



Available online at www.ewijst.org

ISSN: 0975-7112 (Print)
ISSN: 0975-7120 (Online)

Environ. We Int. J. Sci. Tech. 6 (2011) 167-174

*Environment & We
An International
Journal of Science
& Technology*

Nitrogen Use-Efficiency and Crop Production - A Mini Review

Firoz Anwar¹, Siddiqui M.H.^{2*}, Salem S. Alghamdi¹, Al-Wahaibi M.H.² and Abhishek Chandra³

¹Legume Research Unit, Plant Production Department, College of Food and Agricultural Sciences, King Saud University, P. O. Box 2460, Riyadh 11451, Saudi Arabia

²Department of Botany and Microbiology College of Science, King Saud University, P.O. Box 2455, Riyadh- 11451, Saudi Arabia

³Department of Botany, Sri Venkateswara College, University of Delhi, Benito Juarez Marg, Delhi 110021, India

*E mail: manzerhs@gmail.com

Abstract

Nitrogen is an essential macronutrient required by plants. Nitrogen use efficiency of the plants, by increasing the efficiency of mechanisms plants use to accumulate and utilize nitrogen. Development of crop varieties with high nitrogen use efficiency (NUE) is imperative for sustainable agriculture. Worldwide, the NUE for crop plants is of great concern. One of the most significant contributions of the research community is the improvement in our understanding of the relationship between N availability, N uptake, and overall plant development. N uptake is one of the most critical NUE components under N-limiting conditions in a number of crops; the transfer of knowledge should be relatively straightforward when the experimental procedures have been adapted to larger or structurally different systems grown under agronomic conditions. Understanding the molecular basis of NUE will make enable to provide handle for crop improvement through biotechnological means.

Keywords: Abiotic Stresses; Nitrogen Use Efficiency (NUE); Enzyme Activities; Plant Production

Introduction

Plants need plenty of nitrogen to grow properly, as the nutrient plays a role in almost all plant activities. To satisfy this need, farmers worldwide apply about 90 million tonnes of nitrogen fertiliser to croplands each year. The reason farmers have to add this much nitrogen is that many agricultural crops are very inefficient at using it. For example, cereals use only 30 per cent of the nitrogen that is applied as fertiliser. Apart from Carbon and oxygen Nitrogen is one of directly or indirectly main component in the plant cycle and plant development. Nitrate assimilation in plants and related organisms is a highly regulated and conserved pathway in which the enzyme nitrate reductase (NR) occupies a central position. Regulation of the

nitrate assimilation pathway coordinates the incorporation of nitrogen with important processes of plant development, such as cell differentiation and photosynthesis. Genes and proteins of this pathway are induced by nitrate itself (Crawford, 1995; Daniel-Vedele *et al.*, 1998; Forde, 2000; Llamas *et al.*, 2002; Rexach *et al.*, 2002) and are repressed by ammonium or by products of nitrate metabolism, such as Gln and Glu (Stitt, 1999; Crawford and Forde, 2002; Glass, 2003; Fan *et al.*, 2006; Fernandez and Galva' n, 2007). A key component of the nitrate assimilation pathway is nitrate reductase (NR), which catalyzes the reduction of nitrate to nitrite. This enzyme and the gene encoding it are subject to complex regulation mechanisms: not only are gene expression and protein activity regulated by inorganic nitrogen compounds and metabolic derivatives, but they also respond to external cues, such as day and night cycles (Kaiser and Brendle-Behnisch, 1991; Galangau *et al.*, 1998). In plants, NR activity is regulated by phosphorylation of a Ser residue that allows binding of 14-3-3 proteins and leads to subsequent degradation of NR (Kaiser and Huber 2001). Two kinases responsible for the phosphorylation of the Ser residue seem to be activated by calcium, whereas the third, a member of the SNF1 kinase family, is calcium independent (Douglas *et al.*, 1997). CYG56 participates in ammonium-mediated NIA1 repression through a pathway that involves NO, cGMP, and calcium and that similar mechanism might be occurring in plants (Montaigu *et al.*, 2010).

