Nitrogen Metabolism In Developing Wheat Grains

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Abstract: Nitrogen plays a key role in high agricultural production. It is mostly taken up as nitrate which is reduced to ammonia before being assimilated into amino acids and proteins ultimately. The protein content is one of the principal qualities of the wheat grain. The quality and quantity of grain protein in the cereals can be improved by an increase in the rate of assimilation of substrate as nitrogen and its efficient transport to the developing grains. The present study was undertaken to investigate total nitrogen, proteins, free amino acids and a few enzymes involved in nitrogen metabolism such as nitrate reductase and protease.

Key words: Days after anthesis (DAA), Ear heads, Nitrate reductase, Protease.

Introduction

Changes in nitrogen content: Sofield et al (1977) studied wheat grain development in relation to nitrogen metabolism and observed that nitrogen contents of grains increased linearly throughout the grain growth period. Percent of nitrogen fell sharply during the first few days after anthesis (DAA) but rose progressively thereafter. Hence, a continuous steady uptake of nitrogen by grain at maturity suggested that these nutrients did not regulate the duration of dry matter accumulation. Compositional changes were noticed in the developing grain of high- and low-protein wheats (Donovan,1979; Donovan et al,1977) and maximum nitrogen content/grain was reported two weeks before maturity and subsequent losses may be due to the movement of soluble material from grain to other parts of the ear. Further Donovan and Lee (1978) studied in cultured wheat ear heads that nitrogen nutrition does not play a major role in grain dry weight accumulation as there was no relationship between per cent protein or nitrogen and yield. This was in accordance with the commonly held view that application of nitrogenous fertilizer to the crops after heading resulted in little or no increase in yield but did give grain of higher protein content. In intact plant, grain nitrogen is derived either from nitrogen reduced during grain filling or from mobilization of nitrogen reduced during vegetative stages of development or from both sources. They also proved that detached wheat earheads were capable of incorporating NO^{3-} into amino acids and ultimately into proteins and therefore nitrate reduction by nitrate reductase is not a limiting factor for the synthesis of proteins in the developing grains. The kinetics of nitrogen accumulation and electrophoretic pattern of proteins in developing cultivars of wheat was studied by Bollini et al (1981). In all the cultivars, grain nitrogen content increased up to 5th week after anthesis. Campbell et al (1981) also observed a similar trend in developing wheat grains from 6th or 7th day onwards after anthesis.

Protein Synthesis: Flint et al (1975) observed that synthesis of most of the endosperm proteins occurred in wheat grains during maturation. After 20 days of maturation, the rate of synthesis of high molecular weight proteins declined more rapidly than those of low molecular weight proteins. Gupta et al (1976) also noticed synthesis of proteins rich in lysine declined progressively, preferentially at later stages of maturation. Although physiological maturity occurred at 35-40 days but maturation was complete within approximately 48 days after anthesis according to Campbell et al (1981). Synthesis of proteins continued until physiological maturity and was followed by grain dehydration associated with disruption of cellular structures. Loss of water beyond a critical limit is imperative to the cessation of protein synthesis. It is quite possible that accumulation of ABA at that time (may be caused by water stress induced by dehydration) might be associated with the termination of protein synthesis which is pre-requisite for the physiological maturity of seeds. Level of soluble amino acids was also higher during the initial stages of seed development but decreased with increasing magnitude of dehydration (Singh and Vijaykumar, 1981).

Nitrate reductase: It has been suggested that flow of nitrate nitrogen into amino acids can be controlled by regulating the activity of nitrate reductase. Therefore, a quantitative measurement of its activity could provide a quantitative estimation of reduced nitrogen input. Naik et al (1982) have suggested that nitrate reductase (NR) is under a complex regulatory mechanism. Generation of NADH through a supply of metabolites and prevention of oxidation by oxygen creating thereby anaerobic conditions are the major factors regulating nitrate reduction (Subbalakshmi et al, 1979). Demonstration of NR activity in immature seeds is suggestive of a possibility of an immediate supply of reduced nitrogen to be used for the formation of amino acids and proteins.The process of induction of NR in plant tissue is very much sensitive to dehydration and desiccation stresses during developmental stages. Croy and Hageman (1970) correlated increased NR activity in wheat with increased grain protein. In developing wheat grains, an increase in activity of NR was noticed until 15-20 days after anthesis and activity declined thereafter and at about 32-35 days, little or no activity was detectable (Nair and Abrol, 1973). Calculation on nitrite release basis revealed that between 10 and 32 days after anthesis, the ear contributed significantly to nitrate assimilation. A significant positive correlation between NR activity and beginning of accumulation of reduced nitrogen in wheat was suggested by Dalling et al (1976) and was found to be the limiting factor. It was disputed by the contention of Simmons and Moss (1978) and Naik et al (1982) that during the later stages of ontogeny, nitrate supply rather than the enzyme *per se* is the most limiting factor. Lee (1978) suggested that glumes rather than grain were the primary sites of nitrate reduction and amino acids synthesis thus indicating that amino acids were not synthesized *in situ* but were translocated from other parts of the head also.

