AC0221 Annex 7

Potential for minimising fertiliser N requirement through the development of N efficient varieties

Summary

Nitrogen use efficiency (NUE) is the product of two components: nitrogen uptake efficiency (NUpE), the proportion of available N which is taken up by the crop, and nitrogen utilisation efficiency (NUtE), the grain yield (kg/ha at 100% dry matter) achieved per unit of N taken up by the crop (kg N/ha).

It has been shown for both wheat and barley that modern varieties have higher NUE than historic varieties. However, yields under low N conditions have improved by only a small amount, relative to yields under high N conditions, probably because most breeding programmes have taken place at moderate to high N levels. It should be noted that the NUE of barley has been increased by more than that of wheat in the last 30 years of breeding, which may be partly because barley breeding trials are generally conducted at sub-optimal N levels. Studies have found that the relative importance of the two components of NUE (NUpE and NUtE) varies with N supply, so selection for NUE at one extreme of N supply would be likely to limit the type of traits which are being selected for.

Numerous studies have shown that considerable variation in NUE exists in modern elite germplasm, but breeding may also benefit from crosses involving more exotic varieties, from regions where N stress is more common than in the UK. The use of alternative species should also be considered, such as triticale as an alternative to feed wheat. National statistics show that the NUE of triticale in the UK is typically 24% higher than that of feed wheat, and in recent a ADAS trial following a wheat crop, triticale out-yielded feed wheat by 1.5 to 2 t/ha at a range of levels of N fertilisation. Further work is required to test the relative performance of triticale and wheat over a greater range of sites and seasons.

Although NUE can be assessed simply by measurement of yield at known available N levels, the components of NUE and associated traits are more challenging to assess. Distinguishing between NUpE and NUtE requires measurement of whole plant and grain N content, which is a relatively time-consuming process, and the key traits for NUpE include aspects of root morphology, which are also difficult to assess.

One of the most versatile and efficient genetic tools used in breeding is marker-assisted selection. However, relatively few genetic markers have been established for traits associated with high NUE, many of which are likely to be controlled by multiple genes. Greater reductions may be achieved using GM technology, e.g. promoting activity of the enzyme alanine aminotransferase enhances N assimilation in plants and has been shown to reduce the fertiliser N requirement of oilseed rape by 50%.

Recently, remote-sensing devices using spectral reflectance have enabled more rapid assessment of crop parameters including canopy size, N content and photosynthetic capacity. Research has been carried out for wheat, barley and oilseed rape into the optimal spectral reflectance indices for assessing different crop characteristics. To date, spectral reflectance techniques have been developed more for assessing the nitrogen fertiliser requirements of crops than for assessing NUE in a breeding context, but assessing the nitrogen status of different breeding lines in low N conditions could give an indication of their relative N uptake efficiencies.

An important trait for improving NUpE may be root length density (RLD; the length of root within a volume of soil) at depth. Other rooting traits which have been linked to NUpE include expression of nitrate uptake transporters and the control of root proliferation in response to localised sources of N, but further research is needed to establish how useful these traits are in field conditions and whether sufficient variation exists in crop germplasm to enable their improvement through breeding. The duration of N uptake is important, as well as the rate. Recent work for the 'Green Oil' LINK project LK0979 has shown that the relatively slow N

uptake of oilseed rape after flowering may be an important limiting factor for NUpE and that genetic variation exists for this trait, but further work is needed to investigate the mechanisms involved. A similar trait in cereals is the 'stay-green' phenotype, which describes delayed senescence and hence a prolonged period of photosynthesis and nutrient uptake. This effect is particularly strong in stress environments, and has been more commonly investigated in conjunction with drought tolerance. Some progress has been made into finding the genes which control the stay-green phenotype in wheat, which provides potential for marker-assisted selection in breeding for the stay-green phenotype.

