

Recent Advances in CROP PHYSIOLOGY

VOLUME 2

The Editor



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— Editor —

Dr. Amrit Lal Singh

Principal Scientist,

Plant Physiology

Directorate of Groundnut Research,

Junagadh, Gujarat

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Foreword

Agriculture plays a pivotal role for food and nutritional security, and in alleviation of poverty. But, agriculture sector has been confronted with numerous challenges linked to food and energy crisis, climate change and natural resources. With beginning of 21st century, India is being recognized as the global power in the key economic sectors with high economic growth, but its slow growth in agriculture sector is major concerns for the future food and nutritional security, as one-third of the country's population lives below poverty line, and about 80 per cent of our land mass is highly vulnerable to drought and floods. Indian agriculture, with only 9 per cent of world's arable land, contribute 8 per cent to global agricultural gross domestic product to support 18 per cent of the world population. Also, India has nearly 8 per cent of the world's biodiversity and many of these are crucial for livelihood security of poor and vulnerable population. Thus, acceleration of agricultural growth along with natural resources conservation is of supreme importance.

As the Global food demand is expected to be doubled by 2050, world must learn to produce more food with less land, less water and less labour by devising more efficient and profitable production systems that are resilient to climate change. Thus, more than ever, we need to produce more food with less land. Also looking to the demand of 2050 all the institutions and agricultural universities need to redesign their research and teaching programmes for harnessing power of science and bringing excellence in agricultural research and education that ensures food, nutrition and livelihood security for all.

The ICAR with the help of SAUs has brought green revolution in agriculture in India through its research and technology development in past and its subsequent efforts have enabled the country to increase the production of food grains by 4-fold, horticultural crops by 6-fold since 1950-51 which made a visible impact on the national food and nutritional security. Using cutting edge technologies, there is

tremendous development in agriculture during the last two decades and it is hoped that with ingenuity, determination and innovative partnerships among everyone working in the agricultural sector, we can meet the food needs of 9 billion people by 2050 without irreparably harming our planet. However, all these informations are scattered and need to be compiled and circulated widely.

This series on "Recent Advances in Crop Physiology" is a timely effort in this direction, which will act as a reference for directly implementing the available technologies and to help the researchers for planning their future research programme.

Swapan Kumar Datta

Preface

“Food security exists when all people, at all times, have physical, social and economic access to sufficient safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life.”

Global food demand is expected to be doubled by 2050, while production environment and natural resources are shrinking and deteriorating. World cereal production has gone 2525 million tonnes (mt) during 2013-14 and is expected to be 2535 mt in 2014-15. Same time, world cereal utilization which was 2416 mt in 2013-14 is put 2464 mt in 2014-15. To feed the world in 2050, yields on maize, rice, wheat, and soybeans will have to rise by 60-110 per cent, but the present projections show an increase of only 40-65 per cent and most rice and wheat had very low rates of increase in crop yields. In other places, the trajectories of population growth and food production are heading in different directions. The rice, is the central to existence in many nations, feeds the world, and provides more calories to humans than any other food, and more than a billion people depend on rice cultivation for their livelihoods. Changes in the price and availability of rice have caused social unrest in developing countries and in 2008, when rice prices tripled, 100 million people were pushed into poverty. About 90 per cent of the world’s rice is grown in Asia, on more than 200 million small scale farms (about 1 acre), where additionally 8-10 m t of rice need to be produced every year to keep prices affordable with population increase. However, the International Food Policy Research Institute estimates that by 2050 rice prices may increase 35 per cent because of yield losses due to climate change.

Malnutrition in form of under nutrition, micronutrient deficiencies and obesity imposes unacceptably high economic costs and improving nutrition requires a multisectoral approach that begins with food and agriculture. A total of 842 million people in 2011-13, or around one in eight people in the world, are estimated to be suffering from chronic hunger, regularly not getting enough food for an active life.

The agriculture play its fundamental role in producing food and its processing, storage, transport and consumption contribute to the eradication of malnutrition. Because of better agriculture the total number of undernourished during 2013 has fallen by 17 percent since 1990–92. Agricultural policies and research must continue to support productivity growth for staple foods with greater attention to nutrient-dense foods and more sustainable production systems. Traditional and modern supply chains can enhance the availability of a variety of nutritious foods and reduce nutrient waste and losses.

Recently the Intergovernmental Panel on Climate Change (IPCC) predicted that global food production due to climate change will decline 2 per cent per decade for the remainder of this century compared to food production without climate change even as food demand increases 14 per cent per decade. In 2007, the panel was hopeful that gains in agricultural productivity would more than make up for losses due to climate change. But later research revealed in greater detail the impacts of climate change on sensitive crops and raised questions about how much elevated carbon dioxide levels could increase productivity.

The organic material decays without oxygen, in water-logged rice paddies, soil microbes generate methane, a greenhouse gas with 25 times more warming potential than CO₂. In India, rice methane emission accounts for about 10 per cent of the nation's total greenhouse gas (GHG) emissions. Also, nitrous oxide emissions from rice grown under dryer and aerated conditions, can be as significant as methane emissions which has about 300 times more warming potential than CO₂. It has not yet been estimated what percentage of nitrous oxide emissions come from rice cultivation in India, and other rice growing regions in Asia.

If we are unable to double yields on existing cultivated lands, due to food insecurity pressure, we are likely to clear more land for agriculture leaving environmental concerns and efficiency measures a side. This will have a ripple effect, putting additional pressure on already stressed water resources and wildlife habitat, accelerates climate change. This cycle, left unchecked, can only end with farmers competing for increasingly scarce water and arable land in the face of ever more extreme weather – from floods to droughts – brought on by climate change.

