) The course of changes in amino acid nitrogen, amide nitrogen and ammonia nitrogen during the period of high temperature treatment. (*b*) The course of changes in nitrate and nitrite nitrogen and total amino nitrogen during the period of high temperature treatment.

DISCUSSION

The results presented here indicate that the observed changes in nitrogen metabolism were primarily due to heat stress and effects of water stress, if any, were associated only when the plants were left at 48° C for 24 hours. Such high temperature, even in arid areas, is rarely encountered and never known to persist more than 4 to 4½ hours in the afternoons. However, long durations of heat treatment have been given in this investigation only to obtain a magnified and progressive effect on the nitrogen metabolism of *P. typhoides*, which being a crop of arid areas is known to be a fairly heat tolerant plant. Heat tolerance, as such, seems to be extremely variable. The capacity of the seed to resist heat cannot be compared with that of the seedling. In view of

this it seems that information obtained on the effects of hyperthermia on the three-week-old plants of *P. typhoides* may not be strictly comparable with the effects of temperature stress at other developmental stages of this plant.

The results suggest that supra-optimal temperature primarily brings about degradation of proteins causing an increased accumulation of soluble nitrogen. Although protein synthesis and breakdown are independent processes (Yemm 1949), it may be presumed that synthesis of protein, as such, stops completely under hyperthermia. It has been mentioned earlier that the observed increase in protein nitrogen after 24 hours of heat treatment could only be due to variation in the total nitrogen content in the sample. Even at this stage protein nitrogen content was much lower than its initial level at the 0 hour.

Protein degradation caused a direct increase in the level of amino acid nitrogen. The catabolic process, however, was slow during the first six hours which increased manifold during the next six hours. This is evidenced from slight increase in total amino nitrogen between 0 and 6 hours. Moreover, decrease of protein nitrogen was only 6·1 mg/h during first six hours and during the second six hours reduction in protein nitrogen was of the order of 16·6 mg/h.

Absence of detectable ammonia nitrogen even after six hours of heat treatment indicates high heat hardiness of this plant. It is tempting to speculate that during this period the level of ammonia was kept low by its increased incorporation with organic acids, as a detoxicating measure, and thus level of amide nitrogen showed an increase. Although the amide level showed a progressive increase at 12 and 24 hours, indicating an effort by the plant to normalize the level of ammonia, fast catabolic processes and possible limitations of organic acid turn-over brought about an unavoidable increase in the level of ammonia nitrogen beyond six hours. These suggest that ammonia toxicity is the primary cause of heat injury.

In view of these it follows that heat-hardy plants should be efficient in increasing the level of organic acids under temperature stress by high activity of enzymes present in the oxidizing cycle of respiratory substrate. In the chain of enzyme systems that ensures the continuation of oxidizing respiratory processes, dehydroginase activity is known to be a principal regulating factor. Demonstration of greater increase in dehydrogenase activity in heat resistant plants as compared to non-heat resistant plants under high temperature conditions (Petinov and Molotkovsky 1962) further suggests that the capacity of greater reinforcement of organic acid may be main metabolic adjustment of heat-hardy plants. Since this is a preliminary study, it is rather difficult to reach a definite conclusion. Further investigations on this problem therefore seem necessary to establish this action mechanism.

It has again been observed that the nitrate and nitrite nitrogen increased sharply after six hours of temperature treatment. Since the transport of nitrogen from the roots was not possible, this increase in the level of nitrate

and nitrite could be brought about by progressive oxidation of ammonia which was present in excess in the tissue. Under such circumstances oxidation-reduction potential should shift towards oxidation which is contrary to normal chain of events in nitrate reduction process. However, evidences (Petinov and Molotkovsky 1962) of excessive accumulation of oxygen in the tissue and consequently of oxidized compounds under hyperthermia lends support to this possibility.

In view of certain apparent similarities in the action of heat and moisture stress on nitrogen metabolism, it may be pertinent here to compare the action mechanism of both these processes. In this context earlier work (Lahiri and Singh 1968) on the effect of moisture stress on this plant helps us to define the differences. Although accumulation of soluble nitrogenous compounds is the resultant effect of both temperature and moisture stress, hyperthermia primarily triggers off proteolysis while dehydration causes impediment of protein synthesis. Under water stress, protein degradation may occur when the plants have wilted. Thus accumulation of amino acids under two types of stresses is brought about by different means. The tendency to counter the ammonia toxicity by increased amide formation has been found both under temperature and moisture stress. Under moisture stress evidences strongly support unimpaired transport of nitrogen from roots but this process stops under supra-optimal temperature. Nitrate and nitrite accumulation in water-deprived plants is caused by inactivation of reductases but under heat stress this accumulation is brought about by oxidization of ammonia.

Since amide synthesis has been found to be the key mechanism for binding the free ammonia in both moisture and temperature stress, level of organic acids seems to be the main determining factor for the assessment of relative hardiness. Although it is appreciated that divergent and involved problems of heat and drought hardiness may not be resolved by an unidirectional approach, detailed investigations on the organic acid metabolism may throw some light on these complex issues at this juncture.

Acknowledgements

Thanks are due to Dr. B. B. Roy, Head of the Division of Basic Resource Studies and Mr. C. P. Bhimaya, Director, for providing facilities and encouragement in the prosecution of this investigation.

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