Review
Breeding for Higher Yields of Wheat and Rice through Modifying Nitrogen Metabolism

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Abstract: Wheat and rice produce nutritious grains that provide 32% of the protein in the human diet globally. Here, we examine how genetic modifications to improve assimilation of the inorganic nitrogen forms ammonium and nitrate into protein influence grain yield of these crops. Successful breeding for modified nitrogen metabolism has focused on genes that coordinate nitrogen and carbon metabolism, including those that regulate tillering, heading date, and ammonium assimilation. Gaps in our current understanding include (1) species differences among candidate genes in nitrogen metabolism pathways, (2) the extent to which relative abundance of these nitrogen forms across natural soil environments shape crop responses, and (3) natural variation and genetic architecture of nitrogen-mediated yield improvement. Despite extensive research on the genetics of nitrogen metabolism since the rise of synthetic fertilizers, only a few projects targeting nitrogen pathways have resulted in development of cultivars with higher yields. To continue improving grain yield and quality, breeding strategies need to focus concurrently on both carbon and nitrogen assimilation and consider manipulating genes with smaller effects or that underlie regulatory networks as well as genes directly associated with nitrogen metabolism.

Keywords: cereal; biomass; NUE; yield component; nitrate; ammonium; adaptation

1. Introduction
Balancing crop nitrogen and carbon status under changing environmental conditions is essential for sustaining agricultural productivity and food security. Nitrogen constitutes 1 to 2% of plant dry biomass, yet plants allocate a disproportionate amount of their energy to convert inorganic nitrogen forms, especially nitrate (NO$_3^-$) and ammonium (NH$_4^+$), into organic compounds [1]. As much as 50% of total plant protein may be ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco), the enzyme that initiates plant assimilation of CO$_2$ into organic carbon through C$_3$ carbon fixation [2]. Consequently, plant organic nitrogen and organic carbon are inextricably linked [3]. Rice (Oryza sativa L.) breeding has succeeded in increasing both grain yield and grain protein concentration in recent decades [4]. In contrast, long-term wheat (Triticum aestivum L.) breeding has achieved incremental biomass yield gain, but at the loss of grain protein content over time [5]. Plant breeders therefore actively seek to achieve two sometimes opposing goals, maximization both food productivity and quality.

Articles about breeding strategies to improve yield often discuss crop ideotype, outlining and dissecting desirable traits with the potential to achieve the highest theoretical yield or most rapid progress in genetic gains (for example, [6–11]). This review offers commentary presented in four sections: Process, Progress, Prospects and Puzzles. First, we briefly discuss crop inorganic nitrogen uptake, assimilation, and mobilization, topics for which a plethora of reviews already exist (for example, [12,13]). Second, we evaluate recent successful breeding endeavors involving genes within the nitrogen pathways that improve yield, using the framework of yield component analyses. Third, we present key trends among 40 validated genes that enhance crop yield. Highlighted are genes that influence...
tiller number, flowering time, and \( \text{NH}_4^+ \) assimilation. Lastly, we conclude by identifying areas for further research such as homologs across species, responses to different inorganic nitrogen forms, and complexities of natural variation and epistasis.


Plants acquire most of their nitrogen, both organic and inorganic forms, from soil, but reliance on each form varies greatly over time, with location, and under different environmental conditions [14]. Soil microorganisms mineralize organic nitrogen into \( \text{NH}_4^+ \), which subsequently becomes oxidized into \( \text{NO}_3^- \) through nitrification [15]. Plants compete with soil microbes for \( \text{NH}_4^+ \), a form which also serves as a crucial microbial energy source [16]. In temperate aerobic agricultural soils, microbial activities rapidly convert most soil nitrogen into \( \text{NO}_3^- \), and so \( \text{NO}_3^- \) remains the dominant soil inorganic nitrogen compound available to crops [16,17].

Plant nitrogen acquisition relies on a well-coordinated network of transporters [13]. Nitrate transporters are among the most extensively studied groups of proteins and include low and high affinity systems that cover a large range of concentrations in soil; they also have additional functions beyond \( \text{NO}_3^- \) transport [4]. Ammonium transporters are considered high affinity systems because they operate under low \( \text{NH}_4^+ \) concentrations [4]. The primary inorganic nitrogen assimilation pathway involves several reactions: nitrate reductase (NR) catalyzes \( \text{NO}_3^- \) reduction into nitrite (\( \text{NO}_2^- \)), nitrite reductase (NiR) catalyzes nitrite (\( \text{NO}_2^- \)) reduction into ammonium (\( \text{NH}_4^+ \)), and the concurrent actions of glutamine synthetase/glutamate synthase (GS/GOGAT) catalyze the incorporation of \( \text{NH}_4^+ \) into amino acids [18]. The resulting organic nitrogen compounds are transported, remobilized, and re-assimilated in different organs according to sink demand as a plant develops [4]. As plants mature and reach a reproductive stage, nitrogen compounds that have accumulated throughout vegetative stages are directed toward seeds, the organs vital to species survival and the harvestable part for most crops [4].

Our major focus here is wheat and rice for multiple reasons. First, these crops are the two top sources of plant protein that we consume daily according to the Food and Agriculture Organization of the United Nations (FAO) [19] (Table 1). Relative reliance on these two crops as a major protein source varies across geographical regions. Wheat contribution to human protein intake is dominant in Northern Africa (38%), Central Asia (38%), Southern Asia (26%), Western Asia (39%), and Europe (22–29%). Rice prevails in South-eastern Asia (34%), Southern Asia (21%), and Micronesia (18%). Second, wheat and rice are both C\(_3\) plants belonging to the Poaceae family. Such relatedness may facilitate the transfer of knowledge between these two closely related species, although the genome of hexaploid wheat is 40 times larger than that of rice [20]. Third, as model species and major food crops, they both have been the subject of extensive research extending over a broad range of production areas across diverse environmental conditions worldwide [21]. Lastly, under current cultivation practices, wheat and rice may have adapted to different habitats [22], especially to distinct forms of inorganic nitrogen. Wheat is grown in aerobic soils, dominated by \( \text{NO}_3^- \), whereas rice is grown usually under hypoxic conditions with a relatively high \( \text{NH}_4^+ \) presence in the root zone. Understanding how major food crops adapt to different forms of nitrogen should highlight nutrient management strategies to improve grain yield and quality. Three major components contribute to yield of small grain crops: number of tillers, number of grains per tiller (or grains per spike), and grain weight [23]. Number of grains per tiller may be further divided into number of panicles (or spikelets) and number of grains per panicle (or spikelet). Whereas tiller development can be influenced significantly by changes in the environment, grain characteristics are highly heritable [23,24]. Grain number and grain yield are positively correlated with crop nitrogen content [21]. Crops absorption of \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) from soils and assimilation into organic forms reaches a peak at anthesis [25]. During grain production, plants remobilize stored organic nitrogen compounds and translocate them to seeds [26]. Nitrogen supply from before planting until anthesis is more strongly related to vegetative growth and yield
potential, while nitrogen application post-anthesis is more strongly related to improved protein content and grain quality [25,27]. Photosynthesis, a process in which nitrogen-rich compounds play a major role, contributes biomass to fill in grain weight [21]. In other words, nitrogen is fundamental to all processes that determine final grain yield [11]. Therefore, optimizing nitrogen acquisition throughout crop development is crucial for attaining maximum yield potential.