These colliding trends indicate that the world must learn to produce more food with less land, less water and less labour by devising, climate resilient more efficient and profitable production systems. Thus, more than ever, we need to produce more food with less land. Farmers must seek out crop production technologies that will be highly productive and have a smaller impact on water quality and quantity, climate and habitat. To do this, we have the tools and technologies that reduce the need for inputs like fertilizer, pesticides and herbicides; innovative irrigation methods that reduce water demand; and methods that reduce greenhouse gas emissions. Using improved technologies, there has been tremendous development in agriculture and productivity during the last two decades and it is hoped that with ingenuity, determination and innovative partnerships among everyone working in the agricultural sector, we can meet the food needs of 9 billion people by 2050 without irreparably harming our planet on which we all depend. However, all these

informations are scattered and need to be compiled and circulated widely. This series on Recent Advances in Crop Physiology is an effort in this direction, which will act as a reference to the farmers for directly implementing the technologies and also to help the researchers for planning their future research to improve crop productivity.

This second volume of *'Recent Advances in Crop Physiology'* encompasses 13 chapters written by the experts in the field describing production physiology, drought and salinity stresses, nutrient efficiencies particularly P and N, radiotracer and their use in mineral nutrition, nutritional quality of potato and wheat and role of bioregulators in increasing productivity through amelioration of abiotic stressed. Abiotic stresses are the major factors limiting crop productivity worldwide. The chapter one on 'Drought management in pulses and their diversification under new niches' and chapter seven on 'Can water deficit be useful in potato? – Some issues', widely covers the physiological behavior of these crops under water stresses and how best the water stress could be managed to increase productivity and quality of pulses and potato in India. Chapter eight on 'Bioregulators ameliorate water deficit stress in wheat' is an effort on water stress management through bioregulators and new molecules altogether a different approach.

There are plenty of acid soils and the soil salinity problem is increasing in India and worldwide due to faulty irrigation and drainage practices. A comprehensive chapter three on 'Salinity Management in Vertisols: Physiological Implications' and chapter six on 'Physiological basis of Iron toxicity and its management in crops' takes care of soil and crop management in saline soil and iron- toxicity in crops in acid soils and provide a guidelines how to manage these crops under these stresses. The nutrients and fertilizers are the driving force in increasing the productivity of any crop, but in recent years there is an indiscriminate use of nitrogen and phosphorus inspite of the fact that there is limited P sources on the planet. The use of nutrient efficient crop varieties are the best alternative for managing both deficiencies and excess of these nutrients and in chapter two on 'Role of phosphorus efficient genotypes in increasing crop production' and chapter 12 on 'Nitrogen-use efficiency and productivity of wheat crop' discuss these issues in depth with solutions.

The precise study of mineral nutrition in crop plants require use of radiotracer and hence chapter 10 on the 'Radiotracer use in understanding mineral nutrition of crop plants' is fully devoted on the same.

India is emerging as an export hub of several horticultural crops and chapter four on 'Physiological basis for maximizing yield potentials in coffee' extensively covers the major hurdles and list the ways to increase production and quality of coffee for domestic consumption as well as export. Similarly the chapter five on 'Bioregulators improve the productivity and quality of Indian table grapes' list the best practices and use of bioregulators to increase the productivity of indian grapes.

The forest cover majority of the geographical areas of India and world and play an important role in the climate management and environmental protection, but there are no systematic studies on the productivity of forest. The chapter nine on 'Phenology and productivity of forest flora of Gujarat' is an effort in this direction to highlight the issues how the phenological studies can help to increase forest

productivity of Gujarat and reduce the carbon dioxide concentrations on earth through carbon sequestration by forest plant species.

Finally, seed, which is the primary requirement for enhancing crop productivity, plays a vital role in ensuring food security, and a chapter on 'Quality seed- a mega factor in enhancing crop productivity' are well composed by the renowned scientists in the field.

I would like to express my gratitude to all the stalwarts of agriculture and plant biology from various disciplines who has contributed in enhancing agricultural production. Thanks are also due to all the staffs of plant physiology at DGR Junagadh for their help in the various ways. Finally, I would like to express my sincere thanks to Mr. Prateek Mittal for coming forward to take up the responsibility of publishing the series and Mr. Anil mittal and the staff of Astral International (P) Ltd, New Delhi for their care and diligence in producing the book timely.

Dr. Amrit Lal Singh

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Chapter 12

Nitrogen-use Efficiency and Productivity of Wheat Crop

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1. Introduction

Nitrogen is the most important nutrient element essential for growth and development of plants. Nitrogen availability is a major constraint on grain production and the main component of the carbon footprint of cereal production. Large amount of nitrogen fertilizers are being used to increase the crop production. Nitrogen fertilizers are used as an important agronomic tool to improve output, quantity as well as quality in all cultivated crops. However, current agricultural and economic environmental concerns require farmers to constantly optimize the application of nitrogen fertilizers throughout the growing season to avoid pollution by nitrates while preserving their economic margin. Over the past four decades, the doubling of agri-cultural food production worldwide has been associated with a seven fold increase in the use of nitrogen fertilizers (Hirel *et al.*, 2007).

Excessive use of fertilizer increases the cost of production and causes ecological risks like nitrate leaching, volatilized ammonia and greenhouse gas emission (nitrous oxides) (Galloway *et al.*, 2008; Hirel *et al.*, 2007). It has been estimated that nitrogen fertilizer accounts for more than 70 per cent of the greenhouse gases (GHGs) associated

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with the production of wheat (Mortimer *et al.*, 2004). Therefore, efficient use of available nitrogen reduces the cost of production, reduces the harmful impact on environment and increases the yield per unit nitrogen use. Given the cost of N fertilizer and the negative environmental impact of high fertilizer use, efficient utilization of nitrogen resources is vital to sustainable cereal production.

