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Hawkesford, M. J., Buchner, P. H. and Riche, A. B. 2018. Nutrient dynamics in wheat. *Annual Plant Reviews online.* 1, pp. 1-14.

Annual Plant Reviews (2018) **1**, 1–14
doi: 10.1002/9781119312994.apr0663

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NUTRIENT DYNAMICS IN WHEAT

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Abstract: Nutrients are taken up by plant roots in a regulated manner and are then distributed around the plant according to demand. As the plant develops and matures, requirements will change and new sinks for nutrients will replace old. In the case of wheat, the developing grain replaces the canopy as the major sink. Hence, nutrient allocation is a dynamic phenomenon, achieved by nutrient recycling and linked to processes of development including senescence. The effectiveness of these processes strongly influences performance and quality, particularly grain protein and mineral nutrient content, which are important health and quality attributes of the seeds. The effective reuse of nutrients is an essential contributor to nutrient use efficiency, an important sustainability trait. Movement of nutrients is achieved by multiple large gene families encoding for transporters, each family usually specific for transporting individual substrates, but often with family members showing varied distribution and regulatory patterns. There are observed interactions between nutrients resulting in coordinated accumulation within the plant.

Cycling of nutrients refers to the process of internal movements of nutrients between cells, compartments, and organs or their reuse in metabolic processes. It may also refer to processes occurring within the ecosystem including cycling in the soil and between soil and crop. In this article, emphasis is placed on initial uptake and use of nutrients by the wheat plant and the recycling and partitioning of these nutrients to grain tissues.

Keywords: nutrient use efficiency, nitrogen, micronutrients, transporters, senescence, sulfate, nutrient recycling

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1 Introduction

Nutrient use efficiency (NUE) is usually defined as grain or total biomass produced per unit input of a nutrient. This is a particularly important concept for crop production, but will also influence successful competition of a species in any ecosystem. An alternative measure of NUE in crops is fertiliser recovery, and specifically a measure of capture of applied fertiliser components to the consumed product. In crop production, NUE is an economically important trait, and one gaining increasing importance for minimising negative environmental impacts of excess fertiliser use. It has been estimated that for cereals worldwide, recovery of fertiliser nitrogen (N) in the harvested fraction is only 33% efficient, indicative of a huge waste of resource (Raun and Johnson, 1999). Partially offsetting some of these losses is the re-cycling which occurs in the soil as harvest residues break down releasing the minerals for future crops. With minerals not required for the nutritional quality of the food it is important to maximise this recycling and minimise unnecessary mineral export from the field at harvest.

Crops receive fertiliser to maximise performance and ultimately yield or economic return. *NUE* (the amount of biomass or grain produced for the amount of nutrient available; $\mathrm{kg\,kg^{-1}}$) is the product of *uptake efficiency* (NUpE; the amount of a nutrient taken up (in the total biomass at harvest) as a fraction of the amount available; $\mathrm{kg\,kg^{-1}}$) and *utilisation efficiency* (NUtE; kg of grain or biomass produced as a function of amount of nutrient taken up; $\mathrm{kg\,kg^{-1}}$). For wheat, the key product is grain and therefore the grain yield is used to calculate NUE; however, total biomass may be relevant for some crops. Another important consideration is the *nutrient harvest index* (NHI), which is the fraction of nutrient taken up finally allocated to the harvested portion of a crop compared to the total amount taken up. NUE commonly refers specifically to nitrogen, as a major economic input and as a major driver for yield, but may be also applied to other mineral nutrients.

Mineral nutrients are taken up by the roots, and the efficiency of this process is a major determinant for plant growth and development. Factors influencing uptake efficiency include genetic variability of root structure and function, and additionally soil and climatic conditions together with agronomic management. To achieve optimal nutrient efficiency, following uptake, internal processes of nutrient cycling or recycling are also required. The cycling may be at the subcellular level, between cells or between organs; it may involve physical movement of resources or cycling between metabolic pools. Finally, nutrient harvest index will reflect the recovery of a nutrient in the usable biomass. In modern wheat varieties, the harvest index for biomass and many minerals is extremely high (Barraclough et al., 2014).