Table 1. Average contribution of wheat and rice to daily protein intake between 2010 and 2019 [19].

<table>
<thead>
<tr>
<th>Region</th>
<th>Total Intake</th>
<th>From Wheat</th>
<th>% From Wheat</th>
<th>% From Rice</th>
<th>% Wheat and Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>81.39</td>
<td>16.28</td>
<td>20.00</td>
<td>10.08</td>
<td>12.38</td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Africa</td>
<td>60.06</td>
<td>6.31</td>
<td>10.50</td>
<td>3.35</td>
<td>5.58</td>
</tr>
<tr>
<td>Middle Africa</td>
<td>45.17</td>
<td>3.50</td>
<td>7.75</td>
<td>2.39</td>
<td>5.29</td>
</tr>
<tr>
<td>Northern Africa</td>
<td>93.05</td>
<td>35.33</td>
<td>37.97</td>
<td>3.45</td>
<td>41.68</td>
</tr>
<tr>
<td>Southern Africa</td>
<td>78.91</td>
<td>13.76</td>
<td>17.44</td>
<td>2.77</td>
<td>20.95</td>
</tr>
<tr>
<td>Western Africa</td>
<td>63.46</td>
<td>5.02</td>
<td>7.92</td>
<td>7.87</td>
<td>20.32</td>
</tr>
<tr>
<td>America</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caribbean</td>
<td>67.31</td>
<td>9.40</td>
<td>13.97</td>
<td>9.37</td>
<td>27.89</td>
</tr>
<tr>
<td>Central America</td>
<td>83.73</td>
<td>6.86</td>
<td>8.19</td>
<td>2.05</td>
<td>10.64</td>
</tr>
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<td>Northern America</td>
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<td>19.31</td>
<td>17.46</td>
<td>1.44</td>
<td>18.76</td>
</tr>
<tr>
<td>South America</td>
<td>86.53</td>
<td>11.89</td>
<td>13.74</td>
<td>5.53</td>
<td>20.13</td>
</tr>
<tr>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Central Asia</td>
<td>91.29</td>
<td>35.14</td>
<td>38.49</td>
<td>1.26</td>
<td>39.67</td>
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<tr>
<td>Eastern Asia</td>
<td>98.35</td>
<td>17.29</td>
<td>17.58</td>
<td>14.89</td>
<td>32.72</td>
</tr>
<tr>
<td>South-eastern Asia</td>
<td>69.05</td>
<td>4.82</td>
<td>6.99</td>
<td>23.52</td>
<td>41.05</td>
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<td>16.48</td>
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<td>13.09</td>
<td>47.17</td>
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<td>87.67</td>
<td>34.33</td>
<td>39.16</td>
<td>3.72</td>
<td>43.40</td>
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<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>97.68</td>
<td>28.77</td>
<td>29.45</td>
<td>0.69</td>
<td>30.15</td>
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<tr>
<td>Northern Europe</td>
<td>106.60</td>
<td>24.27</td>
<td>22.76</td>
<td>1.13</td>
<td>23.83</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>104.59</td>
<td>26.91</td>
<td>25.73</td>
<td>1.25</td>
<td>26.92</td>
</tr>
<tr>
<td>Western Europe</td>
<td>105.43</td>
<td>23.54</td>
<td>22.33</td>
<td>0.83</td>
<td>23.12</td>
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<tr>
<td>Oceania</td>
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<tr>
<td>Australia/New Zealand</td>
<td>106.23</td>
<td>18.93</td>
<td>17.82</td>
<td>1.73</td>
<td>19.45</td>
</tr>
<tr>
<td>Melanesia</td>
<td>65.03</td>
<td>8.40</td>
<td>12.92</td>
<td>3.74</td>
<td>18.67</td>
</tr>
<tr>
<td>Micronesia</td>
<td>71.28</td>
<td>10.86</td>
<td>15.23</td>
<td>12.60</td>
<td>32.91</td>
</tr>
<tr>
<td>Polynesia</td>
<td>92.76</td>
<td>14.25</td>
<td>15.36</td>
<td>4.17</td>
<td>19.86</td>
</tr>
</tbody>
</table>

3. Progress—Common Breeding Strategies Are Limited to Regulating Expression of Few Genes

Plant breeders achieve genetic gain in breeding populations over generations by selecting and retaining genetic materials with targeted characteristics and superior performance. A more thorough understanding of the molecular biology and genetic basis of specific traits facilitates the rapid development of more desirable genotypes, especially for traits that are controlled by a single or few loci with large effects. Yet, improving complex traits like yield and nitrogen responses remains challenging.

Will breeding for improved nitrogen uptake and assimilation also increase yield? While yield improvement can arise from factors affecting yield components besides increased nitrogen use efficiency (NUE), breeding for this trait should lead to increased biomass production and grain yield [28,29]. Nonetheless, breeding programs for yield seldom monitor nitrogen responses [30–33], and modern cultivars with higher yield demonstrate little improvement in NUE [34].

The genetic basis underlying desirable phenotypes for grain yield and quality often remain obscure, despite genetic gains through selection. For example, in Green Revolution varieties, the genetic variants and mechanisms responsible for the short stature and increased harvest index that underpin the yield boost were identified only several decades after the release of improved cultivars [35]. In rice, the recessive loss-of-function mutation of Semi-Dwarf 1 (SD1) impairs an oxidase enzyme in the synthesis pathway of gibberellin, a key hormone promoting height, whereas in Green Revolution-derived varieties of wheat,
mutations of Reduced Height 1 (RHT-1) encode modified proteins that also diminish height, but are insensitive to gibberellin-induced degradation [36–39]. Dwarfing genes improve yield through several mechanisms that act in concert to both diminish height and significantly increase biomass partitioning to the grain [36]. High harvest index, the fraction of biomass allocated to harvestable organs, is known to be strongly associated with high crop nitrogen status [40]. Unfortunately, many Green Revolution phenotypes, regardless of the mechanisms responsible for decreased gibberellin responses, also limit crop responses to nitrogen [41,42]. Plants with a dwarfing gene often have slower nitrogen uptake [42] and nitrogen accumulation relative to dry matter accumulation after anthesis, thereby decreasing NUE on a grain biomass basis in the field [41]. Insensitivity to increased nitrogen supply may be beneficial because the absence of nitrogen-promoted stem elongation makes plants more resistant to lodging [42], although at the high cost of requiring additional nitrogen fertilizer to maintain adequate yield. This case study from Green Revolution varieties underscores the challenge of improving yield through modifying nitrogen metabolism.

Generally, attempts to improve yield also alter rates or paths of metabolite production [43]. In particular, the enhanced harvest index of widely grown Green Revolution varieties diverts more biomass into harvestable grains. Nonetheless, assuming we have not reached the limits of biomass production, we may coordinate source vs. sink balance and continue to allocate additional crop assimilation of carbon and other nutrients toward yield [44]. Although efforts to increase crop source strength in terms of light capturing efficiency have been long underway [45], this goal seems elusive unless we address water and nutrient limitations [46–48]. Greater emphasis on enhancing nitrogen accumulation upon which biomass production depends may prove more effective in increasing yield in the near future [46,48]. With more extensive knowledge about the genetics of the underlying traits and advanced breeding tools, we could perhaps make even faster progress if we target both enhanced carbon and nitrogen assimilation concurrently.

