

Some Aspects of Nitrogen Assimilation in Wheat Plant (*Triticum aestivum* L.) as Affected by Different Nitrate Levels and Bacteria Inoculation

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Abstract

The N metabolism of bread (yocora rojo) wheat (*Triticum aestivum* L.) plants, grown with different levels of nitrate (0.5, 1, 5 and 10 mM) in the media, was studied in a pot experiment with soil under optimum conditions in a growth cabinet. At all nitrate levels the activity of nitrate reductase (NAR) was higher in wheat shoots rather than the roots, and the proportion of the enzyme in the shoot to root was calculated to be 94 : 6 % respectively, The wheat shoot : root fresh weight ratio was increased (1.3 to 2.6) with elevated nitrate supply. Plants inoculated with *Azotobacter chroococcum* showed not much change in the distribution of the enzyme with alterations in nitrate supply. Most of the TCA-soluble nitrogen (N) was present as amino acids, nitrate and ammonium-N; and the levels found in both shoots and roots confirmed that internal reserves of N depends on approximate space value (ASV) and type of the N pools within the plant. The abundance of free amino acids in shoot pools and root pools fed with nitrate indicate that under experimental conditions the proportion of N being taken up as dissolved inorganic nitrogen (DIN) was released as dissolved organic nitrogen (DON). The free amino acids detected, including Asp, Glu, Asn, Ser, Gln, Thr and Ala, were remarkably high in the shoot and root. In contrast, others (including Arg, Tyr, Trp, Met, Val, Phe, Iso, Leu, Orn and Lys) showed similar variation pattern between shoot and root. The higher ratio of Gln : Glu in root suggesting most probably due to either a reaction involved in the reduction of the nitrate or to the activation of glutamine synthesis (GS). A conclusion was drawn that the higher level of glutamate than that of glutamine, may have been due to the presence of GDH-dependent glutamate synthesis in the shoot.

Key words: Amino compounds, inoculation of wheat, Nitrate reductase, *Triticum aestivum*, Wheat.

List of abbreviation: ASV =approximate space value, N= nitrogen, NAR=nitrate reductase, TCA= trichloroacetic acid, (TAA)= total amino acid, Gln= glutamine, Glu= glutamate

Introduction

Nitrogen (N) is one of the major mineral nutrients required for growth and development of plants, and plant growth rate is often dependent upon N supply (Mattson *et al.* 1991). It has also been shown that when factors other than N supply are limiting growth, there is a dependence between nitrogen uptake and the relative growth during N starvation (Macduff *et al.* 2001). Furthermore, the availability of different forms of N and heathland communities confer an advantage to the root surface to utilize N (Okamoto and Okada 2004). Genes regulating N recycling in barley have been observed to be located on chromosomes 6 (Mickelson *et al.* 2003). The mechanism for sensing

N status in plant cells is not well understood at the molecular level although it may be expected to be similar in all plants (Parsons and Sunley 2001). There is evidence that nitrate, ammonium, or amino acid may serve as signaling molecules in plants (Coruzzi and Bush 2001). It is generally recognized that, although plants may take up N in various forms, the main sources in natural conditions are nitrate and ammonium ions (Haynes and Goh 1978). On the basis of numerous studies, we can state that nitrate is the key form of the nitrogen supply for cereal plants. Almost all tissues of plants investigated are capable of synthesizing the complement of enzymes necessary to assimilate nitrate (Smirnov and Stewart 1985). In this connection, a study should be done to determine the

transfer, deposition, and assimilation of nitrate, which plays an important role in the regulation of synthesis and the function of nitrate reductase (NAR), the key enzyme in the reduction of nitrate, which determine the productivity of plants (Bulgakova *et al.* 1996). In nitrate-grown garden pea (*Pisum sativum* L.cv. Greenfeast), the root has a higher proportion of the total plant NAR activity than in most non-legumes (Hocking *et al.* 1984). This high activity of NAR in the legume root was correlated with the amount of reduced nitrogen. However, some plants contained only 6% of the total NAR activity (Atkins *et al.* 1980). The distribution of NAR in root and shoot may also be influenced by nitrate supply (Wallace 1986). The data in other studies showed that the amount of NAR activity in the root and shoot was relatively constant regardless of the level of nitrate supplied to plants (Andrews *et al.* 1984). The purpose of these studies was to use bread (yocora rojo) wheat (*Triticum aestivum* L.) plants as model of plant N physiology that enabled the implications for N addition and the distribution for in vitro NAR in root and shoot upon the effect of nitrate supply. Experimentation also, examined the potential impact of *Azotobacter chroococcum* on growth, with a view to its influence on the NAR activity and nitrate content. Additionally, most of the TCA-soluble nitrogen (N) was determined as type of N within shoots and roots. Finally, the abundance of free amino acids isolated from shoots and roots were analysed.