Breeding new crop plant cultivars with improved productivity in low nitrogen environments provided an effective approach to increase nitrogen use efficiency (Rengel and Marschner, 2005). Thus, breeding for cultivars that absorb and metabolize nitrogen most efficiently for grain or silage production is becoming increasingly important. Introduction of semi-dwarf wheat varieties which were responsive to nitrogen fertilizer application during 1970's saw an extensive use of fertilizers (Austin, 1999) but the present agricultural system demands improved productivity and profits with reduced inputs. It is well established fact that semi-dwarf wheat varieties have better yield potential over tall varieties due to better partitioning of nutrients to grain at the expense of straw. Semi-dwarf varieties have better grain yield potential for the same total N-uptake and total dry matter production *i.e.* greater grain-NutE (Fischer and Wall, 1976). Such crops would make better use of nitrogen fertilizer supplies giving higher yields with improved protein contents. Cultivars with increased NUE will be of economic to farmers and help reduce environmental contamination associated with excessive inputs of N fertilizers. There is increasing emphasis worldwide in breeding wheat cultivars with NUE (Ortiz-Monasterio *et al.*, 2001; Guarda *et al.*, 2004; LaPerche *et al.*, 2006; Hirel *et al.*, 2007; Li *et al.*, 2008).

Development of Nitrogen use efficient cultivars requires a better understanding of nitrogen metabolism and its regulation, and identification of target genes to monitor N uptake by either direct gene transfer or marker-assisted breeding. Nitrogen uptake is an essential element in crop improvement, either directly for grain protein content or indirectly for photosynthetic production. Nitrogen movement within the plant life cycle may be conveniently divided in two phase's *i.e.* vegetative and reproductive phases. During vegetative phase young developing roots and leaves act as a sink for assimilation of inorganic N to produce amino acids (Hirel and Lea, 2001). During reproductive stage remobilization of accumulated N takes place and shoots and/or roots behave as a source by providing amino acids which are later exported to reproductive and storage organs represented, such as seeds, bulbs, or trunks (Masclaux *et al.*, 2001). In cereal crops such as rice and wheat 60 to 95 per cent of grain N comes from the remobilization of N stored in roots and shoots before anthesis (Palta and Fillery, 1995; Mae, 1997; Habash *et al.*, 2006; Tabuchi *et al.*, 2007). In oilseed crops amount of N taken up by plant during grain filling is low (Rossato *et al.*, 2001) and as a result large quantities of N absorbed by plant and stored in vegetative organs is not used and lost in early falling leaves (Malagoli *et al.*, 2005)

2. Defining Nitrogen Use Efficiency

Nitrogen use efficiency (NUE) can be defined as the grain dry matter yield divided by the supply of available nitrogen from the soil and fertilizer (Moll *et al.*, 1982). Nitrogen-use efficiency is dependent on efficient uptake of nitrogen by crop per unit

nitrogen available from soil and fertilizer (known as nitrogen uptake efficiency) and accumulation of nitrogen by crop in the form of grain and dry matter yield (nitrogen utilization efficiency). Hence, there are two major components of NUE: 1) uptake efficiency ($UPE = N \text{ in plant}/N \text{ applied}$), which is important in low to moderate N environments and 2) utilization efficiency ($UTE = N \text{ in grain}/N \text{ in plant}$), which is most important in high N environments. Environments with moderate N levels appear suited for both components. Therefore, both nitrogen uptake efficiency and nitrogen utilization efficiency play important role in achieving the nitrogen-use efficiency in crop plants.

Identification of metabolic and physiological traits to increase NUE that may be transferred into elite varieties through breeding is therefore imperative. Promising approaches of nitrogen management strategies are also essential to increase NUE. Candidate metabolic and physiological traits with potential for improving NUE and for reducing excessive input of fertilizers, while maintaining an acceptable yield, is essential aspect of breeding for NUE. Improvement of traits through breeding will be most efficiently applied at the farm level when N-efficient cultivars are combined with N management strategies for high NUE.

3. Genetic Characterization of NUE

Characterizing and quantifying recent genetic progress bring meaningful information to researchers. The first decision that breeders have to take is to choose the N level for which they want to breed. Indeed, in numerous studies which analysed agronomic traits, significant genotype \times N ($G \times N$) inter-actions were detected (e.g. Le Gouis *et al.*, 2000; Laperche *et al.*, 2006a; Barraclough *et al.*, 2010), meaning that differential behaviour of variety depends on N treatment. Quantifying $G \times N$ interactions is, therefore, crucial for efficient selection. The identification of traits to improve NUE in wheat and the characterisation of their variability provide useful directions to breeders (e.g. Barraclough *et al.*, 2010; Foulkes *et al.*, 2009; Gaju *et al.*, 2011).