An extensive body of literature is now available about the major transporters and enzymes associated with nitrogen assimilation and remobilization throughout crop growth cycles [4,13,30,49]. Characterized and cloned are key genes that govern metabolic pathways, but successful breeding applications for yield improvement that involve these genes are few, especially those that have reached the stage of commercial field trials [12].

Here, we have tabulated 40 genes that influence nitrogen metabolic pathways and improve grain yield (Table 2). Among these, regulation of gene expression seems to be the most successful approach for translating improved nitrogen metabolism into higher yields. Overexpression of genes [50] is the most common approach. Less common is knocking out [51] or silencing the genes of interest with small interfering RNA (RNAi) [52] or Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) [53] that precisely targets specific genomic regions. Relatively few studies have employed conventional breeding methods and, thus, have avoided transgenic means for introgression or incorporation of a functional allele into a breeding population. Progress in rice overall has been more rapid than in wheat [54]. Below are four different categories of genes involved in nitrogen metabolism that recent breeding efforts have manipulated to improve yield.
Table 2. Breeding applications of nitrogen metabolism genes in rice and wheat that were proven successful in improving yield.

<table>
<thead>
<tr>
<th>#</th>
<th>Gene</th>
<th>Ref</th>
<th>Species</th>
<th>Breeding Application</th>
<th>Yield Component Improvement</th>
<th>Other/Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nitrate transporter 1/Peptide transporter Family 6.1 NPF6.1</td>
<td>[55]</td>
<td>Rice Overexpression</td>
<td>✓ ✓ ✓ ✓</td>
<td>* Effective panicle number. No data on grain number and weight.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Nitrate transporter 1/Peptide transporter Family 6.3 NPF6.3 (NRT1.1A)</td>
<td>[56]</td>
<td>Rice Overexpression</td>
<td>✓ ✓</td>
<td>✓ Shortened maturation time</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Nitrate transporter 1/Peptide transporter Family 6.5 NPF6.5 (NRT1.1B)</td>
<td>[57]</td>
<td>Rice</td>
<td>✓ ✓ ✓ ✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Nitrate transporter 1/Peptide transporter Family 7.1 NPF7.1</td>
<td>[58]</td>
<td>Rice Overexpression</td>
<td>✓ ✓ ✓ ✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Nitrate transporter 1/Peptide transporter Family 7.1 NPF7.2</td>
<td>[59]</td>
<td>Rice Overexpression</td>
<td>✓ ✓ ✓ ✓</td>
<td>Increased root length, root number, root biomass</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Nitrate transporter 1/Peptide transporter Family 7.4 NPF7.4</td>
<td>[58]</td>
<td>Rice CRISPR/Cas9 mutant</td>
<td>✓ ✓ ✓ ✓</td>
<td></td>
<td></td>
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<tr>
<td>7</td>
<td>Nitrate transporter 1/Peptide transporter Family 7.7 NPF7.7</td>
<td>[60]</td>
<td>Rice Overexpression</td>
<td>✓ ✓ ✓ ✓</td>
<td>* Yield presented as g grain/g N. Larger panicle, Higher N content, but not amino acid suggests N accumulation</td>
<td></td>
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<tr>
<td>8</td>
<td>Nitrate transporter 1/Peptide transporter Family 8.20 NPF8.20 (PTR9)</td>
<td>[61]</td>
<td>Rice Overexpression</td>
<td>✓ ✓ ✓ ✓ ✓</td>
<td>Highest improvement at low N</td>
<td></td>
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<tr>
<td>9</td>
<td>High-affinity nitrate transporter 2.1 NRT2.1</td>
<td>[62,63]</td>
<td>Rice Overexpression</td>
<td>✓</td>
<td>Increased Mn accumulation</td>
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<td>10</td>
<td>High-affinity nitrate transporter 2.3b NRT2.3b</td>
<td>[64]</td>
<td>Rice Overexpression</td>
<td>✓</td>
<td></td>
<td></td>
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<tr>
<td>12</td>
<td>Ammonium transporter 1;1 AMT1;1</td>
<td>[68]</td>
<td>Rice Overexpression</td>
<td>✓</td>
<td>Double activation mutants with GOGAT1</td>
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<tr>
<td>13</td>
<td>Ammonium transporter 1;2 AMT1;2</td>
<td>[69]</td>
<td>Rice Overexpression</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Glutamate synthase 1 GOGAT1</td>
<td>[69]</td>
<td>Rice Double activation mutants with AMT1;2</td>
<td>✓ ✓ ✓ ✓</td>
<td></td>
<td></td>
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<tr>
<td>15</td>
<td>Glutamine synthetase 1 GS1</td>
<td>[70]</td>
<td>Rice Overexpression</td>
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<tr>
<td>16</td>
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<td>17</td>
<td>Nitrate reductase 2 NR2</td>
<td>[72]</td>
<td>Rice Transgenic with indica variant</td>
<td>✓ ✓ ✓ ✓</td>
<td></td>
<td></td>
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<td>18</td>
<td>Asparagine synthetase 1 ASN1</td>
<td>[73]</td>
<td>Rice Overexpression</td>
<td>✓ ✓ ♣</td>
<td>* Yield increases only at low N</td>
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<td>19</td>
<td>Amino acid permease 1 AAP1</td>
<td>[74]</td>
<td>Rice Overexpression</td>
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<td></td>
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<tr>
<td>20</td>
<td>Amino acid permease 3 AAP3</td>
<td>[75]</td>
<td>Rice RNAi</td>
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<td>21</td>
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<td>Rice Overexpression</td>
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<td>22</td>
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<td>[77]</td>
<td>Rice RNAi</td>
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<td>23</td>
<td>Nodule Inception-Like protein 1 NLP1</td>
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<td>Rice Overexpression</td>
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<td>[80,81]</td>
<td>Rice Overexpression, Quadrupling the promoter of NiR</td>
<td>✓ ✓</td>
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### Table 2. Cont.

<table>
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<th>Gene Name</th>
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<th>Yield Component Improvement</th>
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<td>26</td>
<td>Growth-Regulating Factor 4</td>
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<td>27</td>
<td>Nitrogen-mediated tiller Growth Response 5</td>
<td>NGR5</td>
<td>Rice</td>
<td>Overexpression</td>
<td>✓ ✓ ? ? ?</td>
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<td>28</td>
<td>Teosinte branched1, Cycloidea, Proliferating cell factor 19</td>
<td>TCP19</td>
<td>Rice</td>
<td>Introgression</td>
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<td>29</td>
<td>NAM/ATAF1/2/CUC2 2-5A</td>
<td>NAC42</td>
<td>Rice</td>
<td>See NPF6.1</td>
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<td>Basic Leucine Zipper 60</td>
<td>bZIP60</td>
<td>Wheat</td>
<td>RNAi</td>
<td>✓ ✓ ✓ ✓ ✓</td>
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<td>Grain Number, Plant Height, and Heading Date 7</td>
<td>Ghhd7</td>
<td>Rice</td>
<td>Overexpression</td>
<td>✓ ✓ ✓ ✓ ✓</td>
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<td>32</td>
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<td>Rice, Wheat</td>
<td>Loss-of-function, CRISPR/Cas9 mutant</td>
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<tr>
<td>34</td>
<td>Dense and Erect Panicle 1</td>
<td>DEP1</td>
<td>Rice</td>
<td>Loss-of-function, gain of function mutant</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>35</td>
<td>Dull Nitrogen Response 1</td>
<td>DNR1</td>
<td>Rice</td>
<td>Loss-of-function mutant</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>36</td>
<td>Dehydration-Responsive Element-Binding Protein 1C</td>
<td>DREB1C</td>
<td>Rice, Wheat</td>
<td>Overexpression</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>37</td>
<td>Nuclear Factor Y A-B1</td>
<td>NFYA- B1</td>
<td>Wheat</td>
<td>Overexpression</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>38</td>
<td>Rice Dof Daily fluctuations 1</td>
<td>RDD1</td>
<td>Rice</td>
<td>Overexpression</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>39</td>
<td>MicroRNA 396</td>
<td>MIR396</td>
<td>Rice</td>
<td>Knock-out mutant</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
</tbody>
</table>

* Depending on genetic background; Tiller number not changed in dep1-driven dwarfism and in Njf-sd1. Grain number not changed in Njf-sd1. Did not show changes in yield components apart from tiller number for NGR5 transgenic plants.