A common strategy in commercial wheat production is to provide fertiliser in multiple applications rather than at a single date. Ideally, application is matched to demand and such an approach should minimise

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environmental losses such as to leaching or volatilisation whilst optimising crop performance.

2 Developmental Processes Influence Nutrient Requirements and Dynamics within the Plant

Demands for nutrients vary during development (Figure 1). As a seed germinates, many of the required nutrients are mobilised from seed reserves; however, the first roots soon become important for acquiring nutrients. A life cycle analysis of nitrogen demands by maize in a hydroponic experimental system confirmed varied uptake during development, mirroring demand, for example during leaf development, and accommodated by regulation of activity of nitrate transporters, and possibly involving multiple regulatory mechanisms (Garnett et al., 2013).

Adequate nutrition is required for canopy development and functionality and the canopy becomes an important sink and reserve of nutrients. This

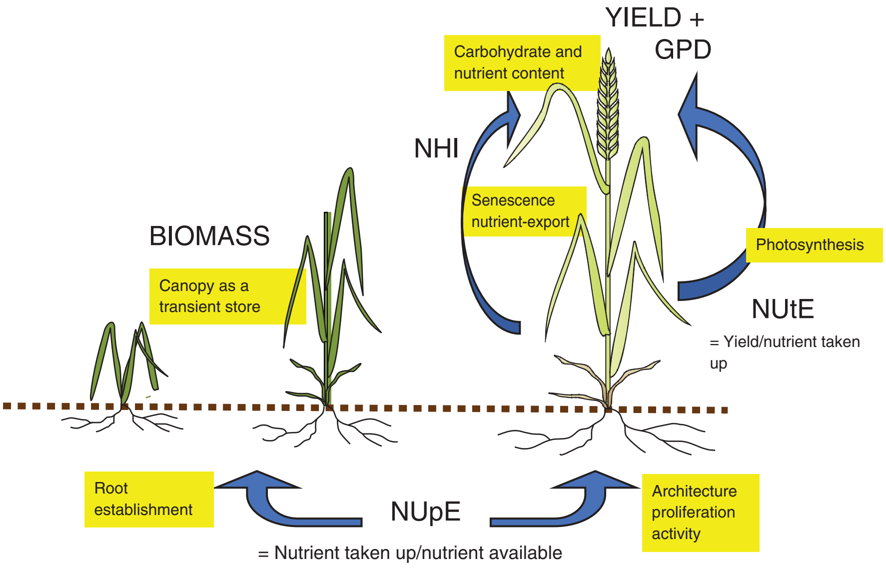

Figure 1 Nutrient use efficiency is composed of several interacting processes, occurring at different developmental stages. Uptake of minerals occurs throughout development; the canopy acts as a sink during vegetative growth; the grain becomes an eventual sink receiving *de novo* taken up nutrients and remobilised nutrients from the canopy. Definition of major components are shown: nutrient use efficiency (NUE) = NUpE × NUtE = yield/available N. GPD: grain protein deviation, i.e. deviation from the negative dilution curve of nutrient concentration as a function of yield.

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is particularly important for micronutrients, which are acquired slowly throughout vegetative development and may subsequently be remobilised efficiently to seed tissues. Biomass will be a critical determinant of uptake and the introduction of short-statured phenotypes in modern wheat as part of the green revolution will likely have contributed to a decreased overall uptake and limited availability for remobilisation to the grain (Fan et al., 2008). After anthesis, the canopy begins to senesce and nutrients are remobilised and delivered to the newly developing sink, namely the grain (Barraclough et al., 2014). During this period, depending on conditions, nutrients will continue to be taken up by the roots and delivered to the grain and in the case of N may be a significant component of its accumulation (Bogard et al., 2010). Processes involved in these nutrient dynamics are discussed in the following sections.