Nitrogen Nutrition Influence Plant Development

N is an essential plant macronutrient required in the largest quantities (1-3% on a dry weight basis) by plants and is most limiting where maximal biomass production is desired (Salisbury and Ross 1992; Hell and Hillebrand 2001). The improvement of N-use efficiency is a major goal of plant improvement under salt stress; such improved plants would make better use of the N-fertilizer supplied, they would also produce higher yields by alleviating the adverse effect of salt stress. Nitrogen is a crucial macronutrient needed in the greatest amount of all mineral elements required by plants.

N availability influences several developmental processes according to the species such as the number of leaves and their rate of appearance, the number of nodes (Snyder and Bunce, 1983; Mae 1997; Sagan *et al.*, 1993), and the number of tillers (Vos and Biemond, 1992; Tra'pani and Hall 1996) are reduced under N-limiting conditions. Moreover, both in spring wheat (Demotes-Mainard *et al.*, 1999; Martre *et al.*, 2003) and in rice (Mae 1997), grain number decreases under N deficiency conditions, a process occurring during the period bracketing anthesis, which is highly dependent on both the intensity and the duration of the N stress*1. The availability of N for yield determination is also important through its direct influence on the sources (leaf area), and consequently the sinks (reproductive organs). Generally, the reduction in photosynthesis of the canopy following N starvation is due to the reduction of the leaf area (radiation interception efficiency, RIE), rather than a decrease of RUE (Lemaire *et al.*, 2007). In grasses, the reduction of leaf area extension is due to a lower cell division in the proximal zone rather than to the final size of the cell (Gastal and Nelson, 1994). In many crops, the relationship between leaf area index (LAI) and N uptake was found to be directly proportional, whatever the environmental conditions.

In contrast, the respective contribution of RIE and RUE in the adaptation to N starvation is variable among species. For example, potato and maize have two different strategies in their response to N-limiting conditions. In potato, the leaf area is reduced and adjusted to the rate of N uptake, keeping the plant leaf-specific nitrogen (g Nm²) and RUE unchanged ('potato strategy'). In maize, leaf area is almost not affected, while photosynthesis and RUE decrease ('maize strategy') (Vos and van de Putten, 1998; Vos *et al.*, 2005). In potato, the adaptation to N limitation results exclusively in a decrease in the amount of light intercepted, the RUE remaining constant, while in maize both leaf area and RUE are decreased. Classifying species and genotypes according to both strategies merits further investigation as it may be another way for selecting crops more adapted to low N fertilization conditions.

According to Lian *et al.* (2006) and Kumar *et al.* (2009) that development of crop varieties with high nitrogen use efficiency (NUE) is imperative for sustainable agriculture. The nitrogen use efficiency (NUE) for crop plants is of great concern globally. The rapid increasing world population needs crop genotypes that respond to higher nitrogen and show a direct relationship to yield with use of nitrogen inputs, i.e. high nitrogen-responsive genotypes. However, for fulfilling the high global demand of organic produce, it requires the low nitrogen responsive genotypes with greater NUE and grain yields. The lack of knowledge about precise regulatory mechanisms to explain NUE in crop plants hampers the goal of agricultural productivity. Understanding the molecular basis of NUE will enable to provide handle for crop improvement through biotechnological means. With the advent of modern genomics and proteomics approaches such as subtractive hybridization, differential display, and microarray techniques are revolutionizing to identify the candidate genes which play a pivotal role in the regulation of NUE. Beside it, quantitative real-time polymerase chain reaction technology is also being used to establish marker-trait association for NUE. The identification of potential candidate genes/proteins in the regulation of NUE will serve as biomarker(s) for screening genotypes for their nitrogen responsiveness for optimization of nitrogen input in agriculture. NUE in plants is a complex trait which not only involves the primary process of nitrogen uptake and assimilatory pathways but also a series of events, including metabolite partitioning, secondary remobilization, C-N interactions, as well as molecular signalling pathways and regulatory control outside the metabolic cascades. Therefore, the selection of plant genotypes with high efficiency in nitrogen use is a reasonable approach for better growth and physio-biochemical performance and yield of plant by regulating enzymes activity of nitrogen assimilation and antioxidant systems under stress. It could serve as an appropriate strategy for nitrogen management in agriculture.