The scope for increasing NUE may be different for different crop species, in part due to the progress which has already been made in breeding crops with high NUE. For example, barley already has a high NUE relative to other crops, probably because barley has been bred for low grain N concentration, which is related to high NUtE. It has been estimated that the N requirement of wheat and oilseed rape may be reduced by 40% and 26% respectively using genetic variation found in modern varieties. Greater reductions in N requirement of oilseed rape of up to 50% may be possible using GM technologies. Realising the full extent of this improvement would require long-term breeding programmes involving crosses with exotic wheat varieties and/or related alien species.

Introduction to nitrogen use efficiency

Nitrogen use efficiency (NUE) is defined as the grain yield achieved per unit of plant available N, including both soil N and applied fertiliser N, and is usually measured in units of kg/ha yield (at 100% dry matter) per kg N/ha. NUE is the product of two components: nitrogen uptake efficiency (NUpE), the proportion of available N which is taken up by the crop, and nitrogen utilisation efficiency (NUtE), the grain yield (kg/ha at 100% dry matter) achieved per unit of N taken up by the crop (kg N/ha).

NUE depends on environmental conditions, agronomic factors such as N application rates and timings, and genetic factors, which are the focus of this review. Increasing the NUE of modern crop varieties could allow current yields to be achieved using lower N application rates, or higher yields to be achieved using current N application rates, either of which would reduce the cost and GHG emissions associated with crop production. Increased NUE could also reduce the N residues remaining in the soil after harvest, which in turn could reduce N leaching into waterways.

This review will examine the crop traits related to high NUE, and the ways in which breeding programmes could be changed to better select for these traits. These characteristics of varieties with high NUE are similar for all crop species, and so will covered by trait rather than by crop species.

Traits related to high N uptake efficiency

Most of the research into crop traits contributing to high NUE has been done on cereal crops, chiefly wheat and maize. Relatively little work has focused on oilseed rape, but it is likely that many of the traits leading to high NUE in cereals will also be relevant in other crop types. Traits can be divided into those which increase NUpE and those which improve NUtE. Given that both of these components have contributed to past improvements in NUE (Ortiz-Monasterio *et al*., 1997; Sylvester-Bradley & Kindred, 2009), it is likely that both have potential for further improvements. Current models for calculating N requirements assume that both wheat and oilseed rape crops take up close to the equivalent of 100% of the N already in the soil at the start of the growing season, and around 60% of applied fertiliser N, up to the optimal N application rate and assuming suitable N timings and other agronomic factors (Stokes *et al*. 1998; Berry & Spink, 2009), again suggesting that there is potential for improvement in NUpE.

NUpE depends mostly on rooting traits, including the quantity and distribution of roots through the soil profile (root morphology), and the expression of N uptake proteins in the root membrane (root physiology) (Foulkes *et al*., 2009). Of these, greater genetic variation is thought to exist for root morphology than for root physiology. When comparing two oilseed rape varieties with contrasting NUpE, Kamh *et al*. (2005) found that the higher NUpE of one

variety was due to higher root growth rather than to any difference in N uptake per unit root length.

The most promising root trait for improving NUpE may be root length density (RLD; the length of root within a volume of soil) at depth: it has been shown for cereal crops that at root length densities above 1 cm/cm^3, the majority of water and N can be extracted from the soil, but that resource capture decreases sharply as RLD falls below this threshold (King *et al.*, 2003). The RLD of a crop is typically well above this threshold near the soil surface, then decreases rapidly with depth such that it falls below the 1 cm/cm^3 threshold at between 40 and 60 cm depth (King *et al.*, 2003). There exists genetic variability in how rapidly RLD decreases with depth, so for some crops RLD remains above the 1 cm/cm^3 threshold to a deeper level, without any increase in total root mass. Avoiding increases to the total root mass is important, as Svečnjak & Rengel (2006) found a high root : shoot ratio to be correlated with poor NUtE in oilseed rape varieties. Siddique *et al.* (1990) showed that modern wheat varieties had a lower root : shoot ratio than older varieties, but that old and new varieties did not differ in rooting depth or water extraction. This was because the reduced root mass of modern varieties was due to reduced RLD in the upper 40cm of soil, rather than at depth. Selecting for increased RLD at depth would not be easy, but is expected to improve uptake of all nutrients, not just nitrogen, without any negative impacts on yield. Increased RLD at depth would be particularly useful in improving drought tolerance because in drought situations water is increasingly available at depth, whereas for nitrogen it must be recognised that availability is highest near the soil surface, due to fertiliser applications and mineralisation from organic matter.