High grain yield with adequate protein content is an important goal in crop improvement, especially for bread wheat (*Triticum aestivum* L.). Unfortunately, it has been shown in various cereals, that these two traits are genetically negatively correlated in extensive (Simmonds, 1995; Oury *et al.*, 2003), although this correlation can be broken down by adequate nitrogen (N) supply late in plant development (Krapp *et al.*, 2005; Laperche *et al.*, 2006). Moreover, it is well known that a negative correlation between yield and protein content exists in wheat (Kibite and Evans, 1984; Simmonds 1995, Oury *et al.*, 2003; Oury and Godin, 2007; Bogard *et al.*, 2010). A yield increase may, therefore, lead to a decrease in protein content which could cause lower end-use quality (Ortiz-Monasterio *et al.*, 1997b; Shewry, 2004). Thus, the question of the genetic improvement in yield or NUE cannot be assessed independent of quality. Studies conducted at different N levels have concluded that genetic progress occurred in both HN and LN conditions, but was higher at HN (Ortiz-Monasterio *et al.*, 1997a; Brancourt-Hulmel *et al.*, 2003; Guarda *et al.*, 2004). Fewer studies have been published on the genetic progress for NUE and its components (Ortiz-Monasterio *et al.*, 1997a; Guarda *et al.*, 2004; Muurinen *et al.*, 2006).

Table 12.1: NUE Definitions and their Units (Foulkes *et al.*, 2009)

Name	Definitions	Units
Nitrogen use efficiency	Kilogram (grain dry mass) at harvest per kilogram available nitrogen (from soil plus fertilizer)	kg kg ⁻¹
Nitrogen-uptake efficiency (UPE)	Kilogram (above-ground nitrogen) at harvest per kilogram available nitrogen (from soil plus fertilizer)	kg kg ⁻¹
Nitrogen-utilization efficiency	Kilogram (grain dry mass) per kilogram (above-ground N) at harvest	kg kg ⁻¹
Nitrogen harvest index (NHI)	Proportion of above-ground nitrogen in the grain at harvest	Unitless
Above-ground nitrogen uptake (AGN)	Gram (above-ground nitrogen) per square metre (ground) at harvest	gm ⁻²
Grain nitrogen concentration (GNC)	Gram (grain nitrogen) per gram (grain dry mass) at harvest X 100	per cent
Root length density (RLD)	Centimetre (root) per cubic centrimetre (soil)	cm cm ⁻³
Green area index (GAI)	Square metre (green area) per square metre (ground)	m ² m ⁻²
Specific leaf nitrogen content (SLN)	Gram (leaf nitrogen) per square metre (leaf area)	g m ⁻²
Radiation-use efficiency (RUE)	Gram (above-ground biomass) per megajoule intercepted global radiation	g MJ ⁻¹
Light-saturated CO ₂ exchange rate (A _{max})	Micromoles (CO ₂) per square metre (leaf area) per second in light-saturated conditions	μmol m ⁻² s ⁻¹
Nitrogen remobilization efficiency (NRE)	Proportion of nitrogen in the whole plant or organ at anthesis which is not recovered in the straw at harvest	Unitless

Various studies worldwide have identified genetic associations between grain yield and NUE components under contrasting conditions of high and low N input supply. In general, these studies indicated that UPE accounts for more of the genetic variation in NUE at low N than at high N supply (Dhugga and Waines, 1989; Ortiz-Monasterio *et al.*, 1997; LeGouis *et al.*, 2000; Muurinen *et al.*, 2006). Few studies have attributed genetic gains in NUE to UTE (Brancourt-Hulmel *et al.*, 2003; Foulkes *et al.*, 1998). Modern cultivars normally had higher yields than old cultivars under low N input conditions (Ortiz-Monasterio *et al.*, 1997; Foulkes *et al.*, 1998; Brancourt-Hulmel *et al.*, 2003). Genetic variation for NUE has been noted in rice (Borrell *et al.*, 1998), wheat (Le Gouis *et al.*, 2000) and barley but improvements in NUE have been limited by expensive and laborious phenotyping (Clark, 1983; Le Gouis *et al.*, 2000; Wang *et al.*, 2011). This variability could help us understand the genetic basis of NUE.

Two major approaches are used to assess genetic progress: (1) historical trial analyses and (2) direct comparison of old and modern varieties in the same environment. But these two approaches suffer from some limitations. (1) When historical trials are analysed, as genotypes are tested in different year \times environment combinations, there is a need to take into account agro-climatic variation. This may induce bias as elimination of 2-yearly effects is often based on variation from year-to-year of common controls leading to inadequate consideration of genotype \times year interactions (Oury *et al.*, 2012; Graybosch and Peterson, 2012). (2) Direct comparisons of old and modern varieties are often limited by the experiment size (Brancourt-Hulmel *et al.*, 2003; Guarda *et al.*, 2004; Muurinen *et al.*, 2006; Green *et al.*, 2012) with few genotypes studied in few environments. This can cause sampling errors. Lopez *et al.* (2012) proposed to base genetic progress assessment only on the highest yielding variety per date of release but still with a quite low number of cultivars. Moreover, the period under study is usually spread out and includes major changes in plant height due to introduction of dwarfing alleles. Indeed, height decrease is one of the major sign of winter wheat genetic improvement between 1946 and 1992 in France (Brancourt-Hulmel *et al.*, 2003) as well as other countries (Ortiz-Monasterio *et al.*, 1997a; Austin, 1999). It is directly linked to NUE through an increase in lodging resistance and nitrogen partitioning (Hedden, 2003). Plant height is now stabilised; therefore, the question of recent genetic gain can be asked independently of this major physiological change using a large panel of recent cultivars grown in the same environments.