Materials and Methods

Growth of plants

Steriled seeds of bread (yocora rojo) wheat (*Triticum aestivum* L.) plants were selected for uniformity in size and appearance. Seeds (8-10 per pot) were planted gently to about 0.5 cm below the soil surface. Plastic pots with sterilized washed air – dried soils were used and placed in a growth cabinet (model 845) from Lab-line, USA, with a photoperiod ranging between 16h/8h light/dark cycle according to Lopez-Millan *et al.*, (2000). The average light intensity (300-500 lux) was provided by fluorescent tubes having a spectral composition similar to sunlight (see Siddiqi *et al.* 1991). The day/night temperatures were

18/21 °C at relative humidity 70-75 %. After 5 days in a germination cabinet all plants were watered daily with 25% L⁻¹ strength Hoagland solution (Hoagland and Arnon 1958) without N in which, KNO₃ and Ca(NO₃)₂ were replaced by K₂SO₄ and Ca SO₄. This replacement was done in order to have both K⁺ and Ca⁺⁺ ions in the solution. Wheat plants (7-day-old) were fed with the same strength of Hoagland solution with or without 0.5, 1, 5 and 10 mM nitrate as KNO₃ (applied in Hoagland solution). At the end of the experiments (22-days-old), plants were harvested, divided into appropriate organs, weighed and immediately processed for enzyme assays. Parts were placed in a freezer at -14 °C and later freeze-dried, ground and stored in sealed vials until analysis. All experiments were repeated 2 -3 times.

Plant inoculation

Wheat plants (7-day-old) were inoculated using a heavy suspension of *Azotobacter chroococcum* (1ml/plant) corresponding approximately 2×10⁷/ml. The suspension was pipetted in the form of drops on the surface of the soil.

Plant sampling

Wheat plants (22-day-old), were carefully removed from the soil and washed gently. Shoot and root were used as source of material throughout this work. plants were harvested from each treatment, their heights were recorded and they were then separated into shoots and roots. Fresh and dry weight samples (ten plant each) at 60 °C were estimated for each part.

Determination of total nitrogen

Samples of each plant part (shoots and roots) were analyzed for total nitrogen. This was carried out using micro Kjeldahl digestion and Markham distillation method described by Mckenzie and Wallace (1954).

Determination of soluble-N

Samples of each plant part (shoots and roots) were analyzed for soluble-N content. The composite of the dry material was extracted with cold 10% w/v aqueous trichloroacetic acid (TCA). The extracts were centrifuged for 15 min, at 3500g (IEC 218A Rotor), and the supernatant was used as source of soluble-

N present and assayed in duplicate according to the method described by Ricketts (1985).

Determination of nitrate-N

Samples of each plant part (shoots and roots) were analyzed for nitrate content. The composite of the dry material was assayed in duplicate according to the method described in Cataldo *et al.* (1975).

Determination of ammonium-N

Samples of each plant part (shoots and roots) were analyzed for ammonium-N content. The composite of the dry material was assayed in duplicate according to the method described by Ricketts (1985).

Determination of total amino acid

Samples of each plant part (shoots and roots) were analyzed for total amino acid (TAA) content. The composite samples of each plant part were recovered in the supernatant obtained and assayed spectrophotometrically in duplicate according to the method described by Matoh *et al.* (1980).

Amino acids analysis

Samples of each plant part (shoots and roots) were analyzed for free amino acids. A tissue sample was extracted by 3 ml HPLC grade water (80 °C for 30 minutes in acid washed glass). The homogenate was centrifuged at 3000g for 10 minutes, and the supernatant (3 ml) was forced down through a sterile 0.2µm Millex (Millipore) filter into each sterile plastic bijoux and analysis by HPLC of fluorescent o-phthalaldehyde derivatives of α-amino acids are described by Flynn and Al-Amoudi (1988).