Nitrogen Influence Enzymatic Activities

A progressive inhibition of the activity of enzymes of N metabolism, i.e. nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase was observed with increasing levels of salinity (Amonkar and Karmarkar, 1995; Dubey, 1997; Nathawat, et al., 2005; Siddiqui *et al.*, 2010). Several physiological and biochemical processes are affected by salinity, particularly nitrate assimilation, which largely influences plant growth (Gouia *et al.*, 1994). N assimilation plays an important role in plant metabolism. It is used for generating glutamine (Gln) that is the precursor of various amino acid syntheses (Bagh *et al.*

2004; Al-Amoudi and Moujahed 2006). Plant growth depends on an adequate supply of N in order to form amino acids, protein, nucleic acid, enzymes, plant growth regulators, chlorophyll, vitamins and other cellular constituents necessary for development. N metabolism is a complex process and varies with species (Garg *et al.* 1993; Mansour 2000; Bertrand Hirel *et al.*, 2007). N and sulphur (S) assimilation is an essential pathway for offsetting salinity. N nutrition has a strong regulatory influence on S assimilation and *vice versa* (Fismes *et al.*, 2000; Koprivova *et al.*, 2000; Siddiqui 2005), and both are involved in protein synthesis (Ceccotti 1996). Reuveny *et al.*, (1980), Smith (1980) and Brunold and Suter (1984) reported that N-deficient *Lemna minor* and cultured tobacco cells exhibited decreased activities of ATP-Sulphurylase (ATP-S; EC: 2.7.7.4), adenosine 5' phosphosulphate reductase (APR; SC: 1.8.4.9) and O-acetylserine (thiol) lyase (OASTL; EC: 2.5.1.47). They were restored when nitrate or ammonia were resupplied. Glutathione (GSH) synthesis is regulated by the N and S supply of plants because GSH contains three moles of N per mole of S and also GSH biosynthesis is regulated by the amino acids cysteine, glutamate and glycine (Kopriva and Rennenberg 2004). Glutamic acid is the initial product of the GS-GOGAT pathway of N assimilation (Geßler *et al.*, 1998). N metabolism is a complex phenomenon and varies from species to species (Mansour 2000). Under salinity, NO_3^- assimilation is not only required for plant growth, but some of its metabolite derivatives can be utilized in osmotic adjustment (Aslam *et al.*, 1984; Siddiqui *et al.*, 2010). Consequently, it is suggested that the higher the sensitivity imposed by salinity on nitrogen assimilation the more severe are the salt-injurious effects on plant growth (Viegas and Silveira, 1999). Nitrate is the main nitrogen source in agricultural soils and most frequently limits plant growth. Nitrate uptake is mediated by a NO_3^- -transporter protein induced by the ion itself (Campbell, 1999). Several studies have shown a close relationship between nitrate uptake and nitrate reduction in higher plants. Activity of NR catalyzes NO_3^- reduction to NO_2^- and its activity is nitrate inducible. The NR activity is the limiting step of N- NO_3^- conversion to amino acid synthesis (Campbell, 1999). NR activity in leaves is largely dependent on nitrate flux from roots (Ferrario *et al.*, 1998) and is severely affected by osmotic shock induced by NaCl (Rao and Gnanam 1990; Viegas and Silveira 1999). On the other hand, the cytosolic NO_3^- seems to protect the NR enzyme against the action of proteases and/or inhibitors besides triggering the *de novo* synthesis of NR protein by induction of NR gene expression (Campbell, 1999). Even so, under conditions of restriction in nitrate flux induced by salt stress or water deficit, NR activity could be lowered initially on account of enzyme degradation/inactivation and the reduction in gene expression and NR protein synthesis (Plaut, 1974; Ferrario *et al.*, 1998). The nitrate assimilation process is relatively more sensitive to water stress than CO_2 -photosynthetic assimilation although there is a close metabolic coordination between these processes by means of sucrose-phosphate synthetase and NR activity (Foyer *et al.*, 1998). The true role of nitrate assimilation in the growth of cultivated plants under salt stress is still unknown (Aslam *et al.*, 1984; Gouia *et al.*, 1994).