Quantitative trait locus (QTL) analysis has proved to be an effective approach to dissect a complex quantitative trait into component loci to study their relative effects on the trait (Doerge, 2002). Nowadays, QTL mapping has become a routine procedure for the identification of genomic regions harboring the genes which control polygenic traits (Saal *et al.*, 2011). Several QTL experiments have been conducted in wheat to study N use efficiency under different N levels in hydroponic culture (An *et al.*, 2006; Guo *et al.*, 2012; Laperche *et al.*, 2006), pot trials (Habash *et al.*, 2007) and field trials (An *et al.*, 2006; Fontaine *et al.*, 2009; Laperche *et al.*, 2007; Quarrie *et al.*, 2005), or to identify QTLs for phosphorus (P) use efficiency in P sufficient and limited conditions (Li *et al.*, 2007b; Su *et al.*, 2006, 2009). Several QTL analyses have been performed during the last two decades on barley (Kjær *et al.*, 1995), maize (Agrama *et al.*, 1999;

Bertin and Gallais, 2001; Hirel *et al.*, 2001), rice (Obara *et al.*, 2001; Lian *et al.*, 2005), wheat (An *et al.*, 2006; Habash *et al.*, 2007; Laperche *et al.*, 2007; Fontaine *et al.*, 2009), and *Arabidopsis thaliana* (Rauh *et al.*, 2002; Loudet *et al.*, 2003). Xu *et al.*, 2014 reported three major QTLs for NUE in wheat located on chromosomes 2D, 4B and 6A which were coincident with *Rht8*, *Rht-B1b* and *TaGW2*, respectively.

Genes encoding major enzymes have been cloned and shown to drive N economy in plants (Mifflin and Habash, 2002; Bernard and Habash 2009). Eleven major chromosomal regions controlling NUE in wheat that co-localise with key developmental genes such as *Ppd* (photoperiod sensitivity), *Vrn* (vernalization requirement) and *Rht* (reduced height) have been identified in wheat (Quraishi *et al.*, 2011). Physical mapping, sequencing, annotation and candidate gene validation of an NUE metaQTL on wheat chromosome 3B allowed them to propose that a glutamate synthase (GoGAT) gene that is conserved structurally and functionally at orthologous positions in rice, sorghum and maize genomes may contribute to NUE in wheat and other cereals.

4. Role of Glutamine Synthetase and Glutamate Dehydrogenase in Nitrogen Assimilation

During the growth and development of plants, nitrogen is moved into and out of proteins in the different organs and transported between organs in a limited number of transport compounds. Some of the organic nitrogen is moved between compounds via the activity of transaminases and glutamine-amide transferases, but a significant portion is released as NH_3 and re-assimilated via Glutamine synthetase (GS) which plays a central role in nitrogen metabolism. Glutamine synthetase (GS; EC 6.3.1.2) was first purified and characterized from plants in 1956. One particular characteristic is its high affinity for ammonia and thus its ability to incorporate ammonia efficiently into organic combination. However, the discovery of NAD(P)H glutamate synthase in bacteria (Tempest *et al.*, 1970) and later ferredoxin-dependent glutamate synthase in plants (Lea and Mifflin, 1974) established a route, the glutamate synthase cycle, for NH_3^{2-} to enter into organic compounds via its assimilation by GS. Evidence based on labelling kinetics, use of inhibitors, in organello studies, and genetics established that this was the major route of primary nitrogen assimilation in plants (Mifflin and Lea, 1980).

The GS is the first key enzyme for N metabolism, as it catalyses assimilation of all inorganic nitrogen incorporated into organic compounds, such as proteins and nucleic acids. This reaction is coupled to the formation of glutamate by glutamate synthase (GoGAT) as part of the GS/GoGAT cycle. Although ferredoxin-GoGAT plays a critical role in the re-assimilation of ammonium released by glycine decarboxylase during photo-respiration, NADH-GoGAT assimilates ammonium from both primary and secondary sources during nitrogen remobilization (Lea and Mifflin, 2003). Genes coding for these two key enzymes of NH_4 assimilation has been cloned in monocots [rice (Tabuchi *et al.*, 2007; Cai *et al.*, 2009), wheat (Caputo *et al.*, 2009), maize (Valadier *et al.*, 2008)] and *Arabidopsis* (Ishiyama *et al.*, 2004; Potel *et al.*, 2009), Brassicaceae (Ochs *et al.*, 1999), *Medicago truncatula* (Lima *et al.*, 2006).

The GS lies at the intersection of C and N metabolic pathways, and its manipulation in wheat plants could potentially raise NUE through more efficient internal recycling of N from older to new leaves. During late leaf senescence NH_3 accumulates in leaves and is liable to be lost from the plant by volatilization and high GS activity reduces these losses (Mattsson *et al.*, 1998). Two major forms of GS exist, comprising: (i) up to five cytosolic isoforms of GS (*GS1*) in root and shoot cells and (ii) a plastidic isoform, GS (*GS2*), in the chloroplasts of photosynthetic tissues and in the plastids of roots and other non-photosynthetic tissue. The *GS1* isoforms are differentially expressed in different plant tissues and specific isoforms appear to play a major role in the synthesis of glutamine in senescing leaves for transport to new tissues. Increases in the activities of *GS1* during leaf senescence have been reported in many plant species (Feller and Fischer, 1994). Masclaux *et al.* (2001) observed a positive relationship between *GS1* activity and UTE and grain yield of maize RILs grown under low N. Similarly, Hirel *et al.* (2001) found positive correlations between leaf nitrate content, *GS1* activity and yield in maize RILs under low N, and co-incident location of QTLs for NUE and a structural gene for *GS1*. Adding an extra GS gene to wheat altered the balance of *GS1* and *GS2* activity in flag leaves of plants (Habash *et al.*, 2001), and in pot trials one such transformed line showed a fourfold increase in leaf *GS1* activity and had more roots, more grain and higher grain N. Two cytosolic *GS1* isoforms have been shown to have major impacts on grain production in maize (Martin *et al.*, 2006) whilst a deletion in single specific *GS1* isoform had a strong impact on growth and grain filling in rice (Tabuchi *et al.*, 2005). QTLs for grain N but not yield were found to map with GS genes in wheat (Habash *et al.*, 2007).