For some plant species, it has been demonstrated that root growth can respond to patches of high nutrient availability in the soil, which is likely to increase NUpE. Drew *et al.* (1973) demonstrated proliferation of lateral roots in barley in response to localised high concentrations of nitrate and ammonium, and numerous more recent studies have investigated this process in Arabidopsis (Lea & Azevedo, 2006). Localised root proliferation in response to nitrogen has also been shown in numerous other species including maize (Qin *et al.*, 2005) and wheat (van Vuuren. 1996). However, there is no evidence to suggest that crop varieties differ in their ability to respond to localised sources of nitrate, or that this ability is correlated to high NUpE or yield in the field (Hodge, 2004).

The duration of N uptake is important, as well as the rate. In oilseed rape, N uptake is relatively slow after flowering, which Teakle *et al.* (2008) suggested may be an important limiting factor for NUpE. It may be that uptake slows due to limited supply, in which case later N applications could increase post-flowering N uptake. Given that late N applications have been shown to improve oilseed rape yields in some situations (Berry & Spink, 2009), this is likely to provide at least a partial solution. Late N uptake may also be 'sink-limited', or demand driven, possibly due to hormonal control of N uptake (Rossato *et al.*, 2002). For 10 oilseed rape varieties tested as part of the 'Green Oil' LINK project LK0979, N uptake after flowering ranged from 33 to 61 kg N/ha, indicating that genetic variation exists for this trait, and N uptake after flowering was correlated with yield at low N supply (Berry *et al.*, 2010). In the same study, high yield at low N supply was also correlated with number of seeds/m^2, but it was not clear whether high N uptake had enabled high seed set in the varieties with high NUE, or whether high seed set due to other factors had driven high late N uptake, by reducing sink-limitation.

In cereals, delayed senescence and hence maintenance of green leaf area for a longer period is known as the 'stay-green' phenotype, and has been associated with increased yield and NUE in milling and durum wheats, although it is more commonly investigated in conjunction with drought tolerance (Foulkes *et al.*, 2007). Delaying senescence can allow a prolonged period of photosynthesis and nutrient uptake, which promotes yield. This effect is particularly strong in stress environments, where source-limitation is more common than it is under optimal conditions. Some progress has been made into finding the genes which control the stay-green phenotype in wheat (Joshi *et al.*, 2007; Silva *et al.*, 2000; Verma *et al.*, 2004), which provides potential for marker-assisted selection in breeding for the stay-green phenotype.

Traits related to high N utilisation efficiency

In wheat, improved harvest index (HI; the proportion of above-ground dry biomass contained in the grain) has been a major contributor to increases in NUtE achieved in 20th century breeding (Foulkes *et al*., 2009). However, increases in HI appear to have slowed in recent decades, with most current wheat varieties having a HI of 50-55% (White & Wilson, 2006). Since Sylvester-Bradley *et al*. (2005) suggest that the ideal structure for wheat, capable of yielding up to 19.2 t/ha, would have a HI of 60% and Austin *et al*. (1980) suggest a theoretical maximum HI of 60%, further increases to HI are unlikely to contribute much to increasing wheat yield or NUtE.

In oilseed rape, there may still be scope to improve NUtE by increasing HI, which is typically 30-50% for current varieties. Svečnjak & Rengel (2006) found that differences in NUtE between several oilseed rape varieties were mainly due to differences in root:shoot ratio and HI. There were no differences in total N uptake and only minimal differences in the N concentrations of certain plant parts.