Extraction and preparation for NAR

Shoots and roots of plant tissue were ground and homogenized at 4 °C with cold mortar and pestle in 0.1M Tris-HCl buffer pH 7.4, containing 0.5 mM EDTA and 5 mM cysteine. The extracts were centrifuged at 3100 g for 30 minutes at - 4 °C and used for NAR assay using the following reaction conversion of NO₃ to NO₂ according to the method of Hewitt (1975). The assay mixture contained 0.1 M sodium phosphate buffer pH 7.0, 0.00136 M NADH, 0.1 M KNO₃, 0.1 M HEPS buffer pH 7.4, and was

extracted in a final volume of 1.9 ml. The reaction was initiated by the addition of enzyme extract, incubated for 20 minutes at 27 °C. The reaction was stopped by the addition of 1ml of 1.0 % (w/v) N-(1-naphthyl) ethylenediamine-dihydrochloride solution. The absorbance was read at room temperature using a spectrophotometer at wavelength of 540 nm. The enzyme activity was calculated as µmoles of nitrite formed per mg fresh weight per hour.

Statistics

Statistical analyses were done with a ANOVA. Mean values ±SD from ten measurements (according to Lohaus *et al.* 2000). Different letters indicate significantly different values at a probability levels of $p < 0.05$. Graph was plotted using microcal origin version 5.0 as gaussian fitting.

Results

The uptake of nitrate was correlated to the amount of total N measured. At all nitrate levels (0.5, 1, 5 and 10 mmol m⁻³ nitrate) the activity was predominantly expressed in the shoot rather than the root. With elevated nitrate supply the proportion of the enzyme significantly ($P=0.05$) increased by the nitrate treatment (the correlation coefficient between the nitrate and enzyme by them was calculated to be 0.98), in comparison with the controls, in the shoot to root represented 94: 6% respectively, over the period of experiment (22-days-old). Thus, as the concentration of nitrate supply was increased, the root component of NAR shows survival rates initiated at both limits of the control and 0.5 mmol m⁻³ nitrate (11% proportion to the total); started to decline even under nitrate sufficient conditions (10 mmol m⁻³ nitrate) contributed only 2% by the end of experiment. The wheat shoot : root fresh weight ratio has increased (1.3 to 2.6) with elevated nitrate supply (Table 1). High levels of nitrate accumulation were observed in the shoot when 5 and 10 mmol m⁻³ nitrate were supplied, whereas <20% of the nitrate accumulated was detected in the root. It was assumed that all nitrate in the shoot came from the cytoplasm of root epidermal and cortical cells. The results indicated that NAR and nitrate content were maximum at 10

Table 1. Influence of nitrate supply on the distribution of NAR activity and nitrate.

Nitrate supply mmol	Plant part	FWg	S/R	Dwg	Nitrate reductase FWg total		Nitrate Fwg total	
0.5	shoot	0.24±0.02	1.33	0.034±0.01	0.71±0.04	0.17 (89)	8.5±0.2	2.0 (79)
1.0		0.33±0.01	1.38	0.49±0.002	1.42±0.09	0.47 (94)	10±0.8	3.3 (82)
5.0		0.40±0.03	1.74	0.062±0.002	1.5±0.07	0.60 (95)	11.2±0.5	4.5 (85)
10		0.88±0.05	2.60	0.16±0.005	3.56±0.3	3.1 (98)	12±0.7	10.6 (92)
0.5	root	0.18±0.01		0.027±0.003	0.1±0.01	0.02 (11)	2.9±0.3	0.52± (21)
1.0		0.24±0.02	ratio as calculated above	0.04±0.03	0.12±0.02	0.03 (6)	3.0±(0.1)	0.72 (18)
5.0		0.23±0.01		0.034±0.01	0.14±0.03	0.03 (5)	3.4±0.2	0.8 915)
10		0.34±0.03		0.055±0.05	0.19±0.01	0.06 (2)	3.0±0.3	0.09 (8)

Mean of 10 plants per replicate with standard errors, FW=Fresh weight, DW=Dry weight, S/R=FW shoot/root ratio.

mmol m⁻³ nitrate in shoot (98 and 92 %) respectively; whereas the ratio between the major and minor NAR in root was approximately 2–3 regardless of nitrate concentration (Table 1). The relative rate of dry matter accumulation was increased over the four treatments and appeared to be correlated with each other ($r = 0.96$) with the result that the highest nitrate treatment resulted the largest plants by the end of the experiment.

