



Review

Identifying traits to improve the nitrogen economy of wheat: Recent advances and future prospects

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ABSTRACT

Nitrogen fertilizer represents a significant cost of production for the grower and may also have environmental impacts through nitrate leaching, use of fossil fuels for manufacture and application, and N₂O emissions associated with denitrification. The development of N-efficient cultivars will be of economic benefit to farmers and will help to reduce environmental contamination associated with excessive inputs of N fertilizers. The physiological, metabolic and physio-chemico processes that may contribute to high N-use efficiency (NUE) and reduced N fertilizer inputs while maintaining an acceptable yield are reviewed and the prospects for application in breeding programs discussed. Promising N management strategies for high NUE are also considered. Traits operating from the cellular to the whole-crop scale, including root traits, nitrate assimilation, N partitioning, leaf and canopy photosynthesis, senescence, grain N remobilization and grain protein content and composition, are discussed in relation to their optimization in both feed and bread-making cultivars. Promising traits for selection by breeders to increase NUE are identified, and approaches for their integration at the whole plant level discussed. It is concluded that: (i) increased root length density (RLD) at depth, (ii) a high capacity for N accumulation in the stem, potentially associated with a high maximum N-uptake rate, (iii) low leaf lamina N concentration, (iv) 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, and (v) a reduced grain N concentration may be of particular value for increasing NUE in feed wheat cultivars; and (vi) for bread-making cultivars, high NUE may be associated with high capacities for uptake and assimilation of N, with high post-anthesis N remobilization efficiency and/or specific grain protein composition.

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1. Definition of N-use efficiency

It is important to define the complex trait of nitrogen-use efficiency (NUE) before considering its improvement through breeding and N management strategies. Moll et al. (1982) defined NUE as grain dry matter yield per unit of N available (from the soil and/or fertilizer) and divided it into two components: (i) N-uptake efficiency (crop N uptake/N available; UPE) and (ii) N-utilization efficiency (grain dry matter yield/crop N uptake; UTE). This definition of NUE has since been found useful by crop physiologists and Table 1 summarizes the definition and abbreviations adopted in the present review. The nitrogen economy of wheat is considered in relation to both NUE and the relationships between grain protein content and composition and N supply.

2. Rationale for improved N economy

Wheat crops with increased NUE will be of economic benefit to farmers and will help to reduce environmental contamination associated with excessive inputs of N fertilizers. Wheat crops with low NUE may be associated with: (i) nitrate leaching polluting ground water, (ii) eutrophication of rivers and lakes and (iii) global warming, due to emission of nitrous oxide derived from denitrification of nitrate by soil bacteria and the use of fossil fuels in the manufacture of N fertilizers (Goulding, 2004). Therefore, there is increasing emphasis worldwide in breeding wheat cultivars (Ortiz-Monasterio et al., 2001; Guarda et al., 2004; LaPerche et al., 2006; Hirel et al., 2007; Li et al., 2008) and in developing wheat N management strategies (Raun et al., 2002; Shanahan et al., 2008) for high NUE. In this review the principal focus is on identifying metabolic and physiological traits with potential to increase NUE that may be transferred into elite varieties through breeding. Initially, future promising approaches for N management strategies to increase NUE are briefly reviewed, since the development of new N-efficient wheat cultivars will benefit from improved agronomy. Evidence is presented for genetic diversity in NUE and its two components. In addition, the candidate metabolic and physiological traits with potential for

improving NUE and for reducing excessive input of fertilizers, while maintaining an acceptable yield, are examined. Approaches for integrating traits to estimate trade-offs and the translation to yield and to identify the most promising target traits for future breeding are discussed. Finally the practical application of traits in breeding is considered including the development of methodologies for high-throughput screening of genotypes for the key traits in segregating generations.

3. Management strategies for improving 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. Management strategies have most potential to increase NUE through raising UPE. An extensive review of the adoption of agronomic practices to meet the challenge of increasing NUE in wheat systems is beyond the scope of this paper. Comprehensive reviews of N management strategies for raising NUE of cereals are available, which examine quantitative understanding of current levels of NUE and future promising agronomic approaches (Cassman et al., 2002; Shanahan et al., 2008). Currently worldwide recovery of N fertilizer in wheat systems is on average low at ca. 30–50% (Raun et al., 2002), although in some countries in high potential conditions higher values for wheat are reported at ca. 50–60%, e.g. in the UK for winter wheat (Sylvester-Bradley et al., 1997). The major managerial causes for low UPE are: (i) poor synchrony between fertilizer N and crop demand, e.g. associated with large application of N fertilizer early in the season (Raun and Johnson, 1999; Fageria and Baligar, 2005), (ii) uniform field applications to spatially variable fields (Hurley et al., 2004; Koch et al., 2004), (iii) use of 'yield-based' N recommendations assuming constant efficiency of fertiliser recovery (Sylvester-Bradley et al., 1990) and (iv) failure to take account of year-to-year weather variations, e.g. the amount soil N mineralized in warm, wet years (Shanahan et al., 2008; Powlson, 1993). Future agronomic options for raising UPE include in-season crop monitoring through ground-based reflectance sensors (Raun et al., 2002) or leaf chlorophyll meters (Blackmer

Table 1
List of physiological variables, their definitions and units.

Physiological variable (symbol)	Definition	Units
Nitrogen-use efficiency (NUE)	Kilogram (grain dry mass) at harvest per kilogram available N (from soil plus fertilizer)	kg kg ⁻¹ (unitless)
Nitrogen-uptake efficiency (UPE)	Kilogram (above-ground N) at harvest per kilogram available N (from soil plus fertilizer)	kg kg ⁻¹ (unitless)
Nitrogen-utilization efficiency (UPE)	Kilogram (grain dry mass) per kilogram (above-ground N) at harvest	kg kg ⁻¹ (unitless)
Nitrogen harvest index (NHI)	Proportion of above-ground N in the grain at harvest	Unitless
Above-ground N uptake (AGN)	Gram (above-ground nitrogen) per square metre (ground) at harvest	g m ⁻²
Grain nitrogen concentration (GNC)	Gram (grain N) per gram (grain dry mass) at harvest × 100	%
Root length density (RLD)	Centimetre (root) per cubic centimetre (soil)	cm cm ⁻³
Green area index (GAI)	Square metre (green area) per square metre (ground)	(m ² m ⁻²) (unitless)
Specific leaf nitrogen content (SLN)	Gram (leaf N) 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	mmol m ⁻² s ⁻¹
Nitrogen remobilization efficiency (NRE)	Proportion of N in the whole plant or organ at anthesis which is not recovered in the straw at harvest	Unitless

and Schepers, 1995; López-Bellido et al., 2004) or aerial or satellite imagery (Shanahan et al., 2001) to target rates that meet but do not exceed crop N requirements.