Most cultivar testing systems worldwide use a relatively high N supply, which means that plant breeders select cultivars that perform well under these conditions. This means that cultivars selected for high yield under high N conditions may not be the highest yielding under lower N conditions. In order to reliably identify which cultivars will yield well at lower N supplies will requires both breeding and testing at low levels of N input (Brancourt-Hulmel *et al.*, 2005) or an understanding of which plant traits are important for yield under these conditions.

One of the key traits that could explain the genetic variability associated with yield variation under low N is leaf senescence kinetics. The capacity of a genotype to retain green leaf area for longer than a standard genotype during grain-filling has been referred to as the 'stay-green' phenotype (Thomas and Smart, 1993). Stay green genotypes may have an increased source : sink ratio during grain filling with an increased fraction of N derived from the soil N. Using stay green genotypes under low N was associated with improved performance in both sorghum (Borrell and Hammer, 2000) and maize (Mi *et al.*, 2003). Genetic variation in functional stay-green lines has been reported in wheat (Silva *et al.*, 2000; Verma *et al.*, 2004; Foulkes *et al.*, 2007), although the underlying physiological mechanisms have not been studied extensively. Christopher *et al.* (2008) found that the stay-green phenotype in spring wheat *SeriM82* was associated with extraction of deep soil water in Australia. In summary, the maintenance of a photosynthetically active canopy in the post-anthesis period under low to moderate N supply should optimize the efficiency of N input use for grain.

5. Traits of NUE

Promising traits and their measurement methodologies for selection to increase NUE are:

- ☆ Anthesis date (AD)
- ☆ Crop dry mass and N content
- ☆ The concentration of N in the straw and ears.
- ☆ Nitrogen Nutrition Index (NNI) could be estimated according to the ratio of the actual above-ground crop N per cent at anthesis and the critical N per cent (N per cent ct), where N per cent ct was estimated according to the 'critical dilution curve' described by Justes *et al.* (1994).
- ☆ The above-ground N and DM per unit ground surface area
- ☆ Number of ears m⁻² changes
- ☆ Crop height measured 14 d after the end of anthesis, estimates of grain number per unit of ground surface area (GN), ear number per unit of ground surface area, grain number per ear and DM of straw, chaff and grain per unit of ground surface area were calculated.
- ☆ The harvest index (HI) and N harvest index (NHI) calculated as the proportion of DM and N in the above-ground plant which is in the grain at harvest, respectively.
- ☆ The nitrogen-use efficiency (NUE) was calculated by dividing the DM grain yield (kg DM ha⁻¹, 0 per cent moisture content) by the amount of N available to the crop from the soil and fertilizer (kg N ha⁻¹).
- ☆ N × cultivar interaction
- ☆ The N-uptake efficiency was calculated by dividing the aboveground N at harvest (kg N ha⁻¹) by the amount of N available to the crop from the soil and fertilizer (kg N ha⁻¹); and the N-utilization efficiency was calculated by dividing the machine-harvested DM grain yield (kg DM ha⁻¹, per cent moisture content) by the above-ground N at harvest (kg N ha⁻¹). The crop N remobilization efficiency (NRE) was calculated as: $\{AGN_H - (AGN_H - GN)\} / AGNA$, where AGNA is the above-ground N at anthesis (kg N ha⁻¹), AGNH (kg N ha⁻¹) is the above-ground N at harvest and GN (kg N ha⁻¹) is the grain N at harvest (kg N ha⁻¹).
- ☆ Lodging was defined as the stems leaning at an angle of at least 45° from the vertical. Close to the time of harvest the proportion of the plot area that was lodged was visually assessed to the nearest 0.05.
- ☆ Senescence kinetics of the flag leaf of five main stems at CF or of the whole canopy at other sites were assessed visually by recording the percentage green area senesced using a standard diagnostic key based on a scale of 0–10 (100 per cent senesced).
- ☆ Increased root length density (RLD) at depth.
- ☆ High capacity for N accumulation in the stem, is associated with high N-uptake rate.

- ☆ Low leaf lamina N concentration,
- ☆ More efficient post-anthesis remobilization of N from stems to grain, but less efficient remobilization of N from leaves to grain, both potentially associated with delayed senescence
- ☆ Reduced grain N concentration may be of particular value for increasing NUE in wheat cultivars used for feed purpose; and
- ☆ Physiological traits relevant for nitrogen-use efficiency: Root length density at depth, GS activity, Alanine aminotransferase (AlaAT) activity, RuBisCo CO₂ specificity factor, Introduction of C₄ 'Krantz' anatomy into C₃ species, specific leaf N content, vertical N distribution with leaf layer, leaf posture, leaf photosynthetic rate post-anthesis, stem N storage, stay-green, N remobilization, efficiency post-anthesis and post-anthesis N uptake.