### 3.1. Nitrogen Transporters (17 Genes)

Modern phylogenetic studies classify major families of nitrogen transporters in land plants based on their substrate: nitrate, ammonium, or peptides [99]. Most characterizations of transporters are in Arabidopsis and rice [100]; relatively limited information is available for transporters in wheat [30,101,102]. Nitrate transporters (11 genes) have received more attention and their potential for yield improvement have been evaluated more thoroughly than transporters of ammonium and organic nitrogen [13,33]. Perhaps this derives from NH₄⁺ being a nitrogen source that only dominates in a few agricultural production systems and from the ability of its counterpart NH₃ to move freely through membranes following electrochemical gradients [1]. Ammonium transporters (2 genes) may also prove to be more elusive as a target for yield improvement because of the potential for toxicity from excessive accumulation of free NH₄⁺ in tissues as discussed below [33,103]. Nonetheless, coupling NH₄⁺ uptake with assimilation by concurrent modification of AMT1;2 and GOGAT can drive yield improvement [69].

Modification of amino acid transporters (AAP, 4 genes), despite receiving less attention than that of NO₃⁻ transporters [12], is another effective strategy for increasing grain yield. These transporters, in contrast to those that transport NO₃⁻ or NH₄⁺, are key players in remobilizing assimilated organic nitrogen compounds, although their exact functions remain largely unknown in cereals [104]. Organic nitrogen transport within plants directly relates to grain nutritional quality at maturity [26]. Of particular interest is that variation
in the promoter regions across germplasm suggest tight expression regulation and local adaptation that may help plants cope with fluctuations in soil nitrogen gradients [75–77]. Overall, while we have some understanding of how transporters contribute to uptake and transport of each nitrogen form across membranes and might improve plant nitrogen acquisition, modification of these transporters has had limited success in crop yield enhancement [33].

3.2. Nitrogen Assimilatory Enzymes (5 Genes)

Assimilation of inorganic into organic nitrogen in plants is well-regulated at transcriptional, translational, and post-translational levels [105,106]. The enzymes GS and GOGAT are central to nitrogen metabolism, but attempts to alter yield by modifying genes coding for these enzymes have achieved only little success. Previous modification to GS1 increased nitrogen partitioning to grain and nitrogen harvest index, but not vegetative yield nor overall shoot nitrogen accumulation [107]. Failure to successfully modify GS1 on its own may derive from the critical functions for which this gene is responsible [108]. By contrast, modifying GS2 can lead to wheat yield improvement in stressful environments [71]. Alteration to GOGAT expression to boost yield was only achieved through changing expression levels of transcription factors upstream of the enzyme (see discussion below). Thus, successful breeding applications coupled GOGAT with changes to ammonium transporter AMT1:2 [69] or simultaneously modulated GS1 and GS2 [70]. Because GS1 and GS2 are involved in crop growth at different developmental stages [109,110], selecting the appropriate developmental time to express each of these enzymes was critical for a positive result [70].

3.3. Nodule INception-like Proteins That Sense NO$_3^-$ and Regulate Downstream Genes (3 Genes)

Legumes when associated with certain bacteria can generate organic nitrogen from dinitrogen gas N$_2$ in air, a process named symbiotic nitrogen fixation [111]. Nodule INception (NIN) genes govern legume root nodule initiation and symbiotic nitrogen fixation [112]. NIN-like proteins (NLPs) that are homologs to NINs found in non-leguminous crops have critical roles in regulating nitrogen signaling and downstream genes within nitrogen metabolism [113].

Multiple highly conserved NLPs are found in Arabidopsis [112], wheat [114], and rice [112]. Arabidopsis NLPs function as transcription factors, and NLP7 also serves as a biosensor responsive to intracellular NO$_3^-$ supply [115]. Rice NLPs generally serve as activators that control expression of nitrogen responsive genes. For example, NLP4 regulates expression of genes underlying key enzymes in nitrogen assimilation pathways [80], thereby affecting activities of Nir [81] and NR [116]. Some NLPs also shows nitrogen form-specific responses with NO$_3^-$ being the major form to which rice NLPs are most responsive. While either NO$_3^-$ or NH$_4^+$ can trigger expression of NLP3, only NO$_3^-$ induces its nuclear retention [79]. Overexpression of these NLPs in rice stimulate yield, whereas reduced expression of NLPs inhibits growth. To date, yield improvements of wheat from modifications of NLPs are lacking, although nitrogen starvation upregulates NLP7 [114] and NLP4 [117].

3.4. Transcriptional Factors and microRNA That Regulate Other Genes (15 Genes)

Transcriptional factors bind to the promoter of target genes to regulate downstream gene functions [118]. System biology is steadily clarifying how a large network of transcription factors regulate nitrogen metabolism and how key transcription factors control expression of multiple proteins in the pathways simultaneously [119]. Whereas modifying expression of individual transporters and enzymes has had only modest success in improving crop performance, altering transcription factors that orchestrate simultaneously systematic changes in multiple nitrogen-related genes may have profound effects on biomass accumulation and grain quality. For example, overexpressing rice DREB1C, which regulates nitrogen assimilation genes, increased nitrogen assimilation and photosynthesis significantly, resulting in increased grain number, grain weight, and harvest index [95].
Together these changes resulted in 68.3% higher yield than wildtypes and in a 13 to 19 days earlier flowering time [95]. Light- and nitrogen-mediated OsDREB1C controlled over 9000 genome-wide putative binding sites, including five gene targets in the carbon and nitrogen metabolism pathways: *rubisco small subunit 3* (OsRBCS3), *nitrate transporter 2.4* (OsNRT2.4), *OsNRT1.1B*, and *flowering locus T-like 1* (OsFTL1). Previous attempts to engineer several individual genes from this list never reached as high a yield gain as manipulating the transcriptional factor gene OsDREB1C alone. For instance, overexpression of transporters led to higher accumulation and efflux of excessive nitrogen because plants were not able to assimilate more nitrogen into protein [120].

Manually coordinating individual genes underlying nitrogen sources and sinks to complete a whole pathway therefore remains a challenge. Although we still have limited understanding about the regulation and function of transcription factors, modifying a single transcription factor appears more effective than manipulating individual genes and proteins in a pathway [121]. This highlights the complexity and tight regulation of nitrogen metabolism. As more genotypic and phenotypic data become available across diverse plant species, the roles of transcriptional factors in nitrogen metabolism should become clearer. Editing genetic networks, rather than individual candidate genes that regulate the balance between carbon and nitrogen metabolism may prove to be a more promising approach for increasing yield.