The long-term effects of management practices on soil quality will also have an important influence on the NUE of the entire agro-ecosystem. Soil organic matter content is a key measure of soil quality and soils that sequester carbon also sequester N, resulting in greater indigenous N supply and a reduction in N fertilizer requirements (Cassman et al., 2002). Therefore, management practices which increase soil organic matter will generally provide efficiency benefits over the long term (see Jenkinson and Smith, 1989; Powlson, 1993). In summary, the better the agronomic basis of NUE is understood the more likely that breeding strategies designed to raise productivity will be applied efficiently. The prospects for genetic improvement of N-use efficiency and for reducing excessive input of N fertilizers are now examined.

4. Genetic diversity in N-use efficiency

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, e.g. amongst 10 spring wheat cultivars in Mexico (Ortiz-Monasterio et al., 1997) and 20 winter wheat cultivars in France (LeGouis et al., 2000). However, Dhugga and Waines (1989) comparing 12 spring wheats in California, found that UPE was the most important component of NUE at all N levels.

Genetic gains in NUE with breeding under low N supply have been related mainly to improvements in UPE in spring wheat in Mexico (Ortiz-Monasterio et al., 1997) and Finland (Muurinen et al., 2006) and to UTE in winter wheat in France (Brancourt-Hulmel et al., 2003) and the UK (Foulkes et al., 1998). In these studies, 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).

Under high N supply, several studies worldwide concluded that wheat breeding did not result in consistent improvements in UPE but in improved UTE associated with higher harvest index, e.g. in Mexico (Fischer and Wall, 1976), Argentina (Calderini et al., 1995a), France (Brancourt-Hulmel et al., 2003) and in various countries (Paccaud et al., 1985; Feil, 1992). In contrast, studies in the UK (Foulkes et al., 1998), Mexico (Ortiz-Monasterio et al., 1997) and Finland (Muurinen et al., 2006) found that increases in NUE were explained approximately equally by UPE and UTE.

Numerous studies of cultivars and segregating populations have shown inverse relationships between grain yield and grain N concentration ($(\text{grain N/grain DM}) \times 100$, GNC) (e.g. Kibite and Evans, 1984; Tribouflet 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 according to Eq. (1).

$$\text{Grain yield (DM) (kg/ha)} = \frac{\text{AGN (kg/ha)} \times \text{NHI}}{\text{GNC}/100} \quad (1)$$

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., 1997; Brancourt-Hulmel et al., 2003). Whereas under high N supply, genetic gains in yield were positively associated with AGN (Ortiz-Monasterio et al., 1997; Brancourt-Hulmel et al., 2003; Foulkes et al., 1998), positively (Ortiz-Monasterio et al., 1997; Brancourt-Hulmel et al., 2003) or neutrally (Foulkes et al., 1998) associated with NHI, and negatively (Ortiz-Monasterio et al., 1997; 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 to date 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., 1997). 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). The potential to identify traits for use as selection criteria to complement direct selection strategies to raise the NUE of wheat is considered in the next section of this paper.

5. Traits influencing N-uptake efficiency

5.1. Root size and morphology

Wheat typically produces about six seminal roots and 10–15 crown roots (also known as adventitious or nodal roots) per plant. Increases in root biomass and total length follow a sigmoidal pattern from sowing to flowering, at which point further increases are not usually observed (Gregory et al., 1978; Barraclough and Weir, 1988). The root dry weight ratio (root DM/total DM; RDW) is ca. 0.3 during early growth, decreasing to ca. 0.1 at harvest (Gregory et al., 1978). Nitrate is readily leached down the soil profile and consequently rooting depth is an important attribute for soil N acquisition. Maximum rooting depth is typically 140–200 cm in winter wheat (Gregory et al., 1978; Barraclough and Weir, 1988) and 80–120 cm in spring wheat (Siddique et al., 1990). The primary root traits for improvement would appear to be root axis number, rooting depth, rooting density and root longevity (especially for post-anthesis N uptake).

Root partitioning and root axis number are affected by root signalling and phenology. Root axis production is also intimately linked to tillering so that factors favouring tillering will increase crown root production. Comparisons of tall and semi-dwarf genotypes suggest that RDW has changed little with breeding for reduced height. Comparisons of cultivars and near-isogenic lines (NILs) have shown positive (Subbiah et al., 1968; Virmani, 1971; MacKey, 1973; Gupta and Virmani, 1973; Brown et al., 1987), neutral (Holbrook, 1973; Welbank et al., 1974; Cholick et al., 1977; Bingham et al., 2002) and negative (Siddique et al., 1990; Haberland et al., 1995) correlations between plant height and rooting depth. Thus, there are no consistent effects of semi-dwarf genes on the relationships between above- and below-ground parts of the crop across environments. Genetic differences in RDW could result from differences in the degree of adjustment to the onset of N stress, i.e. the functional equilibrium between root and shoot (Brouwer, 1963; Van Noordwijk and de Willigen, 1987). The extent of genetic diversity in this adjustment is largely unknown, and further investigations seem justified to quantify differences in feedback effects on the responsiveness of the root: shoot ratio to low N status. Greater root biomass was reported for spring wheat lines with the 1BL.1RS wheat-rye translocation (Ehdaie et al., 2003) and for primary synthetic spring wheats grown in Australia (Dreccer et al., 2004) compared to the recurrent parents, although in each case the RDW was not affected.

Rooting depth is affected by the root penetration rate and phenology. Generally, the longer a crop is growing the deeper it roots (Barraclough et al., 1991). Maximum rooting depth *per se* is rarely measured in the field because of practical difficulties in

sampling roots below approximately 1 m, but it may not be very important. For example, Barraclough et al. (1989) showed that defining 'rooting depth' in terms of the depth at which root length density (root length per unit soil volume; RLD) exceeds a critical value for potential resource capture may be a better indicator of the ability of a wheat crop to acquire subsoil N and water. Differences amongst wheat genotypes in rooting depth have been reported (Siddique et al., 1990; Miralles et al., 1997) but there is little evidence that rooting depth has been changed systematically by breeding.