An alternative way to increase NUtE would be to reduce specific leaf N (SLN; quantity of N per unit area of leaf) while maintaining photosynthetic rate. Foulkes *et al*. (2009) suggest that this is possible, because a significant proportion of the N within leaves is in the form of storage proteins which do not contribute to carbon assimilation, and the SLN of wheat is typically above the threshold SLN required for maximal photosynthetic rate under high light. However, in modelling how reduced SLN could improve yield and NUtE, Semenov *et al*. (2007) assumed that the efficiency of the photosynthetic enzyme RuBisCo would have to be increased to compensate, which is a difficult, long-term goal, probably requiring the use of genetic modification. Tambussi *et al*. (2004) showed that lower SLN was a feature of a high-yielding, high NUtE barley variety, but that it was associated with lower photosynthesis per unit leaf area and compensated for by higher total leaf area. Overall, it seems likely that reducing SLN has some potential for increasing NUtE, but must be balanced by other changes to avoid reducing yield.

Increasing NUtE often involves reducing grain N concentration: across genotypes, negative correlations between yield and grain N or protein concentration occur for all cereal crops (Simmonds, 1995). This a major issue for milling wheats, for which UK bread-makers specify a minimum protein concentration of 13%. In a comparison of historic and modern Italian milling wheats, Guarda *et al*. (2004) found that while yields have almost doubled over the hundred-year period covered, grain protein concentrations declined from 16.1% to 11.4%. It may be that further yield improvements to milling wheats will be limited, if protein concentrations are not permitted to fall further. Breeding for NUE in milling wheat must involve looking for varieties which fall above the usual negative regression line for yield against protein concentration, i.e. those which have a higher yield than would be expected at the required protein concentration.

For milling wheats, it is possible that the relationship between high NUE and low grain N concentration could be countered by improving grain protein quality: lower grain protein levels could be tolerated in milling wheat if grain protein quality was increased. A key protein to target would be glutenin, one of the components of gluten. However, it is not known whether sufficient genetic variation for glutenin concentration exists to enable such a breeding programme (Defra, 2005).

For feed wheat, the negative relationship between yield and grain N is not a problem, as unlike for milling wheat, there is no requirement or premium for high grain protein. For malting barley and wheat grown for ethanol production, it is a benefit, as ethanol yield per tonne of grain increases with falling N content. Selection for barley varieties with low grain N concentration is thought to be a major contributor to the greater improvements in NUE achieved for barley than for wheat over the last 30 years (Sylvester-Bradley & Kindred, 2009). Targets and premiums already exist for barley with low N concentration, and could be introduced for wheat as the bioethanol industry expands. This would encourage the development of distinct breeding programmes for feed and milling wheats, with feed wheats being selected under similar conditions to barley. This would widen the divide between the two classes of wheat. The same negative relationship between yield and seed N content is also likely to occur for oilseed rape, but this is not an issue because the main measure of

quality in oilseed rape is oil content, which at least in N response experiments is negatively correlated with seed N concentration.

Another set of traits linked to nitrogen utilisation are those related to N remobilisation within plants. In all crops, N is first used in shoots and leaves, before being remobilised from the senescing leaves to the developing grain. Efficient remobilisation from leaves before senescence is essential for NUtE, to avoid excessive loss of N in shed leaves. N remobilisation in oilseed rape has been studied as part of the 'Green Oil' LINK project LK0979. Teakle *et al.* (2008) have estimated that to optimise NUE, stem and pod wall N concentration at maturity should be reduced from an average of 1.0% to 0.6%. This should reduce the total crop N requirement by 30 kg N/ha, without reducing seed yield or N concentration. As part of the project, genetic variation in stem and pod wall N concentration has been demonstrated, with the minimum values measured being 0.70% for stems and 0.68% for pod walls. However, correlations were not found between stem or pod wall N content at maturity and NUtE or NHI (nitrogen harvest index; the proportion of crop N contained in the grain at harvest) (Berry *et al.*, 2010).