4. Prospects—Fine-Tuning Yield Component Responses to Transient Nitrogen Supply Can Maximize Yield

Successful manipulation of genes regulating nitrogen metabolism (Table 2) is contingent upon more advanced understanding of how nitrogen acquisition influences growth and vice versa. We now have a better understanding on how nitrogen, especially NO$_3^-$, drives hormonal and physiological changes underlying canopy architecture and development [122]. However, the influence nitrogen has on yield components and the tradeoffs among subcomponents are still uncertain. In this next section, we summarize recent findings, focusing on tiller number, flowering time, and NH$_4^+$ assimilation as key links between carbon and nitrogen metabolism and, therefore, highly relevant to nitrogen-driven yield improvement.

4.1. Tiller Production Contributes to Higher Yields through Multiple Nitrogen-Mediated Signaling Pathways

Tiller number, a key determent of effective number of panicles that contribute to grain filling and grain yield, is the most responsive of all yield components to nitrogen [122]. Tiller number is a routinely measured yield component because its assessment is straightforward. Out of the 40 genes reported to improve yield in this review (Table 2), 19 genes are associated with higher tiller number.

Increasing nitrogen supply generally increases tiller production [82] whereas limiting supply decreases tiller production [122]. Soil NH$_4^+$ concentration correlates linearly with tiller number in rice [123] and nitrogen fertilization levels explain 66% to 96% of the variation in tillering rate, which is significantly correlated with the final grain yield [23]. Similarly, increased nitrogen levels also boost tiller production in wheat [124]. Changing canopy architecture by optimizing nitrogen inputs and increasing tiller number per unit area thus enhances biomass source strength and grain yield in rice [125], and both yield and grain protein content in wheat [126].

Changes in tillering number derive from the interplay between multiple opposing nitrogen-mediated hormonal shifts [122]. High nitrogen availability induces cytokinins to increase tillering, but also induces auxins and strigolactones to inhibit tillering [127]. In rice, multiple amino acid transporters balance the opposite actions of auxins and cytokinins: OsAAP1 and OsAAP4 regulate auxin and cytokinin signaling [74,76], whereas OsAAP5 only influences cellular cytokinin levels [77]. *microRNA393* (OsmiR393), in turn, lowers sensitivity to auxin signaling and increases tillering [128].
Feedback mechanisms between hormones and nitrogen ensure optimized developmental responses to fluctuating external nitrogen pools. As nitrogen supply increased, a negative feedback mechanism driven by DNRI reduced auxin functions to upregulate genes for tiller production and nitrogen metabolism, thereby repressing nitrogen uptake and assimilation as well as tiller production [94]. Conversely, a nitrogen shortage downregulated DNRI, promoting nitrogen acquisition and tiller development [94].

The complex balancing acts of gibberellin, which explain why Green Revolution plant varieties maintain lower height and high yield, but require high nitrogen fertilizer inputs, have been reviewed in great detail elsewhere [106]. In brief, gibberellin and its counterparts DELLA proteins, which are named after their conserved chain of amino acids D-E-L-L-A, have two fates. Under high nitrogen availability, gibberellin can either inhibit tiller development by degrading gibberellin’s downstream transcriptional factor NGR5 protein or promote tiller production via a positive feedback mechanism driven by nitrogen itself to increase nitrogen assimilation and upregulate NGR5, which represses tiller inhibitory genes. Likewise, DELLAAs may sustain tiller promotion by interfering with gibberellin-driven NGR5 destruction [82] or decrease nitrogen accumulation by downregulating nitrogen assimilation genes [42], thereby indirectly limiting nitrogen-driven tiller development. Because most Green Revolution-derived high-yielding cultivars already contain dwarfing genes conferring high DELLA abundance, breeders can further increase tiller production and yield even at low nitrogen levels by increasing NGR5 abundance directly, suggesting a potential decoupling of tillering from nitrogen supply [82].

Modification of transcriptional factors further enhances yield by tipping the balance of proteins and promoting nitrogen-driven tiller production. The coordination for carbon and nitrogen is systematically regulated by the transcription factor GRF4 [82] and its upstream repressor MIR396 [94], both of which modulate nitrogen acquisition and growth via DNRI [94] and modulate nitrogen assimilation genes to counterbalance the inhibitory effects of DELLA [42]. Therefore, increased GRF4 expression alters the balance of GRF4-DELLA, thus enhancing nitrogen assimilation, tiller development, and grain yield [42].

Nitrogen influence on tiller development via the brassinosteroid signaling pathway also remains an active area of research. High NO$\text{$_3$}$ levels decrease rice expression of TCP19, which represses Dwarf and Low-Tillering (DLT), a gene involved in brassinosteroid signaling and tillering promotion, thereby inhibiting tiller bud outgrowth [83]. OsTCP19 overexpression lines exhibit brassinosteroid-deficient phenotypes similar to dlt mutants [83]. In wheat, overexpression of Dwarf4 (DWF4), which encodes a key enzyme in brassinosteroid synthesis, also increases both nitrogen assimilation and tiller number [129]. Furthermore, the proteins of rice DLT and MONOCULM1 (MOC1), which regulate tiller production, are both under control of NGR5 [82].

Interestingly, there are tradeoffs among yield subcomponents. For example, not all yield improvement is associated with increased reproductive tiller number. In fact, fewer tillers is a key characteristic proposed as an ideal canopy architecture for high yields [130]. Mutants with loss-of-function dnri or reduced DNRI abundance develop fewer tillers, but increase auxin, accelerate nitrogen uptake, and exhibit higher yields [94]. In the case of DREB1C overexpression, transgenic rice plants with higher yields have fewer panicle numbers, but instead produce elongated panicles with increased grain weight and number of grains within each panicle [95]. Such coordination between source and sink components appear to shift if carbohydrate supplies increase because these transgenic plants also have higher photosynthetic rates and accumulate more biomass at heading stage. Additionally, reduced branching may also result from a shortened development period to be discussed in the next section. Altogether, regulations of nitrogen-mediated tiller development highlight the importance of evaluating all yield components that contribute to actual yield changes.
4.2. Optimized Flowering Time Maximizes Nitrogen and Carbon Assimilation in Agricultural Settings

Adjustments of flowering time or heading date is an evolutionary adaptation that maximizes seed yield and survivability over generations [131]. Flowering time optimized for each environment can enhance grain yield in staple food crops [132]. The transition from vegetative to reproductive developmental stages determines total nitrogen accumulation over the vegetative growth period [25,32] and shifts the emphasis of nitrogen metabolism to remobilization and reassimilation in maturing grains [26]. While photosynthesis per unit leaf area may remain unchanged, cumulative increases in leaf area, light interception, overall growth period, and vegetative biomass accumulation—all responsive to nitrogen inputs—may together increase yields [133]. Suboptimal or excess nitrogen supply often, respectively, accelerate or slow the transition to reproductive phase [134]. The precise extent to which nitrogen supply influences cereal flowering time, however, is uncertain [135].

