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Changes in Soluble Amino Nitrogen, Protein, Nitrate Reductase Activity and Abscisic Acid during Development of Wheat Grain

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Abstract. Trends in the time course, changes in the moisture, soluble amino acids, proline, abscisic acid contents and nitrate reductase activity determined by *in vivo* method in the developing seeds of wheat were studied. Maximum dry matter augmentation in the seed took place in the period between 10—30 days after anthesis. Per cent moisture and moisture content started declining 15 days and 25 days after anthesis, respectively. Levels of soluble amino acids, proline and nitrate reductase activity were higher during initial stages of seed development, but decreased with increasing magnitude of dehydration and accumulation of abscisic acid (ABA) in the maturing seeds.

The quality and quantity of seed protein in the cereals can be improved by an increase in the rate of the assimilation of substrate nitrogen and its efficient transport to the developing seeds. Nitrate reductase activity of the leaf blade of wheat is positively correlated with seed protein and grain yield (SINGH *et al.* 1977). Evaluation of the complete reduction of NO_3^- reaching up to flag leaf of wheat is suggestive of the operation of a barrier to the movement of NO_3^- from the leaf and stem to the developing ear-heads (NAIR and ABROL 1977). However, DONOVAN and LEE (1978) have unequivocally proved that detached wheat ear-heads are capable of incorporating NO_3^- into amino acids and ultimately into proteins, and nitrate reduction by nitrate reductase is not a limiting factor for the synthesis of proteins in the developing grains. Demonstration of the nitrate reductase activity in the immature seeds is suggestive of a possibility of an immediate supply of reduced nitrogen to be used in the formation of amino acids and proteins (SINGH and SINGH 1976).

The process of induction of nitrate reductase in plant tissues is very sensitive to dehydration and desiccation stresses (MATTAS and PAULI 1965). As a result of a drop in the water potential, certain metabolites, like abscisic acid and proline accumulate in the plant tissues (ASPINALL *et al.* 1973). Unlike other plant structures, seed at ripening and maturation phases

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experiences dehydration induced inhibition in protein synthesis (SINGH and VIJAYAKUMAR 1974) and lipid synthesis (SINGH *et al.* 1971). In this paper we report on *in situ* induction of nitrate reductase and its significance in the synthesis of amino acids and proteins in developing wheat seeds.

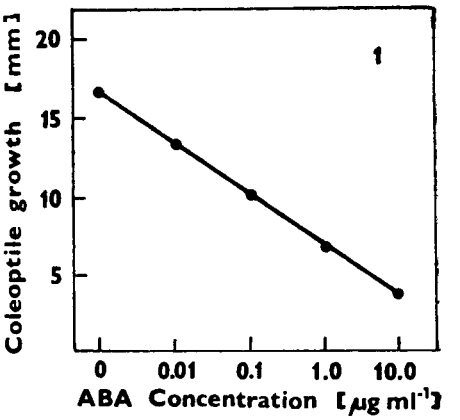

Fig. 1. Standard growth curve of wheat, cv. C-306, coleoptile in response to a range of ABA concentrations.

MATERIAL AND METHODS

A wheat crop (*Triticum aestivum* L.) cv. Kalyan sona which gave 4.56 t ha^{-1} grain yield during 1978—79 at Ludhiana (30° 56N latitude, 75° 52E longitude and 247 m above sea level) has been used in the present investigations. Ear heads on the main culm were tagged at anthesis phase and samples were

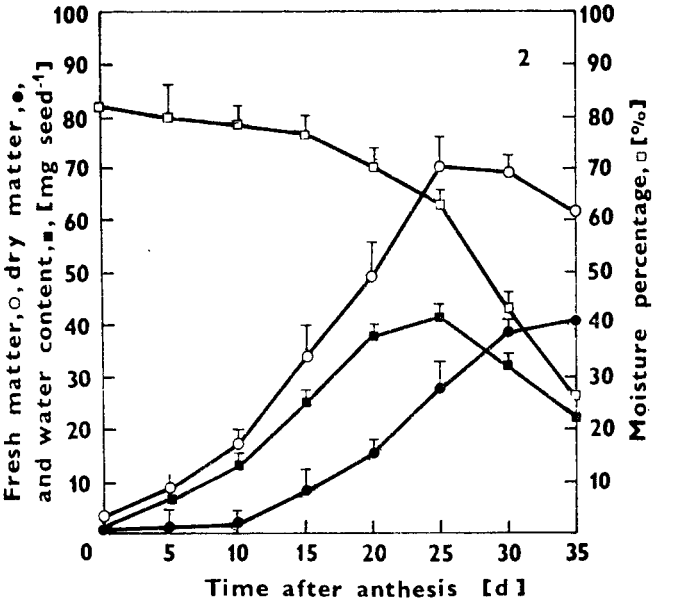

Fig. 2. Time course changes in fresh matter, dry matter, water content and moisture percentage in developing seeds of wheat, cv. Kalyan sona.