Breeding for increased NUE

Through the 20th century, as artificial fertilisers became increasingly available and widely used, the efforts of crop breeders focused on increasing yield and high NUE was not a direct aim. Because most breeding programmes have used high N application rates, it has been suggested that modern varieties are likely to have low NUE and so yield poorly under low N conditions, relative to historic varieties. However, several studies have shown that the opposite is true for both wheat (Guarda *et al.*, 2004; Ortiz-Monasterio *et al.*, 1997; Sylvester-Bradley & Kindred, 2009) and barley (Abeledo *et al.*, 2008; Sylvester-Bradley & Kindred, 2009). While the main factor contributing to high yields in modern varieties is their ability to respond to high N levels, most also achieve slightly higher yields than historic varieties at low N levels. Sylvester-Bradley & Kindred (2009) demonstrated that for spring barley and winter wheat, modern varieties have a higher NUE than old varieties at all N levels but that for winter wheat, economically optimum N levels have also risen, such that NUE at the economic optimum N level has not changed. Both the components of NUE – NUpE and NUtE – have contributed to the increases in NUE achieved through breeding (Ortiz-Monasterio *et al.*, 1997; Sylvester-Bradley & Kindred, 2009).

Despite the unexpected successes in increasing NUE described above, it is likely that considerable scope remains for further improvement. Yields under low N conditions have improved by only a small amount, relative to yields under high N conditions, probably because most breeding programmes have taken place at moderate to high N levels. Assuming an interaction between variety and N level in yield determination (as observed in the Green Oil project), the yield potential of varieties at low N levels can only be determined by either testing them at low N, or screening for traits associated with high NUE. Effective breeding programmes for improving NUE will require the use of breeding material containing sufficient variation in NUE and related traits, appropriate selection conditions and efficient selection criteria.

Sources of useful genetic variation

Numerous studies have shown that considerable variation in NUE exists in modern elite germplasm (Guarda *et al.*, 2004; Le Gouis *et al.*, 2000; Ortiz-Monasterio *et al.*, 1997; Svečnjak & Rengel, 2006), and Foulkes *et al.* (2005) confirm that UK commercial cereal and oilseed rape varieties contain sufficient genetic variation in NUE to enable breeding for improved NUE. However, breeding may also benefit from crosses involving more exotic varieties. Gorny & Ratajczak (2008) crossed modern, high-yielding European barley varieties with lower-yielding varieties from regions where N stress is more common. They found little variation in NUpE, but considerable variation in NUtE, with the highest NUtE in the progeny of crosses including varieties from Syria or Morocco. NUtE and yield were only weakly correlated, suggesting that the high NUtE of low-yielding exotic varieties may be introduced to elite germplasm while maintaining high yields. This process could be aided by marker-assisted selection and breeding under low N conditions.

The use of alternative species should be considered, as well as the use of exotic varieties of wheat, barley or oilseed rape. An example of how this has been done is the creation of triticale, a hybrid of wheat (*Triticum*) and rye (*Secale*) which combines the high yield potential of wheat with the high NUE of rye under low N conditions. From national statistics, Sylvester-Bradley & Kindred (2009) estimate that the NUE of triticale in the UK is typically 31 kg DM/kg N supply, compared to 29 kg/kg for rye and 25 kg/kg for winter feed wheat. Although triticale was first bred over a century ago, it has not been widely adopted: worldwide, the area of triticale production is only 1.7% that of wheat, and in the UK this figure is 0.8%. Consequently, it has received only a fraction of the breeding effort which has been expended on wheat, and varieties can vary considerably in which parent is resembled most closely. However, in recent ADAS trials following a wheat crop, triticale out-yielded feed wheat by 1.5 to 2 t/ha at a range of levels of N fertilisation from nil to super-optimal, thus showing considerably higher NUE then wheat (unpublished data). Further work is required to test the relative performance of triticale and wheat at differing N levels over a greater range of sites and seasons, and to establish the physiology behind the greater NUE of triticale. In experiments on hydroponically-grown seedlings under controlled environment conditions, Paponov *et al*. (1999) found that the high NUE of rye and triticale relative to wheat may be due to higher specific root length and lower allocation of N to the roots, but it remains to be established whether these results apply in field situations. If further work confirms the potential of triticale for high yields and nitrogen use efficiency, work may need to be done to increase its acceptance in the feed and biofuel feedstock markets.