The genetics underlying vernalization and photoperiod pathways in cereals are well-characterized [136], but their interactions with nitrogen remain an open question. Multiple genes regulate flowering time and its influence on grain yield [132]. Indeed, genes underlying developmental timing like Photoperiod (Ppd) and Vernalization (Vrn) appear to co-locate with Quantitative Trait Loci (QTL) associated with NUE [137], suggesting a potential connection with nitrogen metabolism. Several recent studies have identified genes with pleiotropic effects that change both nitrogen responsiveness and crop developmental timing via senescence and flowering time. These include NPF6.3, GS2, Nhd1, Ghd7, ARE1, miR396 (Table 2). Specifically, transcription factors Nhd1, Ghd7 and DREB1C have direct control on genes involved in determining heading date [86,90,95]. Connections of other candidate genes with developmental timing require further validation.

An appropriate flower timing is essential for avoiding stressful conditions and maximizing favorable conditions for seed production [131]. Most genes in Table 2 promote a longer growing season. Prolonged vegetative growth generally allows crops to accumulate and assimilate more nitrogen before a crop reaches maturity and senescence, potentially resulting in increased NUE and biomass accumulation. However, a longer growth season may also increase the chance of experiencing abiotic and biotic stresses [131]. Only NPF6.3, DREB1C, and RDD1 accelerate a transition to the reproductive stage and still show a yield improvement [56,95,97]. For example, OsDREB1C significantly enhanced yield, despite a 2–3 weeks shorter growth period [95]. The ability to accumulate higher biomass under a shorter timeframe indicates a higher capacity for carbon and nitrogen assimilation. Nevertheless, early flowering time in rice with photo-insensitive alleles was previously shown to be associated with reduced grain filling, fewer panicles, and subsequently lower yield [138].

Developmental changes driven by variations in the growth environment determine the extent to which yields can be improved. Varying outcomes from different modifications may derive from the environmental interactions underlying nitrogen influence on growth and development. Late season tiller production may not produce a fertile fluorescence and thus contributes only to vegetative biomass production [35]. Tillers initiated early in the season also tend to have higher yields than late tillers [139]. High tiller production combined with longer maturation time generally contributes to higher rice yield [140]. For OsDREB1C modifications, overexpressing plants grown under long days and temperate climates flower about 50 to 70 days later and have higher yield improvement rates than plants under other experimental conditions [95]. Photoperiod pathways seem to be likely candidates that connect nitrogen responsiveness with flowering time, although no known mechanisms have been confirmed to date [134]. Understanding how carbon and nitrogen assimilation intersect and their environmental interaction in the context of crop developmental timing will be crucial in matching crop demands with resource supplies.
4.3. GOGAT as an Indirect Target—A Case Study from Editing Transcriptional Factors ARE1, Nhd1, and bZIP60

GOGAT, when coupled with GS, catalyze the assimilation of NH$_4^+$ into glutamate, an amino acid central to nitrogen and carbon metabolism [141]. Based on genetic map synteny, a meta-analysis of cereal QTL studies on NUE identified GOGAT as a candidate gene that is conserved among major food grain crops (rice, wheat, sorghum, maize) [137]. Although editing GOGAT directly has little influence on yield, coupling GOGAT with AMT1;2 proved effective in enhancing yield [69]. Modifying transcription factors upstream of GOGAT also has been successful (Table 2): ARE1 and Nhd1 both suppress Fd-GOGAT [87,89,90], while bZIP60 suppresses NADH-GOGAT [85].

Eliminating suppression of GOGAT via these transcription factors, improves yields significantly. Enhancement of GOGAT function seems to be a plausible approach for raising yield because glutamate links carbon and nitrogen metabolism [141]. The role of transcriptional factors suggests that we have yet to characterize additional players involved in the assimilation of NH$_4^+$ into organic nitrogen. Identification and modification of other pathways similar to GS/GOGAT -driven NH$_4^+$ assimilation, in that they influence both nitrogen acquisition and remobilization, may prove most effective for improving yields.

5. Puzzles—Knowledge Gaps about Modifying Nitrogen Metabolism for Yield Improvement

The current body of literature proffers open questions that require further investigation. In particular, studies that compare homeologs across species, crop responses to different inorganic nitrogen forms, and quantitative genetics underlying crop adaptation to natural soil nitrogen gradients should accelerate yield improvement through modified nitrogen metabolism.

5.1. Differences among Homologs across Species Remain Elusive

Comparative studies among species offer unique insights into finding related genes underlying desirable traits [142] such as for genes involved in C$_4$ carbon fixation [95]. Nevertheless, transfer of successful breeding strategies across species remains challenging, even decades after fully characterizing most elements in nitrogen metabolism pathways [143].

To date, studies have identified more candidate genes and generated more breeding applications related to NUE in rice than in wheat [22,54] (Table 2). Translating insights from rice to wheat require herculean efforts, largely because of differences in genomic size and structure [22,144,145]. New mutant resources [146] and transgenic tools [147], however, increase the feasibility of characterizing candidate genes across a polyploid genome. Novel approaches like CRISPR-Cas9 system also further allows more precise editing of targeted loci of interest [148]. Even cross-species gene modifications such as transforming rice with wheat TaGS1 have proved successful in enhancing rice yield [149].

Multiple yield-determining genes are shared among rice, wheat, maize, and barley [22,150]. Identification of orthologous genes offers an alternative to introducing foreign genetic materials. Here, we discuss three examples: GOGAT, DREB1C and Ghd1. First, GOGAT is well-conserved in rice, wheat, sorghum and maize [137]. Editing transcription factors regulating GOGAT, however, seems more effective than modifying individual genes on their own (see discussion above). Second, rice OsDREB1C, whose overexpression increased yields up to 68.3%, has an ortholog in wheat TaDREB1C, whose overexpression results in 22.6% more grain yield than wildtypes [95]; the reason for the large differences in yield enhancement among species is not yet understood. Third, Ghd7 in rice and its ortholog VRN2 in wheat [86] have a high potential to improve agricultural performance. Both genes are well-studied and control flowering time in their respective species [151–153]. To date, however, there is no clear evidence on how VRN2 integrates signaling from nitrogen into regulation of flowering time in wheat. Given the promising yield enhancements attained with Ghd7 in rice, VRN2 might also provide major increases in wheat yields, but this is still unknown.
5.2. Insights on How Inorganic Nitrogen Forms Affect Crop Responses Are Lacking

Each form of inorganic nitrogen, NH$_4^+$ or NO$_3^-$, triggers specific crop responses [154]. In particular, an exposure to high concentration of soil NH$_4^+$ is generally toxic to most plants because root absorption of NH$_4^+$ may exceed the capacity of the plants to sequester the NH$_4^+$ in vacuoles or assimilate it into organic forms [103]. As free NH$_4^+$ accumulates within plant tissues, it can dissipate pH gradients through which mitochondrial and chloroplastic electron transport generate ATP [1]. To avoid such ill effects, plants generally assimilate NH$_4^+$ in roots and transport organic nitrogen compounds to other organs [103]. Optimizing root NH$_4^+$ accumulation and assimilation can enhance plant NH$_4^+$ tolerance and overall nitrogen acquisition [155]. By contrast, plants can store relatively large amounts of free NO$_3^-$ without ill effect [1], and it serves as major signaling molecules for a number of metabolic pathways [156].

Although the importance of each inorganic form as a nitrogen source in crop production is well established [157], information is still meager on how each form induces or suppresses expression of nitrogen responsive genes or how changes in these genes in turn affect uptake and assimilation of each form. For example, NO$_3^-$ transporter genes have a strong influence on NH$_4^+$ metabolism, and vice versa [158]. A more comprehensive understanding of these interactions would be crucial to designing and implementing more effective nitrogen fertilizer management strategies.