3 Nutrient Translocation, the Role of Senescence, and Grain Nutrient Accumulation

Efficient NUE, particularly of nitrogen is dependent on re-translocation from the canopy. Nutrients taken up initially and utilised for canopy synthesis and function are reused for seed formation rather than being lost from the plant with senesced canopy material. The re-translocation of minerals from vegetative tissues to the grain is an important mechanism to ensure seed tissues are loaded with minerals to ensure their vigour upon germination but also has an important consequence to grain seed nutritional quality. Mineral nutrient content decline (Fan et al., 2008) was investigated in a study of 160 years of wheat samples and there was clear evidence for a recent decline in the mineral (Zn, Fe, Cu, Mg) content of grains. This could be partially attributed to higher yields and a dilution of mineral content by increased starch accumulation but may also be attributed to a change in plant architecture. The adoption of short stature plants would limit vegetative index, and although a high harvest index would ensure effective translocation, the total taken up may be reduced.

Remobilisation from canopy to grain is mostly driven by senescence processes in the vegetative tissues (Gregersen et al., 2008; Hortensteiner and Feller, 2002; Lim et al., 2007) and involves processes of autophagy (Guiboileau et al., 2012; Li et al., 2016), which are little studied in wheat. As a consequence of canopy senescence, photosynthetic functionality decreases and continuing contribution of newly generated photosynthate decreases, ultimately limiting yield. All vegetative tissues, including the roots, will contribute to nutrient recycling and usually there is a progression of senescence in tissues, particularly for the green vegetative tissues to simultaneously facilitate remobilisation and continued photosynthesis. N stored mainly

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as protein, as well as other macronutrients and micronutrients in storage pools such as the vacuoles are efficiently remobilised and reused or go into storage pools in the newly developing grain sinks (Howarth et al., 2008). The mechanisms underpinning regulation and control of senescence have been extensively investigated (Hortensteiner and Feller, 2002), particularly in model species including Arabidopsis (Diaz et al., 2008; Have et al., 2017), and crops such as rice and maize. Similar mechanisms are assumed to exist in wheat (Zhao et al., 2015). The timing of onset and the rate of senescence will influence yield and efficiency of remobilisation. One key gene having a major impact on remobilisation efficiency is the NAM, ATAF1/2, and CUC2 domain proteins (NAC) transcription factor, no apical meristem B1 (NAM-B1), present in many ancestral wheats but largely missing from the modern gene pool (Uauy et al., 2006b). The functional NAM-B1 allele accelerates senescence and confers enhanced grain protein, Zn, and iron content; however, this may be at the expense of yield (Uauy et al., 2006a; Waters et al., 2009). Many other genes, including other NAC transcription factors (Zhao et al., 2015), appear to have contributory roles in regulating the kinetics of senescence and hence nutrient remobilisation.

Cereal grain mineral nutrient accumulation is of critical importance for both quality for nutrition but also for seed viability and seedling vigour. Whilst a major influence on mineral accumulation is availability which likely will limit uptake, total accumulation will also depend on total vegetative biomass as discussed earlier. A greater biomass will lead to a greater accumulation. However, total uptake is less important for wheat crop production than grain mineral density. The density of grain N and other mineral accumulation is influenced firstly by crop uptake which determines total uptake, by harvest index which influences partitioning and finally by yield which can cause a dilution and effectively lower concentration. The first two are mediated by transporters (see the following discussion) and the final by yield potential of the crop and environmental factors influencing yield. The effect of dilution by yield resulting in nutrient dilution is well known and subject of substantial efforts to break this inverse relationship (Bogard et al., 2010; Mosleth et al., 2015). Examples of this for both N (and hence protein) and Zn as a representative micronutrient are shown in Figure 2. In all cases, for both N and Zn and at all rates of N-fertilisation, concentration decreases with increasing yield. Application of N fertiliser increases yields and grain N concentration (Figure 2a). Increasing N fertiliser does not increase grain Zn concentration compared to no fertiliser (where yield is very low), however there is a small increase with increasing N, probably due to the higher protein content (Figure 2b). Although Zn concentration is relatively unaffected by N-fertiliser inputs, the total amount of Zn taken up by the crop is much greater at the higher N-inputs because of the higher yield. The greater grain yield is the key sink contributing to this greater uptake and this is facilitated by the greater canopy biomass-enabling