Appropriate selection conditions

Opinions differ about how selection conditions should be changed to allow efficient breeding for high NUE, while maintaining the current focus on high yield and quality. It should be noted that the NUE of barley has been increased by more than that of wheat in the last 30 years of breeding, and that this may be partly because barley breeding trials are generally conducted at sub-optimal N levels, while selection of wheat varieties is usually done at super-optimal N levels (Sylvester-Bradley & Kindred, 2009). This suggests that the NUE of feed wheat could be further improved by the use of breeding programmes more similar to those used for barley, i.e. with lower applied N levels and selection for low grain N concentration. This will not be possible for milling wheats (NABIM groups 1 and 2), which require high grain protein levels.

Studies have found that the relative importance of the two components of NUE (NUpE and NUtE) varies with N level, although there are contradictory results regarding which contributes more at low N and which at high N (Dhugga & Waines, 1989; Ortiz-Monasterio *et al*., 1997; Le Gouis *et al*., 2000). Consequently, selection for NUE at one extreme of N supply would be likely to limit the type of traits which are being selected for. Some researchers suggest the use of parallel trials at low (nil) and high (optimal) N rates (e.g. Le Gouis *et al*., 2000), to select for varieties with robust high yields in a range of N conditions. Wheat breeders at CIMMYT in Mexico at first suggested the use of medium N levels, at which both NUpE and NUtE are likely to contribute to improved NUE (Ortiz-Monasterio, 1997), but more recently have trialled a range of breeding strategies and found the most effective to be alternating high and low N regimes, starting with high N levels in the F2 generation (Van Ginkel *et al*., 2001). A system of alternating high and low N levels has the advantage of not increasing the scale of breeding trials, unlike parallel trials at different N rates.

Selection tools and criteria

Effective breeding for improved NUE requires efficient selection tools, as well as suitable testing conditions. NUE can be assessed simply by measurement of yield at known available N levels, but the components of NUE and associated traits are more challenging to assess. Distinguishing between NUpE and NUtE requires measurement of whole plant and grain N content, which is a relatively time-consuming process, and the key traits for NUpE include aspects of root morphology, which are again not easily assessed.

One of the most versatile and efficient genetic tools used in breeding is marker-assisted selection. This allows selection for any type of trait, without the need for growing a breeding line to maturity, provided that the genes involved have been identified or at least located

within a small region of a chromosome. However, relatively few genetic markers have been established for traits associated with high NUE, many of which are likely to be controlled by multiple genes. Genes which have been identified include uptake transporters for nitrate and ammonium (Lea & Azevedo, 2006) and genes controlling the stay-green phenotype in wheat (Joshi *et al*., 2007; Silva *et al*., 2000; Verma *et al*., 2004).

Recently, remote-sensing devices using spectral reflectance have enabled more rapid assessment of crop N status. These instruments measure the reflectance of specific wavelengths of light from crop canopies. Given that the photosynthetic pigment chlorophyll selectively absorbs certain wavelengths – particularly those in the red and far-red regions of the spectrum – these reflectance measurements can give an indication of canopy chlorophyll content, and hence canopy size, N content and photosynthetic capacity. Many different spectral reflectance indices have been tested on a number of crops, which vary in precisely which wavelengths are compared and are suited for assessing different crop characteristics. For example, Babar *et al*. (2006) have demonstrated that certain spectral reflectance indices, measured at booting, heading or grain-filling, are well correlated with eventual yield in wheat; Muller *et al*. (2008) have established spectral reflectance indices which correlate well with green area index (GAI), biomass and crop N content in oilseed rape; and Petersen et al. (2002) have used spectral reflectance indices to assess N status in barley..