TABLE 1

Time course changes in nitrate reductase activity, soluble amino acids, soluble proline and protein contents in developing seed of wheat, cv. Kalyan sona

Days after anthesis	N.R. activity [μmol NO_2^- g^{-1} d.m. h^{-1}]	Soluble amino acids [mg g^{-1} d.m.]	Soluble proline [mg g^{-1} d.m.]	Protein [mg g^{-1} d.m.]
0	2.84 ± 0.26	88.52 ± 6.5	6.05 ± 0.45	52.68 ± 5.4
5	3.61 ± 0.31	92.83 ± 5.6	8.26 ± 1.15	58.50 ± 6.2
10	5.20 ± 0.41	86.67 ± 4.6	10.58 ± 1.40	65.86 ± 6.8
15	4.62 ± 0.20	79.66 ± 3.4	14.62 ± 1.62	76.56 ± 3.6
20	2.43 ± 0.41	71.26 ± 2.8	4.51 ± 0.60	102.80 ± 7.1
25	2.10 ± 0.15	43.60 ± 4.5	3.22 ± 0.40	123.76 ± 4.8
30	0.62 ± 0.16	12.84 ± 2.2	2.10 ± 0.22	124.40 ± 4.2
35	0.06 ± 0.03	9.38 ± 2.5	0.86 ± 0.12	124.45 ± 4.6

collected at random. Seeds from the central five spikelets and from the outer florets were collected at 5-day intervals, starting from anthesis till 35-day stage of complete seed maturity during March—April 1979. After dissection, fresh weight and dry weight were recorded to find out the moisture and dry matter content of the seeds. Soluble amino acids (LEE and TAKAHASHI 1966), soluble proline (BATES *et al.* 1973) and protein (LOWRY *et al.* 1951) were estimated. Nitrate reductase in the developing seeds was assayed by *in vivo* method of Hageman and FLESHER (1960). Seeds were collected for the extraction and purification of free and bound abscisic acid according to the methods of GOLDSCHMIDT *et al.* (1973). Wheat coleoptile elongation bioassay tests (GOLDSCHMIDT and MONSELISE 1968) were followed for detection and biological activity of growth inhibitors against standard curve prepared with synthetic abscisic acid (Fig. 1).

RESULTS

Time course changes in fresh and dry matter, water content and moisture percentage in the developing seeds are depicted in Fig. 2. Initially a lag phase in the increase in dry weight of seeds existed for 10 days after fertilization. A linear increase in fresh matter as well as dry matter of the seed was recorded in between 10-day and 30-day stages. Water content of the seed attained a peak at 25-day stage and then started declining to the lowest level at maturity.

Maximum concentration of soluble amino acids was obtained at the 5-day stage, thereafter it decreased in a gradual fashion, attaining the lowest level at the 35-day stage. Free proline in the seed rose to the maximum level at the 15-day stage and decreased thereafter. The level of protein in the developing seeds was stabilized by the 25-day stage, which coincided with the start of the dehydration of the seeds.

Nitrate reductase activity was observed in the ovules even at the anthesis phase. Activity of the enzyme was found to increase up to the 15-day stage. At the later stages of seed development, NR activity decreased to a low level at the terminal phase of seed maturation. Content of free abscisic acid continued increasing up to the 25-day stage and started declining after-

TABLE 2

Time course changes in the levels ($2\times$ $\mu g\ g^{-1}$ dry. m.) of free and bound abscisic acid in the developing seeds of wheat, cv. Kalyan sona. Determinations were made by wheat coleoptile bioassay and paper and thin layer chromatography in isopropanol : ammonia : water (10 : 1 : 1. v/v/v). Results based on the analysis of 5 g fresh m. seed, have been reported as $\mu g\ g^{-1}$ dry. m. of the seeds

Component	Days after anthesis							
	0	5	10	15	20	25	30	35
Free ABA	4.0	4.6	5.0	16.3	22.8	24.1	17.1	12.2
Bound ABA	1.0	1.1	1.9	4.4	6.1	17.1	26.3	29.4
Bound: free ABA	2.0	0.2	0.3	0.2	0.2	0.7	1.5	2.4

wards. Maximum level of free abscisic acid was obtained at the time of the start of moisture loss and inhibition in protein synthesis in the seed. A continuous increase in the quantity of bound abscisic acid, from the anthesis to seed maturity was observed. A shift in the ratio of free to bound abscisic acid towards maturity indicated the possibility of their interconversion (Table 2).

DISCUSSION

Post-anthesis changes in the developing seeds need an ample quantity of nitrogen for the synthesis of structural, functional and storage proteins. Various sources through which nitrogen becomes accessible to the seeds are (1) reduction of NO_3^- in the leaf blades by nitrate reductase and the transport of resultant reduced nitrogen (GROVER *et al.* 1978), (2) proteolysis of leaf and stem protein through proteases and transport of the soluble amino acids and amides thus released (NAIR *el al.* 1978), and (3) reduction of NO_3^- in the developing seeds through nitrate reductase activity (SINGH and SINGH 1976). In their study of grain development in detached wheat ear-heads, DONOVAN and LEE (1978) pointed out that nitrogen metabolism of the grain in liquid culture was parallel to that of the intact plants, regardless of the nitrogen source present in the medium. Developing seeds *in vitro* had the potentiality of using nitrate as well as ammoniacal nitrogen in order to synthesize the proteins quite comparable with the seeds developing on the intact plant.