6. Association of NUE with yield

In a number of studies there was inverse relationships between grain yield and grain N concentration ($(\text{grain N}/\text{grain DM}) \times 100$, GNC) (Kibite and Evans, 1984; Triboi *et al.*, 2006). Assuming a constant N harvest index (the proportion of above-ground N (AGN) at harvest in the grain; NHI), grain yield will be positively associated with AGN and/or negatively associated with GNC. Various CSR indices show high correlations with barley and wheat grains yield, biomass and N concentration and are already used in N management strategies. The CSR estimates of biomass and N content can be combined with yield and grain protein to estimate UPE and UTE. The association of CSR with biomass is particularly important, as biomass is a key component of NUE and yield, it is expensive to measure, and harvest index is approaching its theoretical limit, restricting future yield gains to increases in biomass. Under low N supply, genetic gains in yield were positively associated with AGN and NHI and negatively associated with GNC (Foulkes *et al.*, 1998; Ortiz-Monasterio *et al.*, 1997a; Brancourt-Hulmel *et al.*, 2003). Whereas under high N supply, genetic gains in yield were positively associated with AGN (Ortiz-Monasterio *et al.*, 1997a; Brancourt-Hulmel *et al.*, 2003; Foulkes *et al.*, 1998), positively (Ortiz-Monasterio *et al.*, 1997a; Brancourt-Hulmel *et al.*, 2003) or neutrally (Foulkes *et al.*, 1998) associated with NHI, and negatively (Ortiz-Monasterio *et al.*, 1997a; Brancourt-Hulmel *et al.*, 2003) or neutrally (Foulkes *et al.*, 1998) associated with GNC. Essentially, under both low and high N supply, wheat crops with higher NUE compared to current cultivars will require an increase in UPE to raise AGN and/or an increase in UTE. The latter may be achieved by raising NHI and/or lowering GNC. There have been relatively few attempts to use 'direct selection' breeding to improve NUE in wheat. CIMMYT in Mexico adopted a strategy to select for grain yield in medium-to-high fertility conditions, since at this fertility level both UPE and UTE contribute to the observed variation in NUE, resulting in lines which were more N-efficient (Ortiz-Monasterio *et al.*, 1997a). More recently, it has been suggested that this method of selection may not be as efficient as selecting lines under alternating high-low N selection regimes commencing with high N in the F₂ (Van Ginkel *et al.*, 2001).

7. Root Traits Influencing N-uptake

The factors affecting the N uptake efficiency are root axis number, rooting depth, rooting density and root longevity. There is a Root membrane N transporter systems and root proliferation which has a direct relation to N status. Two distinct gene families of nitrate transporters, *NRT1* and *NRT2*, have been identified (Crawford and Glass, 1998; Forde, 2000; Forde and Clarkson, 1999; Williams and Miller, 2001; Hawkesford and Miller, 2004) in the Arabidopsis genome. Some members of both *NRT1* and *NRT2* gene families are nitrate inducible, and are expressed in the root epidermis and in root hairs, and are likely to be responsible for the uptake of nitrate from the soil (e.g., Lauter *et al.*, 1996; Zhuo *et al.*, 1999; Ono *et al.*, 2000; Orsel *et al.*, 2002). Many plant ammonium transporter (*AMT*) genes, which complement a yeast mutant deficient in normal ammonium uptake, have been identified (Von Wiren *et al.*, 2000). There are six *AMT* genes in Arabidopsis, while rice has 10, with two types being distinguishable on the basis of sequence but both conferring high affinity uptake. Like the nitrate transporters, some *AMT* genes are expressed in root hairs (Lauter *et al.*, 1996; Ludewig *et al.*, 2002). Manipulation of N transporters in wheat plants to give higher rates of nitrate transport across the plasma membranes of root cells, thereby improving nitrate and ammonium capture from the soil. Signalling and root proliferation in relation to N status in Arabidopsis (Zhang *et al.*, 1999; Walch-Liu *et al.*, 2006; Rogg *et al.*, 2001; Casimiro *et al.*, 2003; Remans *et al.*, 2006). There are again prospects for transferring this information to wheat for improving UPE in the long term if the root screens used for Arabidopsis could be adapted to the larger and structurally different root system of wheat.

8. Traits Influencing N-utilization

N-utilization efficiency (NUTE) is dependent on the N efficiency of biomass formation, the effect of N on carbohydrate partitioning, nitrate reduction efficiency and remobilization of N from senescent tissues as well as storage functions (Novoa and Loomis, 1981; Good *et al.*, 2004; Lea and Azevedo, 2007; Hirel *et al.*, 2007).

8.1 Nitrate Assimilation

Absorbed nitrate is reduced to nitrite in the cytosol by the enzyme nitrate reductase (NR). Nitrite is transported to the plastid or chloroplast and reduced to ammonium by the enzyme nitrite reductase (NiR). Ammonium is then converted to glutamine and glutamate in the plastid chloroplast by the glutamine synthetase/glutamate synthase (GS/GOGAT) enzyme system (Mifflin, 1980; Ireland and Lea, 1999).

Nitrate reductase was long considered to be the bottleneck in nitrate assimilation and was thought to be pivotal in the growth response to N supply. However, numerous studies altering the expression of NR-encoding genes have resulted in no change to plant growth (Crawford, 1995). Studies of maize (Masclaux *et al.*, 2001) and wheat (Kelly *et al.*, 1995) have also failed to find correlations between leaf NR activity and yield. It appears therefore that N assimilation and yield are not generally limited by the level and activity of the NR/NiR enzymes.

8.2 Manipulating Photochemistry to Improve NUTE

Genetic progress in conversion of N into grain yield could be enhanced by improving the efficiency of fixation of CO₂ (Parry *et al.*, 2007; Long *et al.*, 2006). The key photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) typically constitutes up to 30 per cent of the total N in wheat leaves (Lawlor, 2002) and under field conditions leaf photosynthetic rate is highly correlated with RuBisCo content (Hudson *et al.*, 1992; Lauerer *et al.*, 1993). However, the RuBisCo of thermophilic red algae, *e.g.* *Galderia partita*, is up to three times more efficient than those of C₃ cereals due to greater specificity for CO₂ (Uemura *et al.*, 1997). There are therefore long term prospects of boosting UTE by introducing forms of RuBisCo with greater specificity for CO₂ from red algae into wheat plants, thereby reducing photorespiration.