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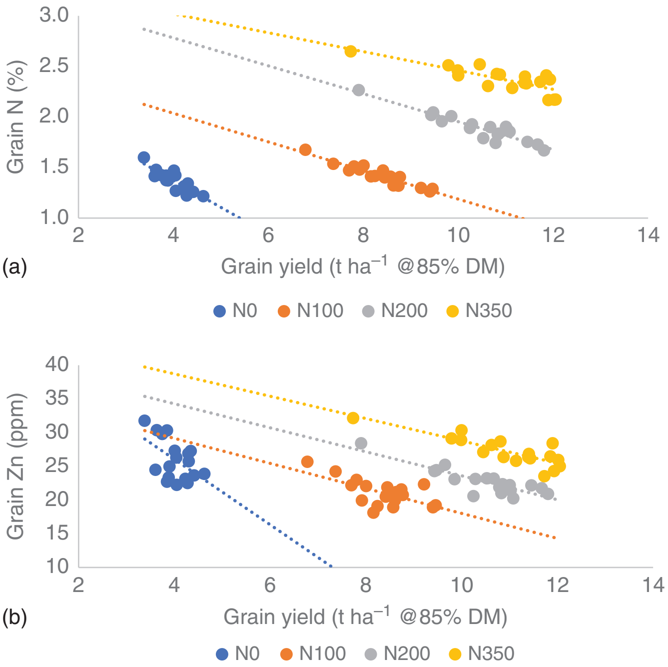

Figure 2 Grain concentrations and yield of, (a) nitrogen and (b) Zn, for a wheat germplasm panel (18 varieties) grown at 4 rates of N fertiliser inputs (0, 100, 200 and 350 kg N ha^{-1}; with mean soil available N of 44 kg N ha^{-1}). Data are the mean of 6 years (2012–2017) and are samples taken from the WGIN trial at Rothamsted, UK (Barraclough et al., 2010). N determined by combustion and Zn by ICP-OES.

uptake during the vegetative phase. Nutrient accumulation and dynamics after acquisition are influenced by many factors including there being inter-dependencies with other nutrients; in this case, Zn uptake being influenced by N-fertiliser.

Delivery of nutrients to grain tissues is an essential step of plant crop maturation. However, the grain is not a homogenous tissue and substantial partitioning of nutrients occurs within the grain. Nutrients must first cross into the endosperm via transfer cells in the nucellar projection and endosperm epithelial tissues (Zheng and Wang, 2011). In the seed, the major destinations are the embryo and endosperm tissues. Substantial differential localisation of elements occurs within the embryo (De Brier et al., 2016). In the relatively homogenous endosperm, there are distinct gradients of proteins (Moore et al., 2016; Savill et al., 2018) and minerals, with, in some cases a strong enrichment in the aleurone or sub-aleurone layers. Mechanisms for much of the differential distribution are unknown but are strongly influenced by environmental conditions (Savill et al., 2018). The variations in density and particularly

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the gradients have substantial implications for the milling process and final product quality.

4 Transporters Are Required for Nutrient Uptake and Cycling

Most mineral nutrients are taken up by root cells, or rarely in the case of agronomic foliar feeds, through the leaf surface, and then distributed around the plant. In addition, nutrients may be stored in intracellular organelles such as vacuoles. These movements of nutrients involve many transmembrane transport steps and many transporters responsible for this movement have been identified by gene sequence. Usually in all plant species, including wheat, large gene families encode for the transporters for specific substrates or groups of substrates. Transporters also exist for metabolites such as amino acids, and these have significance for remobilisation of N and sulfur (S) during crop maturation (senescence of leaves and grain filling). The major transporter gene families have been partially or completely identified in the hexaploid genome of wheat: members of the nitrate transporter 1/peptide transporter family (NPF) responsible for low-affinity nitrate transporter (LATS), high-affinity NRT2 nitrate transporter (HATS) as well ammonium transporter genes have been described (Bajgain et al., 2018; Buchner et al., 2010; Li et al., 2017). At least 89–94 NPF homoeologous NPF genes (Wang, Buchner and Hawkesford, unpublished), 14–17 NRT2-family genes (Buchner and Hawkesford, unpublished), and 7–8 homoeologous ammonium transporter genes (Bajgain et al., 2018; Li et al., 2017) occur in wheat. The complete Pht1 phosphate transporter gene family consists of 16 homoeologous in the three diploid subgenomes (Grun et al., 2018; Shukla et al., 2016); 14 homoeologous members are present for the sulfate transporter gene family (Buchner et al., 2010) as well as 14 ZIP zinc (Zn) transporter homoeologous (Evens et al., 2017).