The survival rates of roots and the low NAR activity prompted us to investigate the potential benefit of inoculating the roots with free-living N₂-fixing bacteria. The controls and plants inoculated with *A. chroococcum* showed a low NAR enzyme activity in the roots at both N levels while inoculation had no change in the relative distribution of the enzyme with an alteration in nitrate supply (Table 2). Root length production increased in all N treatments with *Azotobacter* inoculation. During the course of the experiment, it was noticed that roots were thicker at low N (5 mmol m⁻³) than at high N supply (10 mmol m⁻³) with inoculation (data not shown). Length root / shoot ratio (R/S) also responded to the combined treatment (N & inoculum), and the differences in root/shoot ratio were significantly greater ($P \leq 0.05$) than the control which suggested that the bacteria favoured root growth more than shoot growth. Fresh weights of roots and shoots were significantly ($P=0.02$) increased by the inoculum in comparison with the controls, especially at the higher N level. A greater proportion of total nitrate accumulation occurred in the shoot (60 %) in the 10 mmol m⁻³ nitrate treatment; but the

estimate for the shoot and root, based on NAR data, was approximately 2 to 3 times lower than the actual rate measured (Table 2). This could be related to N availability and the beneficial processes involved in the modification of the cropping environment to enhance productivity through the provision of N to the plant. In order to understand the effect of nitrate (10 mmol m⁻³) supply and assimilation observed over the period of experiment (22-days-old). Measurement of various fractions of soluble N were determined in the shoots and roots (Table 3). The percentage of total soluble N in shoots and roots were less than half of the total N value. However, this reduction could be attributed to large amount of N incorporated into TCA-insoluble compounds, is likely to predominantly be in protein. In association with this effect, most of the remaining TCA-soluble N was present as amino acids, nitrate and ammonium-N (Table 3). Even though ammonium was not supplied to the plant, it was detected in both shoot and root under the fraction conditions; and accumulated to 1.5 to 3 fold higher levels than nitrate. The levels found in both shoots and roots, confirmed that internal reserves of N depends on the size and type of the N pools within the plant (the correlation coefficient between shoot and root total N and TCA-soluble N by them was calculated to be 0.93). For the above reasons, we believe our study has given reasonable estimates of the size N pools with the approximate value of 88.7 mg N/g⁻¹DW, which include amino acids, nitrate and ammonium. This indicated that N was able to enter the tissue and allowing an estimate of the maximum as TCA-soluble

Table 2. Inoculation effects with *Azotobacter chroococcum* on NAR and nitrate content.

Nitrate supply mmol	Inoculation day	Plant part	Length (cm)	R/S ratio	FWg	NAR (FW) total g	Nitrate (FW) total g
0	control	shoot	10.4±1.0	1.49	0.19±0.05	0.3 0.06 (78)	5.3 1.0 (58)
		root	15.5±1.3				
5	15	shoot	12.8±1.1	1.92	0.34±0.07	2.03 0.7 (81)	8.4 2.9 (44)
		root	24.6±1.4				
10	15	shoot	14.7±1.3	2.19	0.86±0.06	6.4 5.5 (70)	11.6 10 (60)
		root	32.2±1.7				

Mean of 10 plants per replicates with standard errors, FW = Fresh weight.

Table 3. The distribution in each part of total-N, amino-N, nitrate-N, ammonium-N, TCA soluble-N and TCA soluble-N in shoots and roots as plant supplied with nitrate (10 m mol).

Plant/part	Total N (mg N/g ⁻¹ DW)	Amino N	Nitrate-N mg N/g ⁻¹ DW	Ammonium-N	TCA soluble-N	TCA soluble-N% accounted for
Shoot	58.2±4.0	7.92±0.6 (38%)*	5.83±0.57 (28%)*	7.21±0.6 (34%)*	21±0.42	36.1
Root	30.5±2.7	5.89±0.4 (42%)*	2.19±0.22 (15.6%)*	6.0±0.5 (43%)*	14.1±0.49	46.2

Mean ± with standard errors of three independent experiments. DW=Dry weight.

*Values in parentheses are percentages of total TCA soluble-N.