Conclusion

Though the afford have been made by many research groups; still need an approach that integrates genetic, physiological, and agronomic studies of the whole-plant N response will be essential to elucidate the regulation of NUE and to provide key target selection criteria for breeders and monitoring tools for farmers for conducting a reasoned fertilization protocol. This prospective conclusion outlines the

main points that will need to be considered in order to develop an integrated research programme for discovering genes by means of a complete and extensive phenotyping, comprising agronomical, physiological, and biochemical studies on crops grown under different concentration of N fertilization applications. In addition to this, modeling NUE through system biology approaches will provide in the near future an avenue to enhance integration of molecular genetics technologies in plant improvement, thus allowing the re-establishment of fundamental and practical research in an intimate and meaningful way that could be beneficial for human beings.

Acknowledgments

This work was supported by Legume Research Unit, Plant Production Department, College of Food and Agricultural Science and Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia. Dr. Abhishek Chandra is thankful to Principal and all faculty members of Department of Botany, Sri Venkateswara College, University of Delhi, for providing the supports and facilities.

Author's contributions: Dr. Firoz Anwar and Dr. Siddiqui M.H. (corresponding author) conducted the field survey, wrote and drafted the manuscript; Dr. Salem S. Alghamdi (Professor) and Dr. Al-Whaibi M.H. (Professor) contributed in editing of manuscript and Dr. Abhishek Chandra (Assistant Professor) contributed in modification and final editing of the manuscript.

References

- Al-Amoudi O., Moujahad H.M. (2006). Some Aspects of Nitrogen Assimilation in wheat plant (*Triticum aestivum* L.) as affected by different nitrate levels and bacteria inoculation. *Saudi Journal of Biological Sciences* 13, 141-151.
- Amonkar D.V., Karmarkar S.M. (1995). Nitrogen uptake and assimilation in halophytes. In: Srivastava HS, Singh RP (Eds) *Nitrogen Nutrition in Higher Plants*, Associated Publ. Co. New Delhi, pp 431-445.
- Aslam, Hukffaker R.C., Rains D.W. (1984). Early effects of salinity on nitrate assimilation in barley seedlings. *Plant Physiology* 76, 321-325.
- Bagh K., Hiraoki T., Thorpe T.A., Vogel H.J. (2004). Nitrogen-15 NMR studies of nitrogen metabolism in *Picea glauca* buds. *Plant Physiology and Biochemistry* 42, 803-809.
- Bertrand Hirell, Le Gouis, J., Ney, B. and Gallais, A. (2007). The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany*, 58(9), 2369–2387.
- Brunold C., Suter M. (1984). Regulation of sulphate assimilation by nitrogen nutrition in the duckweed *Lemna minor* L. *Plant Physiology* 76, 579-583.
- Ceccotti S.P. (1996). Plant nutrient sulphur – a review of nutrient balance, environmental impact and fertilizers. *Fertizer Reserch* 43, 117-125.
- Crawford, N.M. (1995). Nitrate: Nutrient and signal for plant growth. *Plant Cell* 7, 859–868.
- Crawford, N.M., and Forde, B.G. (2002). Molecular and developmental biology of inorganic nitrogen nutrition. In *The Arabidopsis Book*, C.R. Somerville and E.M. Meyerowitz, eds (Rockville, MD: American Society of Plant Biologists), doi/, <http://www.aspb.org/publications/arabidopsis/>.