The ability to capture N depends mainly on the amount of nitrate present in the soil relative to the morphology of the root system. Nitrate is supplied to the root system by mass flow (ions carried along in the transpiration stream) and diffusion (ions moving down a concentration gradient, either through bulk soil water or along water films surrounding particles). About 50% of the N taken up by wheat crops may be transported by mass flow (Gregory et al., 1978). Prolific root systems are more effective at capturing nutrients than sparse systems, but inter-root competition sets a natural ceiling on optimum RLD in cereals, above which further increases require excessive roots which do not have measurable effects on water and nitrate uptake (Van Noordwijk, 1983). Theoretical calculations predict a critical RLD of about 1 cm cm^{-3} for water and nitrate uptake. This figure broadly concurs with the values reported for water uptake of Gregory and Brown (1989) and Barraclough et al. (1989) who showed that a RLD of 1 cm cm^{-3} was associated with the abstraction of all of the available water by both spring barley and winter wheat, respectively. RLD distribution with depth is principally determined by time for growth (residence times are greater in the topsoil than the subsoil), soil porosity and strength, and nutrient and water availability (Barraclough et al., 1991). Root length density in wheat is typically below the critical root density of $ca. 1 \text{ cm cm}^{-3}$ at soil depths below $ca. 80 \text{ cm}$ (Gregory et al., 1978; Barraclough and Leigh, 1984; Barraclough and Weir, 1988; Ford et al., 2006). A recent modelling study concluded that distributing roots relatively deeper in the soil profile and decreasing specific root length (root DM per unit length) would confer greater N capture and yield under low N availability (King et al., 2003). Experimental evidence also supports the strategy of distributing roots relatively deeper to improve below-ground resource capture. Synthetic derivative wheat lines showed increased water uptake associated with a root system that was distributed relatively deeper in the soil compared with recurrent parents (Reynolds et al., 2007), and the drought tolerance of spring wheat SeriM82 was related to its relatively deep root system compared to the check cultivar Hartog (Christopher et al., 2008). Further root traits which could be beneficial in boosting N capture include enhanced root longevity post-anthesis and root penetration ability (Bengough et al., 2006), although there is relatively little information on genetic variation in these traits in wheat.

Methods for phenotyping wheat roots in the field were reviewed by Manske et al. (2001), including the use of rhizotrons and the measurement of root parameters from soil cores (root washing and root counts/image analysis). However, field phenotyping for root traits is not feasible in breeding programmes so genetic progress will depend on the development of high-throughput controlled-environment screens or molecular markers for root traits for marker-assisted selection (MAS). The use of root observation chambers and a non-destructive digital imaging technique offers some promise (Manschadi et al., 2006), but may be less suitable for screening root traits expressed at later stages of crop development. Recent work in rice has demonstrated the scope for developing molecular markers to select for root traits. NILs obtained through marker-assisted backcrossing for four QTLs for root length out-performed the recurrent parent for yield and

biomass when field tested under drought conditions (Steele et al., 2006). Future work in wheat should aim to evaluate the use of marker-assisted backcrossing for root QTLs, and exploit findings in *Arabidopsis* where root screens for mutants have identified genes such as AUX1 and LAX3 that regulate important root architectural traits such as lateral root development (Swarup et al., 2005).

5.2. Root membrane N transporter systems and root proliferation in relation to N status

Nitrate is actively transported across the plasma membranes of plant cells, but net uptake is a balance between active influx and passive efflux. 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).

Ammonium is the other major form in which N is acquired by plants. Many plant ammonium transporter (AMT) genes, which complement a yeast mutant deficient in normal ammonium uptake, have been identified (Von Wirén 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).

Expression of both nitrate and ammonium transporter genes is regulated by supply and demand for N. Uncoupling demand-driven expression and hence uptake may allow for greater influx which in turn may drive increased N assimilation. Alternatively, decreasing the activity of efflux systems (transport mechanisms as yet unknown) could also improve the efficiency of uptake. There are therefore prospects, albeit long term, of manipulating 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.

A further potential contribution may come from studies on 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 (Table 2).

6. Traits influencing N-utilization efficiency

N-utilization efficiency 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). The minimum N% in the biomass or grain at harvest will largely determine how much biomass or grain can be produced per unit of absorbed N. Van Keulen (1977) concluded that the minimum level to which N can be diluted in small grains is approximately 1.6%, and our data for current UK winter wheat cultivars show a minimum value of approximately 1.25% (Barraclough and Hawkesford, unpublished data). If NHI remains constant, then increasing UTE will result in a decreased GNC. UTE will vary according to the type of protein being made and according to changes in dry matter partitioning between plant organs since these vary in their protein concentration. Raising UTE associated

Table 2
Physiological traits relevant for nitrogen-use efficiency.