N as approximate space value (ASV) to be obtained. For the purpose of calculation and comparison we determined the ASV of total N from the data plotted in (Table 3) and using equation of Devienne described by (Devienne *et al.* 1994), and was found to be 75.1 mg N/g⁻¹DW, compare to that value of 88.7 mg N/g⁻¹DW which appeared in (shoot + root) and was estimated by direct method. In any case, even a quite substantial error in our estimates would not significantly alter the conclusions of this study. Thus it seems reasonable to conclude that the nitrate pool was reduced mainly in the cytoplasm of epidermal cells and apparent as ASV of total N occurred in the cytoplasm of cortical cells. In addition, the changes of the free amino acids were a reflection of nutrient status and as such is a likely source of numerous regulatory signals. The abundance of free amino acids in shoots and roots fed with N (10 mmol m⁻³ nitrate) indicate that under experimental conditions the proportion of N being taken up as dissolved inorganic nitrogen (DIN) and released into root pools and shoot pools as dissolved organic nitrogen (DON). From the experiments data (represent one typical experiment out of two) the bulk DON released shown in Fig.1. It was clear that Asp, Glu, Asn, Ser, Gln, Thr and Ala were most abundant amino acids in the shoot and root. In contrast, the

contents of Arg, Tyr, Trp, Met, Val, Phe, Iso, Leu, Orn and Lys showed similar patterns between shoots and roots except for Val and Phe they were lower in the shoot and root. Total concentrations of Asp, Glu, Asn, Ser, Gln, Thr and Ala accounted for 84% of the total amino acids in the shoot and root. Fluctuations in root and shoot Ala are apparent, during growth and given that with Glu are considered to be interconverted by transamination enzyme, transient changes in levels of Ala may be explained by considering Ala as a sink for increases in Glu. Hence, it may be suggested that, the roots pools have a close relationship with absorbed N. The index of asparagine amino was highest in the shoot reaching 3 times the level of glutamine N and may be contributed by its relatively limited place at which it was synthesized from glutamine. The ratio of glutamine : glutamate (Gln : Glu) in the roots was about twice than in shoot, suggesting most probably due to either a reaction involved in the reduction of NO₃⁻ to NH₄⁺ or to the activation of glutamine synthesis (GS). The higher level of glutamate than that of glutamine, may have been due to the presence of GDH-dependent glutamate synthesis in the shoot. The different step of nitrate assimilation in roots may results in different fate in the assimilative path.

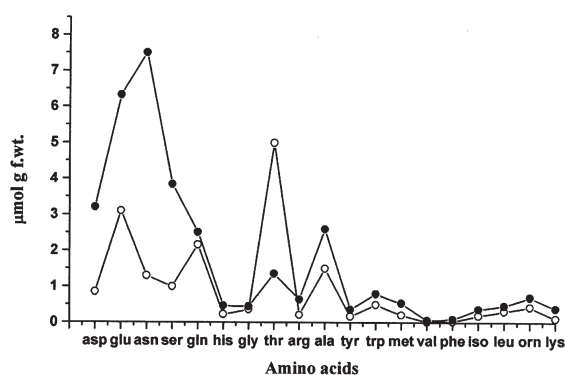


Fig 1. The abundance of free amino acids in shoots (solid circle) and roots (open circle) isolated from fed (22-day-old) wheat plants with 10 m mol m^{-3} nitrate. Data represent one typical experiment (out of two).

Discussion

In the current study, measurements NAR activities indicates that shoot component had about 98% of the total in plant at the concentration of 10.0 mmol m^{-3} but it did appear that the shoots could, under addition of concentrations of N, result in a higher level being available to support nitrate reduction in the root. The accumulation of nitrate in the shoot, under additional nitrate concentration, showed a higher level than those of the root throughout the whole experimental period. Increasing the nitrate supply increased the proportion of NAR activity in the shoot. The proportion of the total NAR activity in the root represented <11 % of the total NAR activity. The simplest explanation for this phenomenon is that a higher spill-over of nitrate to the shoot results an enhanced nitrate assimilation in the leaves. Olday *et al.* (1976), in a comparison with earlier works it was found that other non- legume plant accumulated more nitrate and showed that the highest accumulation was in the shoot. Such effects are normally considered positive, since excess availability of nitrate resulted in enhanced dry weights. The results of inoculated plants showed the highest and a pronounced effect on the value of NAR and there were some differences between inoculates with low and high N concentration and could represent a positive effect. When the activity of NAR was compared with the values of accumulation of reduced N, there was in some cases a reasonable agreement, but in some other cases the estimate value, based on NAR data, was more than 4