8.3 Post-anthesis N Remobilization

During grain filling the N stored as protein in the vegetative tissues becomes important as root N uptake falls short of the grain N demand. Bread-making wheat cultivars in particular require efficient translocation of N during grain filling. Genetic variation in N remobilization efficiency of the vegetative tissues has been reported in wheat in the overall range 0.52–0.92 (Cox *et al.*, 1986; Van Sanford and Mackown, 1987; Papakosta and Garianas, 1991; Barbottin *et al.*, 2005; Tahir and Nakata, 2005), and the genotype effect has been shown to depend on year (Przulj and Momcilovic, 2001) and N fertilization level (Cox *et al.*, 1986; Papakosta and Garianas, 1991).

8.4 The Stay-Green Trait

Several investigations have concluded that the genetic control of N remobilization seems likely to be involved in the regulation of leaf senescence (Sinclair and De Wit, 1975; Masclaux *et al.*, 2001), and an improved understanding of N remobilization could be important in developing cultivars with stay-green properties. Although under optimal conditions wheat crops are in general little limited by the assimilate supply during grain filling (Dreccer *et al.*, 1997; Borra's *et al.*, 2004; Calderini *et al.*, 2006), under low to moderate N fertilizer levels yields may be more limited by postanthesis assimilate supply. The capacity of a genotype to retain green leaf area for longer than a standard genotype during grain filling has been referred to as the 'stay-green' phenotype (Thomas and Smart, 1993). In the grasses, such as *Festuca pratensis*, stay green mutant lines have been identified (Thomas *et al.*, 2002) in which the phenotype is non-functional (delayed senescence not associated with extended photosynthesis). One stay-green mutation of this type was located in the phaeophorbide an oxygenase gene (Thomas *et al.*, 2002), increasing the stability of the light harvesting and reaction-centre thylakoid membrane proteins during senescence. Genetic variation in functional stay-green (delayed senescence associated with extended photosynthesis) lines has been reported in bread wheat (Silva *et al.*, 2000; Verma *et al.*, 2004; Foulkes *et al.*, 2007), although the underlying physiological mechanisms have not been studied extensively. Christopher *et al.* (2008) found that the stay-green phenotype in spring wheat SeriM82 was associated with extraction of deep soil water in Australia. More studies have been carried out on the mechanisms

underlying genetic variation in stay-green in sorghum. Nitrogen dynamics are an important factor in the maintenance of green leaf area in sorghum, with stay-green in sorghum hybrids linked to changes in the balance between N demand and supply during grain filling resulting in a slower rate of N translocation from the leaves to the grain compared with senescent genotypes (Borrell and Hammer, 2000). Stay-green mutants have also been identified in durum wheat (*Triticum turgidum* spp. durum) (Spano *et al.*, 2003), with delayed senescence being correlated with a higher rate and duration of grain filling. A transcription factor (NAM-B1) accelerates senescence and increases N remobilization from leaves to grains in emmer wheat (an ancient cultivated tetraploid species, *T. turgidum* ssp. *dicoccoides*), whereas modern durum wheat varieties carry the non-functional NAM-B1 allele (Uauy *et al.*, 2006).

8.5 Optimizing Grain Protein Content and Composition

Grain proteins can be broadly divided into structural/metabolic and storage proteins (Shewry and Halford, 2002). The embryo and outer layers of the grain (including the aleurone) contain about 30 per cent of the total grain N. They are enriched in albumins, globulins and insoluble proteins, most of which are structural and metabolic in function, but both tissues also store a 7S globulin protein. Structural and metabolic proteins are also present in the starchy endosperm cells, but the predominant protein fraction in this tissue is the gluten storage proteins, comprising a mixture of monomeric gliadins and polymeric glutenins. These groups of proteins are present in approximately equal amounts and together account for about 60–70 per cent of the total N in the endosperm tissue. The gluten proteins are crucial for the processing of wheat into bread, other baked food, pasta and noodles, as they confer viscoelastic properties to dough. A precise balance of gliadin and glutenin proteins is also required, as glutenins are predominantly responsible for dough elasticity (strength) and gliadins for dough viscosity and extensibility. Thus, highly elastic (strong) doughs are required for bread making and more extensible doughs for making biscuits and cakes.

Cultivars for bread making are selected for high protein content and strong gluten properties with appropriate levels of N fertilizer being applied to the crop to ensure that the required protein content is achieved. For example, the Chorleywood Bread making Process, which is the predominant process in the UK and a number of other countries, requires wheat with a minimum protein content of 13 per cent on a dry weight basis. Although “high protein” genes have been reported in wheat, there is in fact limited variation between the grain protein contents of elite wheat cultivars grown under similar agronomic conditions.

9. Summary and Conclusion

Nitrogen is the important nutrient element required for crop growth and glutamine synthetase plays an important role in its assimilation in plants. Increased cost of fertilizers and environmental concerns has forced us to use fertilizers more judiciously. Semi-dwarf rice and wheat varieties released during green revolution possessed higher nitrogen utilization efficiency as they had an ability to translocate most of the absorbed nitrogen to the developing grains. Hence, breeding cultivars

with that absorb and use nitrogen efficiently is becoming important. Present day cultivars of cereals such as rice and wheat possess better N utilization efficiency than old cultivars and further improvement of N uptake efficiency under low N conditions would enhance NUE of the present cultivars.

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