Primarily, nitrate reductase is a substrate inducible enzyme and therefore it is the nitrate level in the metabolic pool which actually determines the level of nitrate reductase (ASLAM *et al.* 1976). It thus becomes pertinent to visualize the reduction of NO_3^- by nitrate reductase in the developing seeds of wheat. A positive relationship between NR activity, soluble amino acids, protein and moisture content of the seed up to the ripening phase, confirms this contention (Table 1). At present, work is in progress to find out the effect of endogenous as well as exogenous supply of NO_3^- on the induction of nitrate reductase in the developing seeds in order to develop

an understanding of the regulatory function of nitrate reductase in NO_3^- reduction and protein synthesis in the developing seeds.

Protein synthesis in non-growing tissue is very susceptible to moisture stress, as in the case of rapidly growing tissues (HUFFAKER *et al.* 1970). It follows that dehydration of the seeds at the maturation dormancy phase should depict the physio-chemical changes transduced by moisture stress. Loss of water beyond a critical limit is imperative to the cessation of protein synthesis in the maturing seed (SINGH and SINGH 1975). Decrease in the NR activity and accumulation of abscisic acid in the maturing seeds confirmed such changes which are consistent with the drop in tissue water potential (PLAUT 1973). It would be a difficult proposition to isolate the individual as well as combined effect of dehydration and abscisic acid on NR activity and protein synthesis in the maturing seed. Quite possibly, accumulation of abscisic acid might be associated with the termination of protein synthesis, which is a prerequisite for the physiological maturity of the seed (KLEIN and POLLOCK 1968). However, the process of attainment of an elevated level of abscisic acid and decrease in that of proline in the maturing seeds is inconsistent with the general contention that decrease in tissue water potential results in the accumulation of both (ASPINALL *et al.* 1973).

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BOOK REVIEW

NEWTON, W. E., OTSUKA, S. (ed.): MOLYBDENUM CHEMISTRY OF BIOLOGICAL SIGNIFICANCE. — Plenum Press, New York—London 1980. 425 pp. US $ 39.50.

As a reflection of the current interest in molybdenum chemistry stemming from its importance in biology, the International Symposium of Molybdenum Chemistry of Biological Significance was held at Lake Biwa, Japan, April 1979. The Proceedings of the Symposium represent one of the most specialized books devoted to the molybdenum chemistry and biochemistry published in the last several years.

As can be expected, most of the contributions presented there were devoted to the role of molybdenum in the enzymes of nitrogen assimilation (*e.g.* nitrogenase, nitrate reductase) and to reactions and properties of molybdenum complexes which have been now frequently used as powerful tools and models for understanding the structure and function of the molybdenum-containing enzymes.

Thirty two contributions, most of them having the character of a short review, have been arranged into three chapters: Molybdoenzymes, Iron-Molybdenum Cofactor and Mo-Fe-S Cubane Clusters, and Reactions and Properties of Molybdenum Complexes. The Proceedings bring a lot of evidence showing molybdenum to be crucial for the activity of at least five enzymes catalyzing redox-type chemical reactions: nitrogenase, nitrate reductase, xanthine oxidase, sulphite oxidase and aldehyde oxidase. However, nitrogenase has been in the centre of the Symposium's interest, ranging from the physiological level of the nitrogenase studies (*e.g.* C_2H_2 Reduction and $^{15}N_2$ Fixation in Blue-Green Algae by M. Ohmori and A. Hattori) through nitrogenase biochemistry (*e.g.* Electrochemical and Kinetic Studies of Nitrogenase by G. D. Watt, or Electron Partitioning from Dinitrogenase to Substrates and the Kinetics of ATP Utilization by R. H. Burris and R. V. Hageman) up to the very deep analysis of the nitrogenase proteins, MoFe-protein subunits, FeMo-cofactor and metal centres of nitrogenase (*e.g.* Chemical Properties of the Fe-Mo Cofactor from Nitrogenase by W. E. Newton *et al.*). A degree of the structure similarity between the nitrogenase and nitrate reductase metal-containing proteins and subunits was studied by W. H. Orme-Johnson *et al.* and by D. J. D. Nicholas.

In order to understand the real structure and function of the molybdenum-containing enzymes in detail a broad spectrum of molybdenum complexes has recently been synthesized. As is seen from the Proceedings these have been intensively studied from the point of view of their structure, redox properties, reactivity, and binding sites for dinitrogen and for additional substrates. Using different molybdenum complexes J. Chatt, for example, has proposed a mechanism for degradation of dinitrogen to ammonia showing some dinitrogen hybride intermediates (*e.g.* Mo$\cdots$N$\cdots$$NH_2$) to be involved in the dinitrogen reduction.

For those interested in the application of different modern methods, a set of them has been described in the Proceedings such as the EPR-, UV-, Mössbauer-, and XAS-spectroscopy, immunoreaction and so on.

Finally, let me recommended this book as a source of new information on the results in this specialized and very important field of research.

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