Protease: Protease activity of developing wheat grains was found to be high at the beginning of growth and activity decreased markedly thereafter with the accelerated loss of moisture from the grain (Rohrlich and Hitze, 1970). Bushak et al (1971) estimated proteolytic activity in grains of three cvs. of maturing wheat from 10 days after flowering to full ripeness and all of them showed a gradual decline in activity during maturation although the ripe grain still retained considerable activity. These changes in activity coincided with the exponential phase of grain growth immediately after anthesis and the cessation of grain growth at 35-40 days after anthesis suggested that grain growth may be implicated in governing the enzyme activity. The reason for fall in enzyme activity at about 40 days may have been simply due to exhaustion of suitable substrate in the maturing tissue. Presumably, the rate of nitrogen loss from a tissue is a function of the rate of protein degradation or the rate of removal of the products of protein degradation into the vascular system or both. Since a highly significant relationship was observed between acid proteinase activity and nitrogen loss, it seems reasonable to conclude that acid proteinase was limiting nitrogen loss.

Material and Methods

An approved variety of wheat (WL-1562) was selected for the present studies. Sowing was done in six replicates in the field. Ear emergence started after 100 days of sowing, followed by anthesis after about one week. Ear heads of the mother shoots were tagged on the day of anthesis (0 day) and collected after 10, 20, 30 and 40 days of anthesis (DAA) from the field. Grains from the outer florets of the spikelets present in the middle part of the earhead were used. Grain samples of known quantity were oven dried at 60^0C and used for biochemical parameters. Enzymatic analysis was done with fresh grains only.

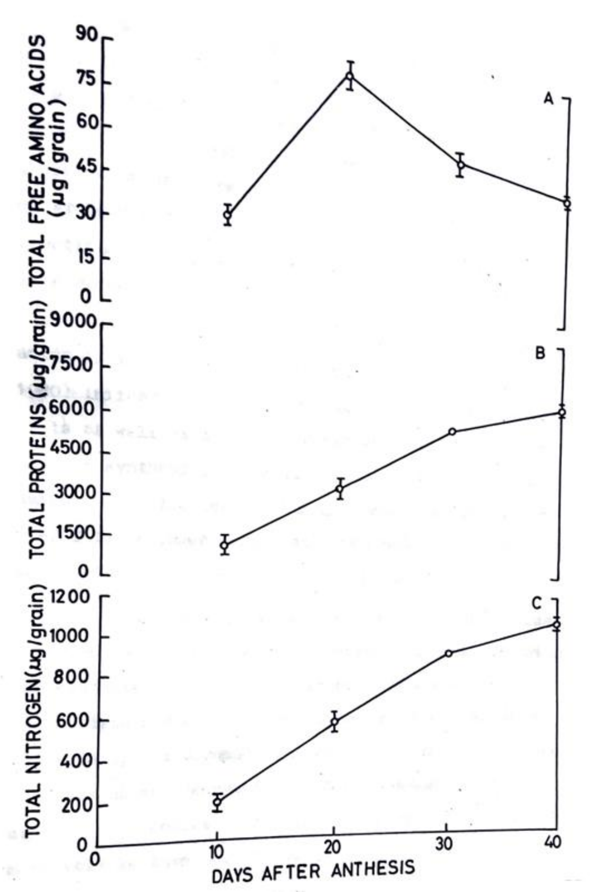
Method of Lee and Takahashi (1966) was adopted for the estimation of total free amino acids. Total nitrogen was determined according to the method of Novozamsky et al (1974). Total protein content was estimated by multiplying the total nitrogen content by conversion factor, 6.25. Nitrate reductase (NR) activity was determined by *in vivo* method of Jaworski (1971) and protease enzyme was assayed by the method of Penner and Ashton (1967).

Results and Discussion

Fig 1A depicts the trend of total free amino acids in the developing wheat grain. Total free amino acids level was minimum at 10 and maximum at 20 DAA followed by decrease thereafter. Amino acids are the precursors of storage proteins in cereal grains. A higher level of these amino acids at 20 DAA will contribute to a faster and greater accumulation of the protein bodies. High level of mRNA carrying the information for a particular protein to be synthesized was reported during the stage of maximum protein synthesis (Cruz et al., 1970).

Protein concentration (µg/grain) increased from 10 to 40 DAA (Fig 1B) High protein content of the grains might be the result of more efficient and complete translocation of nitrogenous compounds from the vegetative parts of the plants to the grains. Campbell et al (1981) reported that maximum protein synthesis continued until physiological maturity and proteins might have been degraded with grain dehydration causing the disruption of cellular structures.