Relatively few studies compare responses to both form of inorganic nitrogen side by side, let alone evaluate the responses to a range of concentrations in diverse genetic materials. Although the model species *Arabidopsis* usually exhibited higher biomass and root production under NO$_3^-$ nutrition, this species showed a wide range of distinct phenotypic responses and gene expression pattern when receiving NO$_3^-$ or NH$_4^+$ as a sole nitrogen source [159]. Wheat growth under either form also demonstrated distinct accumulation and distribution patterns of other essential nutrients [160]. Nonetheless, we do not have sufficient information about the extent to which editing major genes in the nitrogen metabolism pathways changes responses to each inorganic form, and whether responses in wheat and rice are like those observed in *Arabidopsis*.

Most experiments to characterize genes reported in this review have only focused on a single nitrogen form or fail to designate the nitrogen form at all (Table 3). Detailed characterizations of individual nitrogen transporters may show that, not only are they responsible for uptake of both NO$_3^-$ and NH$_4^+$ (for example, NRT 2.3b [64]), but also their functions have expanded and co-evolved to interact with other biotic and abiotic factors [161,162]. For example, NPF6.5 not only regulates NO$_3^-$ uptake, but is also associated with recruitment of root microbes involved in the synthesis of NH$_4^+$ [158]. Understanding balance in crop utilization of both inorganic nitrogen forms will help us improve our crop and fertilization management in response to changing environments [163].
Table 3. Functions, selection, and effects on nitrogen acquisition of nitrogen metabolism genes in rice and wheat that were proven successful in improving yield. Empty cells indicate the lack of information.

<table>
<thead>
<tr>
<th>#</th>
<th>Gene</th>
<th>Ref</th>
<th>Function</th>
<th>Natural Variation and Selection</th>
<th>Effects on NH₄⁺</th>
<th>Effects on NO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NPF6.1</td>
<td>[55]</td>
<td>NO₃⁻ uptake; Must be activated by NAC42 transcriptional factor</td>
<td>Rare allele absent in 90.3% of rice varieties</td>
<td></td>
<td>Increased uptake/concentration</td>
</tr>
<tr>
<td>2</td>
<td>NPF6.3 (NRT1.1A)</td>
<td>[56]</td>
<td>Upregulate N utilization and flowering genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>NPF6.5 (NRT1.1B)</td>
<td>[57,158]</td>
<td>NO₃⁻ uptake, transporter; Upregulate NO₃⁻ responsive genes</td>
<td>Directional positive selection. Indica has a functional variant.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NPF7.1</td>
<td>[58]</td>
<td>Determine axillary bud outgrowth; NO₃⁻ uptake</td>
<td></td>
<td>Increased influx/concentration</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>NPF7.2</td>
<td>[59]</td>
<td></td>
<td>Increased influx/concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NPF7.4</td>
<td>[58]</td>
<td></td>
<td>Increased influx/concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>NPF7.7</td>
<td>[60]</td>
<td></td>
<td>Increased influx/concentration for both variant, Higher for variant 1 only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>NPF8.20 (PTR9)</td>
<td>[61,164]</td>
<td></td>
<td>Increased uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>NRT2.1</td>
<td>[62,63]</td>
<td>High affinity NO₃⁻ transporter; Responsive only to NO₃⁻; Interact with NAR2.1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>NRT2.3b</td>
<td>[64,165]</td>
<td>Buffering pH; NO₃⁻ uptake; Increase NH₄⁺ uptake even though it does not transport NH₄⁺</td>
<td>Under selection. Expression ratio of two variants correlated with vegetative N content.</td>
<td>Increased uptake</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>NRT2.1</td>
<td>[63,65–67,165,166]</td>
<td>NO₃⁻ uptake, interacting with NRT2.1, NRT2.2, NRT2.3a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>AMT1;1</td>
<td>[68,167,168]</td>
<td>NH₄⁺ uptake under low and high NH₄⁺ conditions; N/K homeostasis</td>
<td>Increased uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>AMT1;2</td>
<td>[69,167]</td>
<td>NH₄⁺ uptake and remobilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>GOGAT1</td>
<td>[69]</td>
<td>NH₄⁺ uptake and remobilization Coordinate N metabolic balance and remobilization; Confer tolerance to abiotic stresses; Must be expressed concurrently with GS2.</td>
<td>2 haplotypes in A genome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>GS1</td>
<td>[70,169]</td>
<td>Increase root N uptake before and after flowering, N mobilization and N harvest index; Prolong leaf photosynthesis post-anthesis; Increase expression of NRT2.1 and NPF 6.3. In rice, must be expressed concurrently with GS1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>GS2</td>
<td>[70,71]</td>
<td></td>
<td>2 haplotypes in A genome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>NR2</td>
<td>[72]</td>
<td>Encode NADH/NADPH-dependent NO₃⁻ reductase; Interact with NPF6.5 to control NO₃⁻ uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>ASN1</td>
<td>[73]</td>
<td>Upregulate AMT1;1, AMT1;2, AMT1;3, GS1;1, NADH-GOGAT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>AAP1</td>
<td>[74,170]</td>
<td>Facilitate amino acid transportation to reproductive organs</td>
<td>25 haplotypes. Promoter sequence differs between indica and japonica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>AAP3</td>
<td>[75]</td>
<td>Reduced expression promotes tiller bud elongation, relatively more than formation, via balancing basic and neutral amino acid to maintain higher cytokinin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>AAP4</td>
<td>[76]</td>
<td>Higher expression in indica produce more tiller and grain yield</td>
<td>5 haplotypes. Promoter sequence differs between indica and japonica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>AAP5</td>
<td>[77]</td>
<td>Reduced expression regulate tiller bud via balancing basic and neutral amino acid to maintain higher cytokinin</td>
<td>11 promoter variants. Sequence differs between indica and japonica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#</td>
<td>Gene</td>
<td>Ref</td>
<td>Function</td>
<td>Natural Variation and Selection</td>
<td>Effects on NH(_4^+)</td>
<td>Effects on NO(_3^-)</td>
</tr>
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<td>---</td>
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<tr>
<td>23</td>
<td>NLP1</td>
<td>[78]</td>
<td>Regulate transcription of N related genes and transcriptional factors (both NO(_3^-) and NH(_4^+))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>NLP3</td>
<td>[79]</td>
<td>Bind to NO(_3^-) -responsive cis-elements in promoters of N uptake and assimilation genes; Overlaps with NLP1 and NLP4</td>
<td>Regulate expression of known N genes by binding to NO(_3^-) - responsive cis-element in promoter, Activate NiR</td>
<td>2 haplotypes</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>NLP4</td>
<td>[80,81]</td>
<td>Counteracts DELLA to promote N assimilation both NO(_3^-) and NH(_4^+); Upregulate expression of AMT1.1, GS1.2, GS2, NADH-GOGAT2, NRT1.1B, NRT2.3a, NPF2.4, NIA1, NIA3, NIR1 and genes related to photosynthesis, C metabolism and cell division to maintain stable C:N ratio; Highest expression at low N; Recruit PRC2 upon increased N supply to promote H3K27me3 modification that represses shoot branching inhibitory genes; DELLA proteins stabilize NGR5 and sustain tiller promotion by competitively inhibiting gibberillin-driven destruction of NGR5.</td>
<td>3 haplotypes.</td>
<td>Increased uptake</td>
<td>Increased uptake</td>
</tr>
<tr>
<td>26</td>
<td>GRF4</td>
<td>[42]</td>
<td>Regulate expression of NO(_3^-) - transporter and GS; Activate NPF6.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>NGR5</td>
<td>[82]</td>
<td>Repress tiller promoting gene DLT, the product of which can interact directly with NGR5, to negatively control cellular bud growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>TCP19</td>
<td>[83]</td>
<td>Regulate expression of NO(_3^-) transporter and GS; Activate NPF6.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>NAC42</td>
<td>[55]</td>
<td>Negative regulation on NADH-GOGAT usage</td>
<td>No effects reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>bZIP60</td>
<td>[85]</td>
<td>Regulate expression of NT2, NRT2.4, NPF6.5, NRT2.3a, NPF2.4, NIA2.</td>
<td>3 haplotypes.</td>
<td>Increased uptake</td>
<td>Increased uptake</td>
</tr>
<tr>
<td>31</td>
<td>Ghd7</td>
<td>[86,171]</td>
<td>Repress ARE1 to positively regulate N utilization; Under selection</td>
<td>No effects reported</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>ARE1</td>
<td>[87,88]</td>
<td>Suppress Fd-GOGAT; Activate Hdt3a for flowering time; Control negative feedback on N assimilation (loss-of-function increases Fd-GOGAT and LHT1 activities); Activate AMT1.3, NRT2.4</td>
<td>At least 10 allelic variants. Allelic frequency correlates with N deposition rate.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Nhd1</td>
<td>[89,90]</td>
<td>Negative regulator of auxin-regulated N metabolism; N supply lowers DNR1, thereby inducing Auxin Response formation; Upregulate GS/GOGAT usage</td>
<td>3 haplotypes.</td>
<td>Increased uptake</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>DEP1</td>
<td>[91–93,172]</td>
<td>Negative regulator of auxin-regulated N metabolism; N supply lowers DNR1, thereby inducing Auxin Response formation; Upregulate GS/GOGAT usage</td>
<td>Under selection during japonica domestication.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>DREB1C</td>
<td>[95]</td>
<td>Factors to upregulate NPF6.5, NRT2.3a, NPF2.4, and NIA2.</td>
<td>3 haplotypes.</td>
<td>Increased uptake</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>DREB1C</td>
<td>[95]</td>
<td>Regulate NT2, NRT2.4, NPF6.5</td>
<td>3 haplotypes in promoter sequence.</td>
<td>Increased uptake</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>NFYA- B1</td>
<td>[96]</td>
<td>Control root development and N, P usage</td>
<td>3 haplotypes in promoter sequence.</td>
<td>Increased uptake</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>RDD1</td>
<td>[97]</td>
<td>Upreregulate AMT1.3, GS1.1; Uptake of N, P, K, Na, Mg, Cl, S, Ca</td>
<td>Highly conserved in wild rice relatives.</td>
<td>Increased uptake/accumulation</td>
<td>Increased uptake</td>
</tr>
<tr>
<td>39</td>
<td>MIR396</td>
<td>[98]</td>
<td>Only isoform e and f; Upregulate GRF4, GRF6, GRF8, NIP1, NIP2, GOGAT2, GS1.2, AAPs</td>
<td>Increased uptake/accumulation</td>
<td>Increased uptake</td>
<td></td>
</tr>
</tbody>
</table>