times the *in vitro* rate. The simplest explanation for this phenomenon is that the *in vitro* NAR activity reflects the potential for nitrate reduction in different tissues. The assimilation of N may not always be directly dependent on the level of N supplied to the plant. Indeed a significant part of N either nitrate or ammonium does occur in the root (Atkins *et al.* 1980; Wallace 1986). It has been demonstrated in the current study that the root had relatively low NAR activity and only accounted for less than 11% of the total NAR in the whole plant. Andrews *et al.* (1984) found that in several plant species the proportion of NAR activity was predominantly in the shoot. For cowpea (*vigna unguiculata* L. Walp) on low nitrate there was a substantial increase in proportion of NAR in the root (Atkins *et al.* 1980). It has also been reported in maize (*Zea mays* L.) that NAR activity in the root increased with increasing nitrate supply (Robin *et al.* 1978). In our study, the shoot had more NAR activity which increased with increasing nitrate and in most cases the activity was related to the level of nitrate concentration supplied to the plant. Dry weight of roots and shoots showed a steady increase throughout the whole experimental period compared with the control. This is consistent with the results in other papers (Walker *et al.* 2001, Okamoto and Okada 2004). Microbial activity can act either positively or negatively on plants. In the current study, a marginally significant increase in root length was found, and the differences in root : shoot ratio indicated that the inoculum encouraged root growth more than shoot growth, especially at the lower concentration of nitrate. The combination of *Azospirillum* inoculation and nitrogen increased plant height, number of leaves per plant, branches per plant and total dry mass accumulation than other uninoculated control (Gadagi *et al.* 2004).

Stimulation of wheat growth by inoculation with bacteria has been previously reported (Fayez 1990). Some reports suggest this may be the result of the presence of certain microbial hormones in the root rhizosphere. For example, the microbial production of stimulating substances in the root supports root growth and also possibly is involved in changes in the patterns of N-assimilation (Ferreira *et al.* 1987). On the other hand, the results in the current study, have

showed negative correlation between the potential of the inoculated bacteria to produce more activity of NAR and more assimilated-N in the root. Even though such effects are considered positive, high N levels resulted in more accumulation of N and high organic N as amino acid -N (Okamoto and Okada 2004). The increase of NAR at 10.0 mmol m⁻³ nitrate confirms a role for the bacteria in N metabolism of the plant (Fernandes *et al.* 1978). However, inoculation of wheat with *Azospirillum brasilense* seems to increase fluxes of nitrate into the roots possibly due to changes in the root structure caused by growth (stimulating) substances produced by the bacteria (Ferreira *et al.* 1987). The enhanced root growth possibly was involved in the changes in N-assimilation as a positive correlation between the root / shoot ratio with the NAR in shoots (Ferreira *et al.* 1987).

The present study indicates an additional bacterial effect besides that on root growth, which seems acts as biofertilizers for wheat due to their intensification and their potential for N₂-fixation, and/or in the production of plant growth regulators. The experiment in this study was finished too early to make any firm conclusions about root longevity or turnover; but the results may be taken as an indication that treatment interactions were likely to occur. The use of such bacteria needs further research, especially in relation to the contribution of N-fixation and also to the nature of interference of NAR activity. The future challenge is to define their contribution to the plant, N nutrition and signaling processes (Williams and Miller 2001). Effects of feeding nitrate -N to the seedlings under laboratory conditions much of N incorporated into the plant tissues (shoot and root) had been assimilated into TCA-soluble-N (36 – 46% of total N) compounds. Evident by the observation of soluble-N fractions in plant (shoot and root) the results confirm the differences presumably some relatively macromolecular compounds (such as protein) into the TCA-insoluble (55 – 65%) of total N as plant demand, probably for structural and storage (Marshall and Ellis 1998). Such TCA-insoluble (presumably protein) needs further research, especially in relation to the ASV of N requirement for vegetative growth. In the current study it was noticed that the fractions contained an increasing proportion

of the total assimilated N as time progressed (age of plant after feeding). During the experiment, however, the proportion TCA-soluble N (in root and shoot) was found becoming significant (14 – 21 %) which suggests the ability of the plant part to assimilate N into low molecular-weight compounds (mainly nitrate, ammonium and amino acids) during the period of plant development. Studies on subcellular distribution of N metabolites showed that free amino acids in photosynthetic tissues are located mainly in the chloroplast stroma and in the cytosol; while in non-photosynthetic tissues the vacuole is the major site of accumulation (Riens *et al.* 1991). As the proportion of amino acid in the vacuole may change together with the total concentration of free amino acid in the cell, although the cytosolic pool remains constant, it has been suggested that their distribution in the subcellular compartments is a controlled process (Sakano and Tazawa 1984, Broadley *et al.* 2003). Our results showed that large amounts of N were exported from the root to the shoot during the course of the experiment and this may be regulated in relation to N and physiological status of plant, as well as through the turnover of various N pools.