Trait	Species	Mode of impact ^a	Effects relevant for N-use efficiency	References
Root length density at depth	<i>T. aestivum</i> , <i>H. vulgare</i> , <i>O. Sativa</i>	UPE	Increased root length density at depth leads to improved below-ground resource capture under abiotic stress	Gregory and Brown (1989), Steele et al. (2006), Reynolds et al. (2007), and Manschadi et al. (2006)
Glutamine synthetase (GS) activity	<i>T. aestivum</i> , <i>Z. mays</i>	UPE and UTE	GS activity is positively correlated with leaf nitrate content and yield under low N	Habash et al. (2001), Hirel et al. (2001), Masclaux et al. (2001), and Martin et al. (2006)
Alanine aminotransferase (AlaAT) activity	<i>O sativa</i> , <i>B. napus</i>	UPE and UTE	AlaAT activity is positively correlated with N uptake, biomass and seed yield under low N	Shrawat et al. (2008) and Good et al. (2007)
RuBisCo CO ₂ specificity factor	<i>G. partitida</i>	UTE	High specificity factor leads to reduced photorespiration and increased photosynthetic efficiency	Uemura et al. (1997)
Introduction of C4 'Krantz' anatomy into to C3 species	<i>Oryza</i> spp.	UTE	Genetic variation for morphological features that could support an efficient two-cell C4 pathway	Hibberd et al. (2008)
Specific leaf N content	<i>Triticum</i> spp.	UTE	Increased SLN leads to increased leaf photosynthetic rate, whereas lower SLN predicted to increase NUE	Austin et al. (1982) and Semenov et al. (2007)
Vertical N distribution with leaf layer	<i>T. aestivum</i> , <i>S. altissima</i>	UTE	Distributing N so that leaves receiving the greatest photon flux densities have the largest SLN predicted to increase canopy photosynthesis per unit N	Hirose and Werger (1987) and Critchley (2001)
Leaf posture	<i>T. aestivum</i>		Erect leaf posture reduces light saturation of upper leaves and increases RUE hence NUE	Araus et al. (1993)
Leaf photosynthetic rate post-anthesis	<i>Z. mays</i> , <i>T. Aestivum</i> , <i>O. Sativa</i>	UTE	Enhanced photosynthetic rate in the post-anthesis period as a result of increased sink strength; increased RUE	Reynolds et al. (2001, 2005), Wang et al. (2002), and Ding et al. (2007)
Stem N storage	<i>T. aestivum</i>	UPE	High capacity for true-stem N storage predicted to increase maximum N-uptake rate	Critchley (2001)
Stay-green	<i>T. aestivum</i> , <i>T. durum</i> , <i>S. bicolor</i>	UTE	Delayed onset/rate canopy senescence associated with increased leaf photosynthesis and/or yield hence increased NUE	Borrell and Hammer (2000), Spano et al. (2003), and Verma et al. (2004)
N remobilization efficiency post-anthesis	<i>S. bicolor</i>	UTE	Low N remobilization efficiency associated with stay-green phenotype	Borrell and Hammer (2000)
Post-anthesis N uptake	<i>T. aestivum</i>	UPE	High post-anthesis N uptake correlated with positive departures from the overall negative relationship between grain yield and grain N concentration	Triboi et al. (2006)

^a UPE = N-uptake efficiency; UTE = N-utilization efficiency.

with lower grain N% is feasible in end-use markets for which a high grain starch to protein ratio is desirable, e.g. the feed, distilling or biofuel markets. A lower GNC implies more photosynthesis per unit of absorbed N, and the metabolic and physiological mechanisms by which this may potentially be achieved are now discussed further below. For bread-making cultivars, for which lower grain N% is undesirable, high NUE may alternatively be associated with large capacities for uptake and assimilation of N and with a high post-anthesis N remobilization efficiency.

6.1. Nitrate assimilation

Absorbed nitrate is reduced to ammonium and then to amino compounds in roots and leaves in a series of enzyme-mediated reactions. These reactions may present a bottleneck to growth and yield and could potentially be modified to improve UTE. The basic mechanisms for converting nitrate to ammonium, amino compounds, proteins, pigments and nucleic acids are well established (Mifflin and Habash, 2002; Good et al., 2004; Lea and Azevedo, 2007; Hirel et al., 2007). Briefly, 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.

Glutamine synthetase 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 NH₃ 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). In summary, GS isoforms are critical for N assimilation and remobilization and specific manipulation of some isoforms in a developmentally controlled manner may offer prospects for gains in UTE in wheat.

Another approach showing some promise is the overexpression of an alanine aminotransferase using a root epidermal cell promoter. In studies in both oilseed rape (*Brassica napus*) and rice (*Oryza sativa*) significant gains in NUE have been reported (Good et al., 2007; Shrawat et al., 2008); whilst the mechanism is unclear, the improved NUE may be the result of local relief of negative feedback on uptake.

Manipulating the level of expression of regulatory proteins influencing organic acid metabolism may offer an alternative route for improving UTE. The overexpression of the Dof1 transcription factor, involved in the activation of several genes encoding enzymes associated with organic acid metabolism in *Arabidopsis*, has revealed that both plant growth and N content are enhanced under low N conditions (Yanagisawa et al., 2004). A marked increase in amino acid content and a reduction in the glucose level were found in transgenic *Arabidopsis* expressing Dof1.

6.2. Manipulating photochemistry to improve UTE

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% 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). There is very limited variation in the catalytic rate of RuBisCo amongst higher plants, although the catalytic rate for RuBisCo from more diverse sources, e.g. cyanobacteria and algae, is significantly greater. However, transgenically introduced RuBisCo large sub-unit and small sub-unit genes from such diverse species have failed so far to produce the RuBisCo holoenzyme in flowering plants (Parry et al., 2003).

In addition to its role as a carboxylase for CO₂ RuBisCo also catalyses a competing and wasteful oxygenase reaction with O₂ (photorespiration) which consumes energy and releases fixed CO₂. Up to 60% of the carbon fixed by RuBisCo can be lost in this way (Guillard and Hellebust, 1971). The lower the CO₂ concentration the greater is the loss through the oxygenase reaction. RuBisCo has an inherent ability to discriminate between CO₂ and O₂ which is described by the specificity factor. Limited genetic variation in the specificity factor has been reported amongst C₃ plants, in the range 80–110 (Delgado et al., 1995; Galmes et al., 2005). However, the RuBisCo of thermophilic red algae, e.g. *Galderia partita*, are up to three times more efficient than those of C₃ cereals due to greater specificity for CO₂ (Uemura et al., 1997). If RuBisCo of wheat had a

similar specificity factor to those of red algae, the net CO₂ fixed per unit area of leaf under light-saturated conditions (A_{\max}) would be increased by about 20% (Austin, 1999). 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. However, transformation of the plastid genome is still difficult and only routinely possible for model species. Other potential targets for the improvement of photosynthetic rate include the capacity for regeneration of RuBisCo and other Calvin cycle enzymes, principally sedoheptulose bisphosphatase (Long et al., 2006).