Total nitrogen content (µg/grain) also increased till 40 DAA (Fig 1C). Slight decrease in N content towards maturity may be due to its redistribution to other plant parts. There are two possible sources of grain nitrogen in wheat: soil nitrate reduced during grain filling and redistribution of reduced nitrogen during the vegetative stage of development (Dalling et al, 1976). After anthesis, the developing grains become the dominant sink. Nitrogen nutrition probably changes the level of free amino acids in the developing grain, rather than the capacity of the grain to incorporate amino acids.



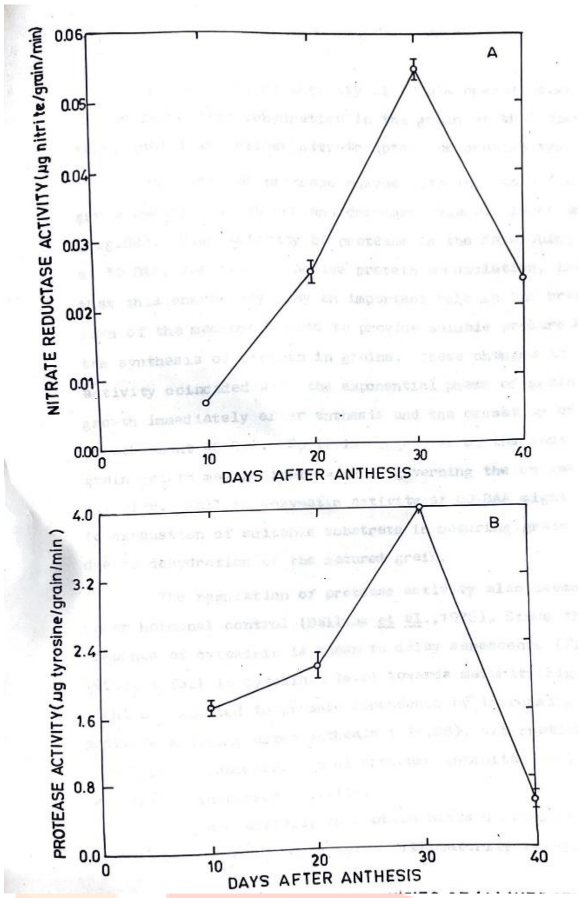


Fig 1: Time course changes in the contents of (A) Total free amino acids (B) Total proteins (C) Total nitrogen in grains
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Fig 2: Time course changes in the activities of (A) Nitrate reductase (B) Protease in the developing wheat developing wheat grains.

Nitrate reductase (NR) is one of the most important enzymes in the assimilation of nitrate, the predominant form of nitrogen available to the plants in soil. Activity of NR can give a good estimate of the nitrogen status of the grain (or any other plant part) available for protein synthesis. Level of N can be correlated with NR activity in the grain. The time course changes in the level of NR depicted in Fig 2A show that the activity increased with the age of the grains. Maximum activity was observed at 30 DAA which declined later on. Presence of NR in the developing wheat grain is of significance due to contribution factor towards the synthesis of amino acids (Nair and Abrol, 1973). There are two separate pools of nitrate in the tissues. One, the metabolic pool concerned with the regulation of the level of NR and other, the storage pool, with the supply of nitrate as the substrate. Christensen et al (1981) reported that photosynthetic capacity was inadequate for ear demands. Phytohormones are also known to exert their effect on NR activity. Gibberellins in combination with cytokinins increase NR in leaves and can also days substitute light for the maintenance of NR. Auxins also play a role in increasing NR. Decrease in NR activity at 30 DAA onwards might be due to prevailing dehydration in the grain at that time thus, inhibiting either nitrate uptake or protein synthesis. Low levels of NR at 40 DAA can also be correlated with the peak value of ABA at that time. Therefore, CK/ABA ratio is very important for the regulation of NR activity.

Activity of protease enzyme also decreased after 30 days of anthesis (Fig 2B). High activity of protease at 30 DAA, the time of active protein accumulation, indicates that this enzyme may play an important role in breaking of macromolecules to provide soluble precursor for the synthesis of proteins in the developing grains. These changes in activity coincided with the exponential phase of grain growth immediately after anthesis and the cessation of grain growth at about 40 DAA. Decrease in protease activity at 40 DAA might be due to exhaustion of suitable substrate in maturing grain or dehydration of the mature grain. Fall in cytokinin level towards maturity might be expected to promote senescence by increasing protease activity after anthesis. Alternatively, a decline in concentration of protease inhibitor could explain the increased activity.

Conclusion

Maximum accumulation of proteins occurred at 30 DAA and changed almost a little afterwards. Nitrate reductase and protease activity also increased up to 30 DAA and declined sharply later during development of wheat grains. Thus, the knowledge of grain development can be used as a tool for the study of the regulation of developmental processes.

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