One of such family is illustrated in Figure 3. Phylogenetic analysis clearly indicates the existence of clusters of related genes, here indicated as Groups 1–5, suggested to have functional specialisation in terms of location of expression (organ-specificity or tonoplast-located in the case of Group 4 (Kataoka et al., 2004)), functional characteristics (high and low affinity for sulfate, Groups 1 and 2, respectively), specificity of substrate (preferentially molybdate in the case of Group 5) or responses of expression in relation to external factors such as nutrient availability (Hawkesford, 2003). This model is too generalist but is a useful starting point in unravelling the complexity of these large gene families. In the case of the SulP family, all members of one of the five clades (Group 5) has been demonstrated in Arabidopsis to have a specific role in molybdenum (Mo) transport (Baxter et al., 2008;

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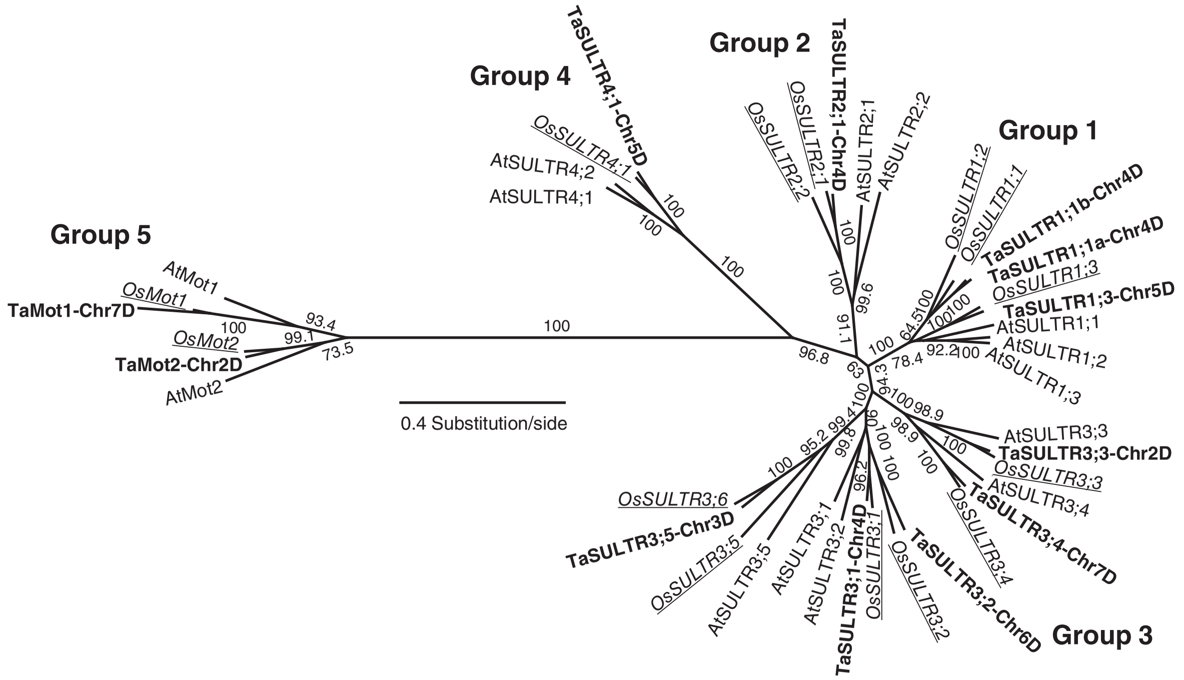

Figure 3 An example of a multigene family for ion transporters: the sulfate transporter gene family for wheat. Phylogenetic relationship of protein sequence of the sulfate transporter gene family in wheat D-genome (Ta, *Triticum aestivum*, bold), rice (Os, *Oryza sativa*) italic, underlined) and Arabidopsis (At, *Arabidopsis thaliana*). A neighbour-joining (Saitou and Nei, 1987) unrooted tree was generated with Geneious software (Kearse et al., 2012) from the multiple alignment (ClustalW) (Larkin et al., 2007) using protein sequences. The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) The analysis involved 40 amino acid sequences. All positions containing gaps and missing data were eliminated.