In this study however, we consider that cellular estimates of ASV of total N derived by equation method was found to be 75.1 mg N/g⁻¹DW, whereas value estimated by direct method was found to be 88.7 mg N/g⁻¹DW. Fluxes and compartmental analyses as tool for studying N transport from root to shoot has been intensively studied using labelled approaches (Devienne *et al.* 1994; Siddiqi *et al.* 1991). Additional fluxes analysis is that of Michaelis-Menten kinetic values (K_m , V_{max}) which have a mechanistic meaning (Kronzucker *et al.* 1996). However, our understanding of these processes at the transport system level is largely hypothetical. Nevertheless, it is useful for modeling purposes to describe uptake in terms of transport kinetics with terms such as (K_m) and (V_{max}), but a comprehensive understanding of the regulation of nitrate developmental process and be transport into the root pools has not yet been achieved. Although some of nitrate may remain unchanged in the root, or be reduced via NAR and subsequently synthesised into amino acids, much of it is likely to be loaded into the xylem. The N

required for stem growth is transported from the roots via the xylem as a mixture of inorganic and organic N, the proportion varying with species and culture conditions. Amino acids can also be subsequently cycle between shoots and roots via the phloem and xylem (Cooper *et al.* 1986). The rate of N cycling, as well as the sink strength of the root for the cycling N can be affected by the external N supply (Agrell *et al.* 1994). The sensitivity of the free amino acid concentration to the changes in the plant N status, and their ability to cycle between different organs, make them suitable messengers between shoot pools and root pools depending on the ASV for N demand of the plant. Besides the occurrence of amino acids in shoot and root of the plant, as the most cellular N pools, it was found that the remaining TCA-soluble N is present as nitrate-N and ammonium-N. The work, observing increased translocation of N to the roots under N-limiting conditions, assumed that the N distribution between roots and shoot is adjusted in the shoot (Lambers *et al.* 1982). Although most of the nitrate taken up by the plants was transported to the shoot (Peuke *et al.* 1996), a smaller fraction was retained in the root; but it is not known where in the plant the bulk of the reduction of nitrate takes place. The nitrate in the root is either stored in the root for a short time or reduced since plant with N-restricted growth do not store nitrate for long periods. Additionally, since ammonium is toxic to cells, it can not be stored in large quantities within the cells. On the other hand, measure of ammonium concentration in xylem sap were not a result of excessive N supplies, as even plants grown under N-deficient conditions contained ammonium (Husted *et al.* 2000). Thus, it can be concluded that the quantities found as nitrate or ammonium within the shoots and roots may be essentially needed for loading transport. Although there have been numerous studies of many processes related to N metabolism of plant, information is also needed with respect to the possible effects of the N supply on amino acid synthesis. However, a shortage in N supply to the plant has decreased the amino acid concentration in the cytoplasm (Barneix *et al.* 1996). On the other hand, the concentration of individual free amino acid often presents and apparently resides in a different compartment, presumably the plant

cytoplasm (Scharff *et al.* 2003). Ammonium-grown plants often present a higher concentration of tissue free amino acid than nitrate-fed plants (Allen and Smith 1986). The present study may partly explain this difference. However, some of the reported differences in observations may be due to different methods of analysis rather than the physical properties of the soil N-content. So it is clear that the free amino-acid composition shows a fast and sensitive response to the addition to the ambient N availability and source. In general the results of this work may be widely applicable. However, total amino acids in shoot differed from that of the root. Earlier observation with many cases plant species, a high N supply increases the total free amino acid concentration in shoot but not in root (Haynes and Goh 1978) and more recent experiments (do Amarante *et al.* 2005) indicate the composition of nitrogenous compounds transported in the xylem to the shoot can reflect some changes in root amino acids. The pool of amino acids cycling between the roots and shoots is considered to serve as a signal for plant internal N status (Aslam *et al.* 2001). In the present study we confirm the comparison, shoots showed higher proportion of amino acids than roots. Processes of amino acids transamination were probably restricted in the absence of photosynthesis. The result was an increase in the proportion of GLN, probably because pathways leading to synthesis of some amino acids require products of photosynthesis. A variety of different levels in GLU/GLN, suggest that the plant may have used (GS) and (GDH) as functional enzymes for primary N-assimilation and amino acids synthesis in the wheat tissues. Less is known about the regulation of amino acid synthesis in response to changes in the N-supply, further studies are needed to investigate the effect of N-on the transcription and activity of a wide range of enzymes in the amino acid biosynthesis pathways.