To date, spectral reflectance techniques have been developed more for assessing the nitrogen fertiliser requirements of crops (e.g. Mistele & Schmidhalter, 2008) than for assessing nitrogen use efficiency in a breeding context. However, assessing the nitrogen status of different crop lines in low N conditions could give an indication of their relative N uptake efficiencies. Given that N uptake efficiency depends largely on rooting traits, which are difficult and slow to assess, this could be a very useful screening technique. One potential problem is that varieties can show differences in spectral reflectance which are not correlated with differences in N status or other physiological measures, as demonstrated by Behrens *et al*. (2004) for oilseed rape. Further work must be done to investigate this issue, before spectral reflectance can been used with confidence in breeding programmes.

Another trait related to N use efficiency which spectral reflectance could screen for is delayed canopy senescence, or 'stay-green'. Delayed senescence can allow prolonged photosynthesis and N uptake, as described in more detail above. Adamsen *et al*. (1999) found strong correlations between certain spectral reflectance indices and canopy senescence, although good results were also obtained by simply using digital cameras and image analysis software.

Breeding technologies

A recent discovery by Good's group in Alberta (Good *et al*. 2004; Lea and Azevedo 2007) showed that that promoting activity of the enzyme alanine aminotransferase enhances N assimilation in plants. This trait has been transferred successfully to oilseed rape in California, using GM technology, causing fertiliser N requirements to halve (Good *et al* 2007), and it will now be transferred to rice, maize and wheat (Etter 2007; Arcadia Biosciences 2007)

Another prospect involves introgressing from a wild relative, the capacity to produce root exudates that inhibit conversion of ammonium to nitrate in soil (Subbarao *et al.* 2007). In combination with ammonium nutrition (from urea fertilisers, or in anaerobic soils), this has the potential to reduce leaching and denitrification losses of N. However, it remains to be seen whether this trait represents a significant advantage under field conditions, particularly in the relatively dry and aerobic soils on which wheat is normally grown.

Scope of potential improvements

The scope for increasing NUE may be different for different crop species, in part due to the progress which has already been made in breeding crops with high NUE. For example, barley already has a high NUE relative to other crops (Table 1). The higher NUE of barley than wheat is probably because barley has been bred for low grain N concentration, which is related to high NUtE. The crop which stands out for having a low NUE is oilseed rape, which

produces only 15.3 kg seed per kg fertiliser N, compared to 35.8 kg/kg for wheat and over 40 kg/kg for barley. The low NUE of oilseed rape is partly due to the high energy content of the seed, but even if NUE is considered in terms of MJ energy per kg fertiliser applied, the NUE of oilseed rape is more than 40% lower than that of wheat (Teakle *et al*., 2008). The remaining difference may be partly because oilseed rape has been the subject of intensive breeding efforts for a much shorter period than wheat or barley.

Table 1 Average yield, N application rate, fertiliser nitrogen use efficiency and overall NUE of major UK crops.

Crop	Average yield 2006-08 (t/ha) (Defra, 2010)[†]	Average fertiliser application rate 2006-08 (kg N/ha) (Defra, 2009)	Fertiliser NUE (kg yield / kg fertiliser applied)	Overall NUE (Sylvester-Bradley & Kindred, 2009)
Winter wheat	7.85	187	35.8	25 (feed) 22 (milling)
Winter barley	6.52	135	41.0	23 (feed) 21 (malting)
Spring barley	5.36	98	46.6	27 (feed) 21 (malting)
Winter oilseed rape	3.23	192	15.3	10

[†] Yields quoted at 85% dry matter for cereals and 91% dry matter for oilseed rape. NUE is calculated at 100% dry matter.

As part of the Green Grain LINK project (LK0959) (Sylvester-Bradley *et al*. (2010) a model was formulated to explain NUE in terms of N capture, N distribution between stem, leaf and ear, and N redistribution to grain (i) as is normally observed for high-yielding wheat crops in the UK, (ii) as was proposed for the GREEN grain ideotype at the outset of the project, and (iii) according to genetic variation in key traits as observed through the project. In the latter case, it was assumed that traits observed in individual cultivars could be combined with minimal interaction to form a 'composite cultivar' bred to approach as far as possible the GREEN grain ideotype. The changes in the wheat ideotype were better capture of applied N, slightly less N per unit area of leaf, smaller leaf sheaths, significantly smaller stems with less N content, and less N in chaff. At harvest, the ideotype was taken to have the same grain yield, but with less straw and smaller N contents overall. These changes were incorporated into the model to compute impacts on target outcomes of the project for these three scenarios (Table 2). Whilst the assumptions necessary to compute emissions and performance in alcohol processing are robust, feeding value and seed performance were better tested empirically, by comparing large grain lots for which the only difference was the fertiliser N used to grow them.