Reproducing the locally high CO₂ environment responsible for the photosynthetic efficiency of C₄ plants in C₃ plants by altering the structural and metabolic characteristics may also offer scope to increase photosynthetic efficiency. In C₄ plants metabolic reactions are divided between bundle sheath and mesophyll cells with C₄ acids being generated in the mesophyll and then diffusing to the bundle sheath where the Calvin–Benson cycle operates. The primary carboxylating enzyme of photosynthesis in C₄ plants in the mesophyll is therefore phosphoenolpyruvate carboxylase rather than RuBisCo. The replacement of non-functioning silent C₄ genes of rice with functioning equivalents from maize resulted in high expression of phosphoenolpyruvate carboxylase in the transformed plants and a decrease in oxygenation by a third (Ku et al., 2000; Matsuoka et al., 2000). A major challenge is to introduce the C₄ (Kranz) anatomy into C₃ plants. Current work at the International Rice Research Institute (IRRI) is focusing on screening a wide range of germplasm including wild relatives of rice for such C₄ characteristics (Hibberd et al., 2008). Previous work on the physiology of interspecific hybrids (*Flaveria* × *Atriplex*) found that these C₄ anatomical features must be well developed in the hybrids before much advantage in CO₂ assimilation is attained over C₃ (Brown and Bouton, 1993; Brown et al., 1986).

6.3. Manipulating leaf and canopy photosynthesis per unit N to improve UTE

6.3.1. Leaf photosynthesis per unit N

Increasing the rate of photosynthesis for a given concentration of leaf N could improve UTE. In C₃ cereals, A_{\max} typically increases to values of the order of 20 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at leaf N concentrations of 2 g N m⁻² under favourable conditions. Assuming an asymptotic relationship between A_{\max} and leaf N concentration (Evans, 1983; Sinclair and Horie, 1989), one possible strategy to raise UTE is to decrease specific leaf N (N content per leaf lamina area; SLN) whilst maintaining A_{\max} . The SLN required for potential photosynthetic function under high light levels has been estimated to be ca. 1.5 g N m⁻² (Jamieson and Semenov, 2000). Since leaves of modern wheat genotypes accumulate more N than this under favourable conditions (Critchley, 2001), UTE could be increased by selecting for lower SLN to decrease the transient 'storage' N components of leaves. A sensitivity analysis using the wheat Sirius model (Semenov et al., 2007) predicted that decreasing leaf SLN in the range of 1–2 g m⁻² increased NUE by 10–15% when N was limiting. Additionally, Tambussi et al. (2005) in barley demonstrated the potential advantage of a low SLN and its possible translation to yield. An alternative strategy of selecting for SLN above values of ca. 2 g N m⁻² to increase A_{\max} seems unlikely to be advantageous overall, since radiation-use efficiency (biomass production per unit radiation interception; RUE) only increases at a low rate as A_{\max} increases above values of about 20 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in cereals (Monteith, 1977; Sinclair and Horie, 1989). This is because individual leaves may operate well below light saturation in the canopy and due to the need to account for dark respiration (Reynolds et al., 2000). There may also be pleiotropic trade-offs with greater SLN being associated with

reduced leaf size and light interception (Austin et al., 1982). Genetic variability in SLN was observed from 1.4 to 2.6 g m⁻² for 144 durum wheat genotypes (Araus et al., 1997) and 2.1–2.4 g m⁻² for 17 durum wheat cultivars (Giunta et al., 2002); and in leaf N concentration from 43.7 to 47.6 mg g⁻¹ for eight bread wheat cultivars (Fischer et al., 1998). The heritability of SLN or leaf N concentration in wheat is largely unknown. However, it is encouraging that the heritability for straw (leaf lamina, leaf sheath and stem) N at anthesis for winter wheat was >0.60 under low N (LaPerche et al., 2006) indicating that breeding to manipulate the amount of global canopy N should be possible.

It has been suggested that to maximize carbon gain by a wheat canopy, N should be optimally distributed so that leaves receiving the greatest photon flux densities have the largest SLN (Grindlay, 1997). Decreases in SLN in successive leaf layers deeper in the canopy have been reported in wheat (Grindlay et al., 1995; Critchley, 2001). In a stand of *Solidago altissima*, Hirose and Werger (1987) showed SLN tended to be greater in shaded leaves at the bottom of the canopy than the optima predicted from their N photosynthesis/canopy light distribution model, possibly because the predicted optimum SLN levels were below that required for maintenance of structural N in the supporting tissue of the leaf base. There is relatively little information on genetic diversity in the vertical distribution of N in the canopy between or within *Triticum* species, despite the large amount of work published on light interception and attenuation by crop canopies. Small differences were observed in the distribution of N in the top four leaves at anthesis between two UK winter wheat cultivars, Soissons and Spark, but overall the distributions were close to that predicted for optimum photosynthesis by Hirose and Werger (1987) in both cultivars (Critchley, 2001). Similarly, the vertical distribution of N at anthesis was close to the optimum, as defined in the optimization theory (Hirose and Werger, 1987), and did not differ significantly for two French winter wheat cultivars, Apache and Isengrain, until almost the end of grain filling (Bertheloot et al., 2008). Further investigations would seem to be justified to test whether this is more generally the case in a wider range of wheat germplasm.

6.3.2. Canopy photosynthesis per unit N

It could be easier to raise canopy photosynthesis per unit N by altering traits operating at the whole-crop scale, such as N and dry matter partitioning amongst plant organs and/or canopy architecture, than by altering single metabolic processes such as leaf photosynthesis.

With regard to N partitioning, about 35% of the total N in a wheat canopy at anthesis is in the leaf lamina. The remaining approximately 65% of the crop N is partitioned between the leaf sheath, true stem (i.e., stem with the leaf sheath removed) and the ear (Critchley, 2001; Jamieson and Semenov, 2000). The leaf sheath typically contains about 15% of the canopy N at anthesis and has a similar photosynthetic function to the leaf lamina. The true-stem N accounts for up to 30% of total canopy N at anthesis (Critchley, 2001) with structural, transport, reserve/storage and metabolic functions. The absolute amounts of N required for transport and metabolic functions are small, with the majority of true-stem N allocated between the structural and reserve pools. However, there is relatively little information on the relative size of the structural and reserve N pools of the true stem. Results of field experiments on four UK winter wheat cultivars indicated that approximately 60% of the true-stem N is in the reserve N pool under optimal conditions (Pask, 2009). Genetic variation in stem N content is reported in winter wheat (Triboi and Ollier, 1991; Critchley, 2001) and in rice (Tirrol-Padre et al., 1996). A high capacity to absorb N in the true stem before flowering could theoretically favour a high maximum rate of N uptake, hence higher UPE. So true-stem N

absorption may be an important trait to maximize the rate of N uptake, especially during warm, wet conditions when large amounts of N mineralization occur and N availability is high. Some studies in maize report early remobilization of N from the stem before the leaf lamina (Beauchamp et al., 1976; Friedrich and Schrader, 1979) consistent with the use of stem N as a buffer for the flow of N from the leaf lamina to the grain. In this case, a high stem N absorption capacity coupled with a high stem N remobilization efficiency (proportion of N in the plant organ at anthesis which is not recovered in the organ at harvest; NRE) would potentially favour high UTE through delayed senescence of the leaf lamina. A high stem N absorption coupled with a low stem NRE, however, would be associated with an inefficient use of absorbed N and a low UTE.