Tomatsu et al., 2007) with suggested locations on either plasma membranes or tonoplast (Bittner, 2014). There is a suggestion that some members of the Group 3 clade, at least in rice, may transport phosphate (Yamaji et al., 2017; Zhao et al., 2016).

Remobilisation of nutrients, particularly N and S is often accomplished by protein degradation (Gilbert et al., 1997; Howarth et al., 2008) followed by transport of constituent amino acids and peptides directly or following interconversion. Members of the NPF gene family are also involved in peptide transport and recently the gene family for wheat amino acid transporters has been described (Wan et al., 2017). More than 100 distinct groups of homoeologous of amino acid transporters were identified showing specific temporal and spatial patterns of expression.

5 The Transporter Systems Are Regulated by Nutrition

The transporter systems show nutritional regulation of gene expression to optimise efficient nutrient capture in relation to changing supply in the environment and demand during development (Garnett et al., 2013). For example, increased levels of expression of sulfate transporters under sulfur-limiting conditions is common although not universal for all isoforms and tissues and has been shown to occur not only under laboratory conditions (Buchner et al., 2010) but also in the field (Shinmachi et al., 2010). Similar regulation of phosphate (Grun et al., 2018) and Zn (Evens et al., 2017) transporters has been shown in wheat.

Some low-affinity NPF as well as high-affinity NRT2 nitrate transporters are induced by nitrate (Orsel et al., 2002). Many, however, have a complex pattern of expression in relation to tissue specificity, growth stage, and sink/source demands (Buchner and Hawkesford, 2014).

Induction is a response to maximise efficiency of capture of N subject to availability as this is the major driver for protein synthesis and growth. Uptake of other nutrients needs to be balanced with N-uptake and growth and is thus demand driven. For optimum uptake of these nutrients, excess uptake, which would be energetically inefficient, is avoided by repression of expression.

6 Prospects to Improve Mineral Accumulation

Recent progress in wheat genome sequencing and analysis, coupled with rapid and cheap genotyping and improvements in phenotyping will accelerate crop improvement, including traits such as NUE. Targets for improvement will be both at the level of capture, possibly with the modification of root architecture (Atkinson et al., 2015) and distribution at the level of internal partitioning. The roles of specific transporters responsible for nutrient dynamics and the identification of new alleles involved in NUE will be important underpinning knowledge for both target areas. Improvements will be through traditional breeding but may also encompass novel biotechnological approaches. One example is the enhancement of seed sink strength for nutrients via specific over-expression of an iron transporter located on the tonoplast, with consequent vacuole accumulation (Connorton et al., 2017). Overexpression in the endosperm, using an endosperm-specific promoter, improved overall iron accumulation but also targeted this nutritionally important micronutrient to the key tissue eaten by consumers, making this an effective strategy for biofortification. Expression of key transporters such as NRT1.1B is associated with high NUE contributing to both uptake and root-to-shoot transport in indica as compared to japonica rice (Hu et al., 2015). Overexpression of specific ion transporters to further enhance uptake would appear to be a good strategy for improving uptake; however, the complex gene families and expression patterns present a challenge. However, the overexpression of a specific pH-sensitive nitrate transporter (OsNRT2.3) in rice, with a variety of constitutive promoters, resulted in a substantial improvement in NUE in the field (Fan et al., 2016). In this case, it was suggested that the transporter is involved as a pH sensor in the phloem and high expression results in increased pH-buffering capacity enhancing N, Fe, and P-uptake, resulting in an extremely attractive strategy. Further enhancements to this strategy may use a more tissue-specific expression as well as application of this technology to a wide range of crops, including wheat.

Acknowledgements

Rothamsted Research receives strategic funding from the Biotechnological and Biological Sciences Research Council (BBSRC) as part of the 20:20 Wheat (BBS/E/C/00005202) and Designing Future Wheat projects (BB/P016855/1), and by the Defra sponsored Wheat Genetic Improvement Network project.

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