- Daniel-Vedele, F., Filleur, S., and Caboche, M. (1998). Nitrate transport: A key step in nitrate assimilation. *Current Opinion in Plant Biology* 1, 235–239.
- Demotes-Mainard SD, Jeufroy MH, Robin S. 1999. Spike and dry matter accumulation before anthesis in wheat as affected by nitrogen fertilizer: relationship to kernel per spike. *Field Crops Research* 64, 249–259.
- Douglas, P., Pigaglio, E., Ferrer, A., Halfords, N.G., and MacKintosh, C. (1997). Three spinach leaf nitrate reductase-3-hydroxy-3-methylglutaryl-CoA reductase kinases that are required by reversible phosphorylation and/or Ca²⁺ ions. *Biochemistry Journal* 325, 101–109.
- Dubey R.S. (1997). Photosynthesis in plants under stressful conditions. In: Pessaraki M (Ed) *Handbook of Photosynthesis*, Marcel Dekker, New York, pp859-875.
- Fan, X., Gordon-Weeks, R., Shen, Q., and Miller, A.J. (2006). Glutamine transport and feedback regulation of nitrate reductase activity in barley roots leads to changes in cytosolic nitrate pools. *Journal of Experimental Botany* 57, 1333–1340.
- Fernandez, E., and Galvan, A. (2007). Inorganic nitrogen assimilation in *Chlamydomonas*. *Journal of Experimental Botany* 58: 2279–2287.
- Ferrario S., Valadier M., Foyer C.H. (1998). Over-expression of nitrate reductase in tobacco delays drought-induced decreases in nitrate reductase activity and mRNA. *Plant Physiology*, 117, 293–302.
- Fismes J., Vong P.C., Guckert A., Frossard E. (2000). Influence of sulphur on apparent N-use efficiency, yield and quality of oilseed rape (*Brassica napus* L.) grown on a calcareous soil. *European Journal of Agronomy* 12, 127-141.
- Forde, B.G. (2000). Nitrate transporters in plants: Structure, function and regulation. *Biochimica et Biophysica Acta* 1465, 219–235.
- Galangau, F., Daniel-Vedele, F., Moureaux, T., Dorbe, M.F., Leydecker, M.T., and Caboche, M. (1998). Expression of leaf nitrate reductase genes from tomato and tobacco in relation to light-dark regimes and nitrate supply. *Plant Physiology* 88, 383–388.
- Garg B.K., Byas S.P., Kathju S., Lahiri A.N., Mali P.C., Sharma P.C. (1993). Salinity-fertility interaction on growth, mineral composition and nitrogen metabolism of Indian mustard. *Journal of Plant Nutrition* 16, 1637-1650.
- Gastal F, Nelson CJ. 1994. Nitrogen use within the growing leaf blade of tall fescue. *Plant Physiology* 105, 191–197.
- Geßler A., Schneider S., Weber P., Hanemann U., Rennenberg H. (1998). Soluble N compounds in trees exposed to high loads of N: a comparison between the roots of Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees. *New Phytology* 138, 385-399.
- Glass, A.D.M. (2003). Nitrogen use efficiency of crop plants: Physiological constraints upon nitrogen absorption. *Critical Review Plant Sciences* 22:453–470.
- Gouia, H., Ghorbal M.H., Touraine B (1994). Effects of NaCl on Flows of N and Mineral Ions and on NO₃- Reduction Rate within Whole Plants of Salt-Sensitive Bean and Salt-Tolerant Cotton. *Plant Physiology* 105: 1409-1418.
- Kaiser, W.M., and Brendle-Behnisch, E. (1991). Rapid modulation of spinach leaf nitrate reductase activity by photosynthesis: I. Modulation in vivo by CO₂ availability. *Plant Physiology* 96, 363–367.
- Kaiser, W.M., and Huber, S.C. (2001). Post-translational regulation of nitrate reductase: Mechanism, physiological relevance and environmental triggers. *Journal of Experimental Botany* 52, 1981–1989.