About 20% of the above-ground N at anthesis is located in the ear (Critchley, 2001; Jamieson and Semenov, 2000; Pask, 2009). Approximately a quarter (Demotes-Mainard et al., 1999) to half of this ear N (Abbate et al., 1995) ends up in the grain at harvest. Thus, the N in the ear at anthesis is high relative to the total amount of N that will eventually be incorporated into the developing grains. This raises the question of whether N concentration in the grain at anthesis is important for floral development. There is current debate on this topic with some physiologists highlighting the close relationship between grain number per ear and ear N content in field data sets (Demotes-Mainard et al., 1999; Demotes-Mainard and Jeuffroy, 2004; Sinclair and Jamieson, 2006); and others suggesting there is no evidence for effects of N on grain number apart from those operating via dry matter accumulation (Fischer, 2007). Ears have a capacity to accumulate large amounts of N in the pre-anthesis period in the glumes at a time when there are few alternative sinks around (Lopes et al., 2006). Again, this could be a mechanism of buffering leaf lamina senescence in the post-anthesis period similar to that suggested above for true-stem N. For example, glumes were observed to lose twice as much of their total N content as that lost by the flag leaf between the milk and early dough stages (Lopes et al., 2006).

Turning to consider dry matter partitioning, increasing ear partitioning at anthesis to raise grains per unit area has been demonstrated to raise post-anthesis RUE in wheat through manipulating feedback regulation of photosynthesis. Greater RUE was observed in response to manipulating the crop by opening rows in the booting/ear emergence phase to increase light interception, ear growth and grain number (Reynolds et al., 2005). Similarly, enhanced RUE was found in response to a pre-anthesis shading treatment in winter wheat (i.e. increasing sink relative to source size) compared to the control treatment (Beed et al., 2007). Such strategies to enhance RUE could also raise NUE in breeding programs. However, this may not lower the N fertilizer requirement (i.e., the economic fertilizer N amount) *per se*. Indeed, there is evidence that genetic progress in yield potential has been associated with higher economic N optima in winter wheat in the UK (Foulkes et al., 1998). Nevertheless, the absolute yield under low N supply could be expected to be positively associated with gains in yield potential (Foulkes et al., 1998; Ortiz-Monasterio et al., 1997; Muirinen et al., 2006).

Finally, effects of canopy architecture on RUE have been observed with RUE being higher for large wheat canopies with more erect leaves, associated with reduced light saturation of the upper leaves (Evans, 1973; Araus et al., 1993). Thus, a decrease in the light extinction coefficient (*k*) (Monsi and Saeki, 1953) associated with more erect leaves might in theory raise NUE. However, most modern wheat cultivars worldwide already have semi-erect or erect flag leaves so there is probably limited scope for further improvement of yield potential and NUE via this approach. Alternatively, it has been suggested that increasing *k* (more prostrate leaves) could reduce the green canopy area hence canopy

N required to maximize light interception (Sylvester-Bradley and Kindred, 2009). However, there are inter-dependencies since RUE could not be maintained if k was increased; and further work is required to review these inter-dependencies before prioritising breeding strategies to optimise canopy architecture.

6.4. 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). A functional explanation for the regulation of grain N accumulation in cereals by genetic factors remains illusive. There is debate as to whether grain N content is regulated by differences in grains per unit land area with protein synthetic capacity influencing grain N 'demand' or by N 'supply' from the vegetative organs, or is co-limited by both. Studies comparing in vitro-cultured grains from low/high protein genotypes of wheat (Donovan et al., 1977) and maize (Wyss et al., 1991) indicated that differences in grain N concentration resulted from differences in protein synthetic capacity. In contrast, more recent experimental evidence suggests that grain N accumulation is mostly source regulated for wheat (Ma et al., 1996; Martre et al., 2003). The flow of nitrogen from the vegetative tissue to the grains may also be strongly affected by the weather (temperature, drought, etc.) since this will affect the source–sink ratio and by intrinsic properties of the organ (Dalling, 1985; Triboi and Triboi-Blondel, 2002).

6.5. 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; Borrás et al., 2004; Calderini et al., 2006), under low to moderate N fertilizer levels yields may be more limited by post-anthesis 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 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).

In summary, a better understanding of the mechanisms underlying the genetic variation in leaf and stem NRE would offer scope to raise NUE in wheat and further investigations with this objective seem justified. For cultivars targeted at the feed, distilling or biofuel markets (high grain starch to N ratio requirement), a low N lamina NRE potentially associated with the stay-green trait, would be a strategy to boost UTE. For bread-making cultivars (low grain starch to N to ratio requirement), on the other hand, a high lamina NRE is still required to maintain acceptable GNC.

6.6. 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% 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% 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 breadmaking and more extensible doughs for making biscuits and cakes.

As mentioned above, an inverse genetic relationship is usually reported between grain yield and protein concentration in wheat cultivars (Kramer, 1977; Kibite and Evans, 1984; Ortiz-Monasterio et al., 1997; Calderini et al., 1995b; Triboi et al., 2006), which may be attributed in part to the higher glucose costs for synthesis of protein than of carbohydrates (Penning de Vries et al., 1974). This negative relationship is also in part due to the balance between the processes of carbon capture and N remobilization from the vegetative tissues to the grain. Efforts to overcome the inverse relationship may concentrate on increasing grain protein accumulation per unit land area (Feil, 1997; Triboi and Triboi-Blondel, 2002; Triboi et al., 2006). In essence, to increase the grain N:C ratio either the capacity of plants to store N at anthesis and/or uptake more N after anthesis must be increased (Triboi et al., 2006).