Table 2. Trait values and impacts estimated by modelling of normal high-yielding wheat, the GREEN grain ideotype, and a theoretical 'composite cultivar' with the best combination of individual canopy and grain traits, as demonstrated by individual cultivars tested in this project. From Sylvester-Bradley et al. (2010).

Trait / Impact	*units*	Normal wheat	GREEN grain ideotype	Composite cultivar[1]
Canopy N content at GS61	*kg/ha*	200	158	152
Grain N content at harvest	*kg/ha*	153	122	115
Protein in dry grain	*%*	11.4	9.1	8.6
Requirement for applied N	*kg/ha*	217	133	129
Fertiliser N cost at £0.50/kg	*/ha*	£108	£66	£65
N Use Efficiency[2]	*ratio*	31	45	46
N emissions from land[3]	*kg/ha*	54	30	29
GHG (CO_2 equiv.) emissions	*kg/t*	415	279	282

[1] Traits were tested with sub-optimal N, so these results are slightly under-stated.

In general the results of analysing UK and associated germplasm indicate that in the long term it should prove possible to breed a new GREEN wheat type needing 40% less fertiliser N. The most contentious element of this conclusion is the assumption that grain protein concentration with optimal N supplies could be reduced by 2-3% i.e. from 11.5% to 9%. This is based on observations of several cultivars, particularly those from Denmark, which were tested at three sites (but only in 2008). It is supported by a recent analysis of grain N% with optimum N in the UK compared to Denmark (Sylvester-Bradley & Clarke, 2009). Accepting these and other observed traits as being repeatable, it appears possible to increase N capture by 10%, reduce canopy N by ~40 kg/ha and grain N by 30-40 kg/ha, with a net effect of reducing requirements for applied N by 80 kg/ha, or 40%. This should have equivalent large effects on growing costs, on direct N emissions of nitrate, ammonia and nitrous oxide, and thus on GHG emissions. Advantages are also expected from the increased grain starch and reduced gliadin in the grain when it is used for feed, for seed and for distilling, but these are relatively small compared to effects on growing costs.

Semenov *et al*. (2007) have used modelling to predict that the NUE of UK wheat could be increased by around 17%, chiefly by increasing phyllochron (the thermal time required for the development of successive leaves) by 20%, i.e. reducing the rate of leaf development, and reducing specific leaf N by 0.5 g/m^2 without reducing photosynthetic rate. However, Semenov *et al*. did not demonstrate whether these changes are achievable. Foulkes *et al*. (2009) estimated that wheat NUE may be improved by up to 20%, but that realising the full extent of this improvement would require long-term breeding programmes involving crosses with exotic wheat varieties and/or related alien species.

For oilseed rape, as part of the ongoing 'Green Oil' LINK project (LK0979), Berry *et al*. (2008) have estimated the scope of the potential for improving the NUE of oilseed rape, using genetic variation found in modern varieties. They estimate that as a result of reducing the N concentration of stems and pod walls and increasing root length density at depth, the typical fertiliser application rate could be decreased from 191 to 142 kg N/ha (a 28% reduction) without reducing yield. If post-flowering N uptake was also improved, yield could also be increased from an average of 3.2 t/ha to 3.6 t/ha, at this lower N rate. This would give a fertiliser NUE of 23.1 kg/kg, a 47% increase on the current average.

Oilseed rape has been the been the subject of the most progress in improving NUE using GM techniques. Promoting the activity of the enzyme alanine aminotransferase has been estimated to reduce N fertiliser requirement by 50% (Good *et al*., 2007).