- Kaoprivova A., Suter M., den Camp R.O., Brunold C., Kopriva S. (2000). Regulation of sulfate assimilation by nitrogen in *Arabidopsis*. *Plant Physiology* 122, 737-746.
- Kopriva S., Rennenberg H. (2004). Control of sulphate assimilation and glutathione synthesis: interaction with N and C metabolism. *Journal of Experimental Botany* 55, 1831-1842
- Kumar A., Gupta N., Gupta A.K., Gaur V.S. (2009). Identification of biomarker for determining genotypic potential of nitrogen-use-efficiency and optimization of the nitrogen inputs in crop plants. *Journal Crop Science and Biotechnology* 12, 183-194.
- Lemaire G, van Oosterom E, Sheehy J, Jeuffroy MH, Massignam A, Rossato L. 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? *Field Crops Research* 100, 91–106.
- Lian X, Wang S, Zhang J, Feng Q, Zhang L, Fan D, Li X, Yuan D, Han B, Zhang Q. (2006). Expression profiles of 10,422 genes at early stage of low nitrogen stress in rice assayed using a cDNA microarray. *Plant Molecular Biology* 60, 617-31.
- Llamas, A., Igeno, M.I., Galvan, A., and Fernandez, E. (2002). Nitrate signaling on the nitrate reductase gene promoter depends directly on the activity of the nitrate transport systems in *Chlamydomonas*. *Plant Journal* 30, 261–271.
- Mae T. 1997. Physiological nitrogen efficiency in rice: nitrogen utilization, photosynthesis, and yield potential. In: Ando T, ed. *Plant nutrition for sustainable food production and environment*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 51–60.
- Mansour M.M.F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. *Biologia Plantarum* 43, 491-500.
- Martre P, Porter JR, Jamieson PD, Tribor E. 2003. Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen utilization in wheat. *Plant Physiology* 133, 1959–1967.
- Montaigu, A.D., Sanz-Luque, E., Galvan, A. and Fernandez, E. (2010). A Soluble Guanylate Cyclase Mediates Negative Signaling by Ammonium on Expression of Nitrate Reductase in *Chlamydomonas*. *The Plant Cell*, 22: 1532–1548.
- Nathawat N.S., Kuhad M.S., Goswami C.L., Patel A.I., Kumar R. (2005) Nitrogen-metabolism enzymes: effect of nitrogen sources and saline irrigation. *Journal of Plant Nutrition* 28, 1089-1101.
- Plaut Z. (1974). Nitrate reductase activity of wheat seedlings during exposure to and recovery from water stress and salinity. *Plant Physiology* 30, 212–217.
- Rao K.R., Gnanam A. (1990). Inhibition of nitrate and nitrate reductase activity by salinity stress in *Sorghum vulgare*. *Phytochemistry* 29, 1047–1049.
- Reuveny Z., Dougall D.K., Trinity P.M. (1980). Regulatory coupling of nitrate and sulfate assimilation pathways in cultured tobacco cells. *Proceedings of the National Academy of Sciences USA* 77, 6670-6672.
- Rexach, J., Llamas, A., Fernandez, E., and Galvan, A. (2002). The activity of the high-affinity nitrate transport system I (NRT2;1, NAR2) is responsible for the efficient signaling of nitrate assimilation genes in *Chlamydomonas reinhardtii*. *Planta* 215, 606–611.
- Sagan M, Ney B, Duc G. 1993. Plant symbiotic mutants as a tool to analyse nitrogen nutrition and yield relationship in field-grown peas (*Pisum sativum* L.). *Plant and Soil* 153, 33–45.

- Siddiqui M.H. (2005). *Study of the effect of N, P and S application on the performance of rapeseed-mustard*. PhD thesis, Aligarh Muslim University, Aligarh, India, 125 pp
- Siddiqui M.H., Al-Whaibi M.H., Basalah M. O. (2010). Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma*, DOI:10.1007/s00709-010-0206-9.
- Siddiqui M.H., Mohammad F., Khan M.N., Al-Whaibi M.H., Bahkali A.H.A. (2010). Nitrogen in relation to photosynthetic capacity and accumulation of osmoprotectant and nutrients in brassica genotypes grown under salt stress. *Agricultural Sciences China*, 9, 671-680.
- Smith I.K. (1980). Regulation of sulfate assimilation in tobacco cells: effect of nitrogen and sulfur nutrition on sulfate permease and *O*-acetylserine sulphydryllase. *Plant Physiology* 66, 877-883.
- Snyder FW, Bunce JA. 1983. Use of the plastochron index to evaluate effects of light, temperature and nitrogen on growth of soya bean (*Glycine max* L. Merr.). *Annals of Botany* 52, 895–903.
- Stitt, M. (1999). Nitrate regulation of metabolism and growth. *Current Opinion in Plant Biology* 2, 178–186.
- Tra`pani N, Hall AJ. 1996. Effects of leaf position and nitrogen supply on the extension of leaves of field-grown sunflower (*Helianthus annuus* L.). *Plant and Soil* 184, 331–340.
- Viegas R.A., Silveira J.A.G. (1999). Ammonia assimilation and proline accumulation in young cashew plants during long-term exposure to salinity. *Raz. Journal of Plant Physiology* 11,153-159.
- Vos J, Biemond H. 1992. Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life span of leaves and stem branching. *Annals of Botany* 70, 27–35.
- Vos J, van de Putten PEL. 1998. Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in potato. *Field Crops Research* 59, 63–72.
- Vos J, van de Putten PEL, Birch CJ. 2005. Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* L.). *Field Crops Research* 93, 64–73.