6.6.1. Optimising grain protein content and composition in breadmaking wheats

Cultivars for breadmaking are selected for high protein content and strong gluten properties with appropriate levels of N fertiliser being applied to the crop to ensure that the required protein content is achieved. For example, the Chorleywood Breadmaking

Process, which is the predominant process in the UK and a number of other countries, requires wheat with a minimum protein content of 13% 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. For example, *Snape et al. (1993)* reported that the UK varieties, Avalon and Hobbit, which had been selected for breadmaking and feed, respectively, differed in their protein contents by only about 2%. A major determinant of grain protein content is the availability of nitrogen to the plant which is consistent with grain protein content being source-limited. In simple terms this means that the transport of N into the grains determines the level of storage protein gene expression and hence the grain protein content rather than vice versa (*Rahman et al., 1983; Bartels and Thompson, 1986; Sorensen et al., 1989*). In addition to this “transcriptional” regulation there may also be a “fine tuning” of storage protein synthesis at the level of translation of the mRNAs to synthesise protein (*Rahman et al., 1983*). For example, N is still transported into the grain even if storage protein synthesis is severely restricted, for example by limiting S availability, but may accumulate as free amino acids (notably asparagine) rather than be incorporated into proteins (*Shewry et al., 1983*). The conclusion is that the most effective way to modify grain protein amount would be to manipulate the amount of N transported into the grain.

An alternative approach to producing high protein wheats for breadmaking is to improve the protein quality to increase the dough strength, allowing lower protein grain to be used for breadmaking. This would avoid the current situation in which increasing grain protein content is generally associated with an increased proportion of gliadin proteins in the gluten fraction, resulting in increased dough extensibility (*Jia et al., 1996; Panozzo and Eagles, 2000; Zhu and Khan, 2001; Kindred et al., 2008*). Although it may be possible to select for low gliadin content (*Kindred et al., 2008*) it is unlikely that this will lead to the development of low protein varieties suitable for breadmaking. However, transgenesis may offer a more promising route, by increasing the proportion of the high molecular weight subunits of glutenin (*Jones et al., 2009*) in a low protein background.

6.6.2. Optimising grain protein content and composition in feed wheats

Another strategy to increase the N economy of the overall agro-ecosystem is through modifications to grain protein of feed cultivars to reduce N excretion in faeces and urine in livestock systems hence N emissions to the environment. Possible modifications can be suggested for grain included in either ruminant or non-ruminant livestock diets.

In ruminants, typically about 70% of daily N consumption is excreted and reducing the dietary N content generally increases the efficiency of feed utilization. N excretion may be reduced by using cereal grain supplements formulated to provide lower amounts of rumen-degradable N (*Castillo et al., 2001*). Therefore, reducing the percentage of grain N as rumen-degradable N could contribute to improved N utilization by ruminants, although it may only make a small difference compared with the large amounts of rumen-degradable N from other dietary sources, e.g. grass and grass silage. Changes in the amino-acid profile of wheat grain proteins are unlikely to have a major impact on N retention by ruminants because of the modifying effects of rumen fermentation (*Rulquin and Verite, 1993*).

When non-ruminant livestock are fed on high quality diets about 12–15% of the N consumed is non-digestible and appears in the faeces while a further 25% is lost as urea (mammals) or uric acid (avians). The well-established concept of the ‘ideal protein’ in diet formulation for non-ruminants is based on the principle that

animals have requirements for specific amino acids, both qualitatively and quantitatively (*ARC, 1981*). Any departures from the ideal will lead to increased N excretion. Cereal grain is the predominant raw material employed in non-ruminant diets but is considerably removed from the ‘ideal’, being deficient in several nutritionally essential amino acids (notably lysine but also threonine) and containing surpluses of others which are non-essential (e.g. glutamine). This imbalance results essentially from the proportions of these amino acids present in the gluten proteins. To date, breeding for improved grain protein quality to improve N utilization in non-ruminants has not been undertaken extensively in wheat, although there has been extensive work on maize, and barley (reviewed by *Shewry, 2007*). This may be partly because it has been estimated that the genetic component of lysine varied by only 5% (*Vogel et al., 1973*). Furthermore, the greatest success with other species has been achieved using mutant “high lysine” genes, most of which are recessive and therefore difficult to identify and exploit in a hexaploid species such as bread wheat. Nevertheless, there could be potential economic and environmental benefits from improving content of lysine and other essential amino acids in grain of feed cultivars in future breeding programs.

7. Screening for traits associated with N-use efficiency

The capacity for precise phenotyping under reliable conditions probably represents the most limiting factor for the progress of genomic studies on traits underlying NUE. Furthermore, the ability to accurately measure the physiological components of NUE has clear direct application in breeding. Several ground-based sensors offer promise for screening large numbers of genotypes for canopy N status with high precision including those based on measuring spectral reflectance, chlorophyll content and leaf photosynthetic activity.

Spectral reflectance is a remote-sensing technique for screening genotypes for canopy light reflectance properties based on the absorption of light at specific wavelengths. The reflectance in the visible (VIS) wavelengths (400–700 nm) is lower than in the NIR wavelengths (700–1300 nm) because of the high absorption of light energy by leaf pigments. Spectral characteristics are affected by canopy size, crop N status and senescence. Spectral reflectance indices, e.g. NDVI = $(\text{NIR} - \text{VIS}) / (\text{NIR} + \text{VIS})$, have proved useful in the assessment of early biomass of different wheat genotypes (*Elliott and Regan, 1993; Bellairs et al., 1996; Babar et al., 2006*). Spectral reflectance may be of particular use in obtaining an objective measure of senescence. NDVI has been correlated with senescence in cereals (*Idso et al., 1980; Adamsen et al., 1999*). *Gupta et al. (2001)* suggested that comparison of wavelengths at which chlorophyll-beta (640 nm) and chlorophyll-alpha (673 nm) have maximum absorbance may give good sensitivity to maturity.

Plants with greater leaf N concentrations typically have more chlorophyll. Chlorophyll in leaves absorbs most strongly in the blue (around 450 nm) and red (around 670 nm) light, and reflects in the green (around 550 nm) region of the light spectrum. The Minolta SPAD 502 CM measures light transmission in the red (650 nm) and near-infrared (940 nm) parts of the spectrum and has been used to estimate leaf chlorophyll content in maize (*Blackmer et al., 1994*) and to predict grain N requirements in wheat (*López-Bellido et al., 2004*).