Acknowledgements

Azotobacter chroococcum used in this work was prepared and kindly provided by Professor A. R. El-Shanshoury. Wheat grains, *Triticum aestivum* yocora rojo, were obtained from college of agriculture and veterinary medicine, King Saud University, Al-Qassim. We wish to thank Dr. K. J. Flynn, University College of Swansea, for amino acids

analysis. H. M. M. acknowledges the receipt of financial support from Umm Al-Qura University which made the studies possible.

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بعض المظاهر المصاحبة للتمثيل النتروجيني في نبات قمح الخبز (*Triticum aestivum* L.) المدروسة تحت تأثير مستويات مختلفة من النترات والبكتريا الملقحة

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الملخص

تهدف الورقة العلمية إلى إظهار نتائج دراسة التمثيل النتروجيني لنبات قمح الخبز (يوكورا روجو) النامي في بيئة مضافاً إليها مستويات متفاوتة من النترات (٥ ، ١٠ ، ٥٠ ، ١٠٠ مليمول) وتحت ظروف تجريبية مثلى في وحدة النمو. وقد أظهرت الدراسة تباين في مستوى نشاط أنزيم نترات ريدكتيز (NAR) لتلك النباتات المغذاة بالنترات عند جميع المستويات المختارة في الدراسة، حيث أظهرت الدراسة أن السويقة تحتوي على قدر أعلى من الإنزيم مقارنةً بما احتوته الجذور، وأن نسبة الإنزيم في السويقة إلى الجذر قدرت ٩٤ : ٦ على التوالي. كما أن النسبة للوزن الغض في السويقة إلى الجذور أظهرت زيادة بمعدل ٣ ، ١ إلى ٦ ، ٢ مع زيادة مستوى النترات. لم يؤد تلقيح النباتات بازوتوباكتر كروكوكوم إلى أي تغير ظاهري على مستوى توزيع نشاط إنزيم نترات ريدكتيز (NAR) حتى في حالة تغير مستويات من النترات. شكل الجزء الذائب من حمض ثلاثي الكربوكسيل (TCA) الموجود على صورة نتروجين ذائب، على أحماض أمينية، و نترات، وأمونيوم- نتروجين لتؤكد أن النتروجين المخزون داخلياً يعتمد اعتماداً على مقدار كمية ونوعية الحمض، ومساحة الحوض الأيضي النتروجيني بداخل النبات. أما معدل تشكيل وتنوع الأحماض الأمينية الحرة المقدر في هذه الدراسة كمحصلة من جراء الإمداد النتراقي في كلاً من منطقة السويقة والجذير فإن الدراسة تشير إلى أن النتروجين قد تحول من الهيئة الغير عضوية إلى هيئة ذائبات النتروجين العضوي. وقد شملت الأجزاء المتحصل عليها قدرأً وفيراً من أحماض أمينية حرة منها - أسبرتيت، جلوتاميت، السيرين، سيرين، جلوتامين، ثيرونين بالإضافة إلى الألبين (*Asp, Glu, Asn, Ser, Gln, Thr and Ala*). بالمقارنة اوضحت تقديرات كلاً من الأحماض الأمينية المشتمة على - أرجنين، تيروسين، تربتافين، ميثونين، فالين، فينيلالنين، أزيليسين، ليوسين، أورثين بالإضافة إلى لاسين - نماذج متشابهة في التباين في كلاً من منطقة السويقة والجذير (*Arg, Tyr, Trp, Met, Val, Phe, Iso, Leu, Orn and Lys*). يبين التقدير الكمي قيمة معنوية لنسبة حمض الجلوتامين إلى الجلوتاميت في منطقة الجذير، مما يعطي مؤشراً عن إحتالية توفر قدرأً من الأمونيا بسبب الإختزال الحادث في النترات أو ربما قد يعود الأمر بسبب حدوث تنشيط لإنزيم جلوتامين سنثيتيز (GS). كما تعزز زيادة تواجد حمض القلوتاميت عن حمض الجلوتامين بصورة عامة في مجمل التقديرات، مما يوحي بجلاء في غالب الأمر مشاركة إنزيم جلوتاميت ديهيدروجينيز (GDH) كمفتاح رئيسي للجلوتاميت في منطقة السويقة.