While direct measurement of photosynthesis is not conducive to the demands of high-throughput phenotyping at the breeding scale, surrogates can be used such as leaf porometry, canopy temperature, carbon isotope discrimination, integrated stomatal conductance over time, and above-ground biomass/photosynthetic capacity, respectively (*Foulkes et al., 2009*). Although to date such techniques have found more application in breeding programs aimed at increasing adaptation to drought (*Condon*

et al., 2008), there are future prospects for the application of these technologies in programs aimed at increasing NUE.

8. Summary of target traits

Improvement of traits by breeding will require studies to identify the genes underlying the key traits, to identify superior alleles under low and high N conditions and to develop molecular markers to allow these to be selected in breeding programmes. Current avenues for elucidating the molecular controls of N assimilation in cereal crops including the use of quantitative genetics and forward and reverse genetics approaches were comprehensively reviewed by Hirel et al. (2007). Of the candidate physiological, metabolic and physio-chemico traits discussed in this paper and summarized in Table 1, several offer promise for breeding for high NUE and reducing excessive N fertilizer inputs in the 'short term' (5–10 years), e.g. relatively deeper roots, lower specific leaf N content, optimized post-anthesis N remobilization and the stay-green trait. Other traits are more intractable but nevertheless of high potential impact in the medium to long term, e.g. improving RuBisCo properties and incorporation of C₄ metabolism into wheat.

The priority traits for selection by breeders in the short term to increase NUE are summarized in Fig. 1. For feed wheat cultivars, the objectives are to increase UPE and UTE. High UPE should be favoured by: (i) increased RLD at depth, (ii) a high capacity for N accumulation in the stem (= high maximum N-uptake rate) and (iii) increased activity of key N assimilation enzymes (e.g. GS and alanine aminotransferase = alleviation of negative feedback on UPE). High UTE should be favoured by: (i) low specific leaf N content, (ii) low leaf lamina NRE (= stay-green), (iii) high true-stem NRE (= stay-green, associated with buffering of remobilization of leaf lamina photosynthetic N) and (iv) a low grain N concentration.

For bread-making cultivars, the objectives are to reduce excessive N fertilizer inputs whilst maintaining high yields with acceptable grain N concentration of approximately 2.3%. This will require a high accumulation of N in vegetative material before anthesis and efficient translocation to the grain of this N and any newly acquired N, during grain filling. High N absorption will be favoured by the combination of traits described above for the feed wheat ideotype favouring high UPE. High post-anthesis N remobilization will be favoured by high activity of key N assimilation enzymes, e.g. glutamine synthetase. Additionally, increasing the proportion of grain protein as glutenin proteins should facilitate the maintenance of acceptable bread-making quality whilst reducing excessive N fertilizer N inputs.

This paper has been concerned with identifying strategies to increase the efficiency of nitrogen use in wheat systems. Future progress will depend on identifying traits at the biochemical, cellular and plant level and integrating towards field performance. These events are highly dynamic and complex and are not yet fully understood. A central problem in integration at the whole plant level is to understand what consequences the metabolic activities in one organ have on the balance of the system (Novoa and Loomis, 1981). So future progress will depend on integrating expertise from whole plant molecular physiology approaches to breeding and agronomy to aid understanding of the regulation of N uptake, N assimilation and N recycling and remobilization in the different plant organs through growth and development under contrasting N levels. Such integrated studies will require input from computer scientists and bioinformaticians, as well as the development of large populations segregating for target traits. In particular, it will be important to increase understanding of the extent to which the genetic control of pre-anthesis N accumulation and post-anthesis N remobilization is intrinsically linked, and whether there is independent genetic control of these processes in the different plant organs.

More advanced crop simulation models should also be developed with particular emphasis on the incorporation of genotype-specific N allocation parameters. The most widely used crop models for calculating N demand and distribution in wheat, e.g. SIRIUS, CERES, and APSIM (Ritchie and Otter, 1985; Jamieson and Semenov, 2000; Asseng et al., 2001), assume minimum, critical and maximum N concentrations within whole shoots or plant organs that change with ontogeny. However, these parameters are generally not genotype-specific in the models. Therefore, there is a requirement for genotype × N × environment experiments to define genetic ranges for the N allocation parameters, their inter-dependencies and their stability across environments and with changing climatic patterns. The development of more advanced crop simulation models may be a way to link model parameters with molecular physiological traits and thus facilitate genetic and genomic research to identify the genes involved in determining the key traits.

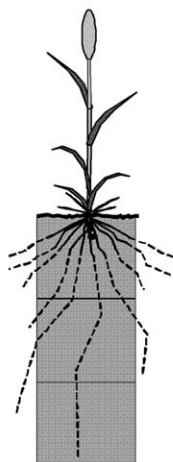
The preliminary analysis presented in this paper has highlighted some of the key physiological traits that may underlie N-efficient genotypes and has indicated that there is scope to improve the NUE within adapted germplasm by up to 20%. For some traits, however, it may be necessary to exploit a wider gene pool, utilising either exotic wheat lines or wide crosses with related alien species. Long term success will depend on a multi-disciplinary approach, linking fundamental and strategic research

MAXIMIZE PHOTOSYNTHEIC CAPACITY PER UNIT N:

- Leaf and stem N storage
- Vertical distribution of canopy N
- RuBisCo catalytic properties
- C₄ metabolism

MAXIMIZE N CAPTURE:

- Distribute roots deeper
- Decrease specific root weight
- Optimize root to shoot ratio
- N transporter systems



OPTIMIZE GRAIN PROTEIN AND N REMOBILIZATION:

- Optimize N remobilization efficiency and stay green
- Optimize grain N%
- Optimize gliadin to glutenin ratio

OPTIMIZE NITRATE ASIMILATION:

- Glutamine synthetase activity
- Alanine aminotransferase activity
- Organic acid metabolism

Fig. 1. Strategies to improve N economy in wheat.

on the one hand, with applied plant breeding on the other, to develop new genotypes with high NUE for sustainable agriculture. It will also depend on the application of a systems view in which genetic improvement is combined with efforts at improvement in management practises. This review attempts to provide the background for such an integrated systems approach.

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