Nitrogen interacts more strongly with carbon assimilation as nitrogen supplies limit crop responses to enriched atmospheric CO\(_2\) levels [173]. Meta-analyses demonstrate that nutritional quality of wheat and rice—especially protein and micronutrients such as iron and zinc—decline significantly under elevated CO\(_2\) levels [174]. Among several alternative explanations for the declining crop protein at elevated CO\(_2\) levels [175–178],
direct inhibition of shoot nitrogen assimilation [179] is most consistent with observations under a wide range of experimental conditions [180–182].

Photorespiration provides energy for shoot NO\textsubscript{3}\textsuperscript{−} assimilation in C\textsubscript{3} plants [3]. Photorespiration generates reductants when atmospheric CO\textsubscript{2}, but not light levels, limits photosynthesis and enables C\textsubscript{3} plants to convert low energy nitrogen sources that most other organisms avoid like NO\textsubscript{3}\textsuperscript{−} into organic nitrogen compounds. This confers an evolutionary advantage to C\textsubscript{3} plants, which remain dominant among plant species [3]. Under the current rapid surge in atmospheric CO\textsubscript{2} level, a condition which slows photorespiration, C\textsubscript{3} species using NO\textsubscript{3}\textsuperscript{−} as a nitrogen source suffer most from decreased organic nitrogen production [179,183]. N\textsubscript{2}-fixing legumes and C\textsubscript{4} plants with CO\textsubscript{2} concentrating mechanisms, are more resilient to changes in CO\textsubscript{2} [174] because their inorganic nitrogen acquisition does not depend on photorespiration.

The use of NH\textsubscript{4}\textsuperscript{+}-based nitrogen fertilizer and breeding for genotypes with improved NH\textsubscript{4}\textsuperscript{+} assimilation and tolerance may offer a solution for sustaining plant protein levels under future CO\textsubscript{2}-enriched atmospheres [184,185]. Biological Nitrification Inhibitors (BNI), which allow certain plant species to regulate their rhizosphere pools of inorganic nitrogen by releasing root exudates that specifically inhibit nitrifying bacteria that convert NH\textsubscript{4}\textsuperscript{+} into NO\textsubscript{3}\textsuperscript{−}, may be beneficial [186]. Application of artificial BNI chemicals or incorporation of this trait into new cultivars may enhance crop growth under NH\textsubscript{4}\textsuperscript{+} nutrition [185,187].

Surprisingly, given the chemical differences between NO\textsubscript{3}\textsuperscript{−} and NH\textsubscript{4}\textsuperscript{+}, relatively little is known about how various nitrogen supplies shape crop adaptation and yield in a field setting (Table 3; see discussion below). The ability of the current germplasm to employ a specific nitrogen form as their predominant nitrogen source and maintain productivity at elevated CO\textsubscript{2} levels thus remains an open question.

5.3. Little Information about Natural Genetic Variation and Genome-Wide Interactions Limits Breeding Applications

Despite an increasing understanding of physiological adaptation of roots and shoot to nitrogen supply [122], less is known about genetic adaptations [188]. Recent advances in genetic approaches greatly facilitate the identification of genes responsible for specific physiological traits. Of particular interest are Genome Wide Association Studies (GWAS) that use extensive sets of molecular markers to explore genetic variation resulting from historical recombinant events and from adaptation to changes in environmental conditions over evolutionary time [189]. Genetic architecture of traits also strongly influences GWAS robustness such that traits with rare alleles are more difficult to identify [190,191].

To date, a combination of GWAS and linkage mapping have identified many loci that underlie nitrogen responses of agricultural crops [192,193]. Importantly, GWAS enables deeper understanding of how environments may have shaped crop adaptation [194]. As such, natural variation of functional alleles can help inform breeding applications to achieve a better match between genotype and location [195]. Haplotype analyses in global germplasm quantifies allelic frequency of different breeding and natural subpopulations [196] and can offer practical strategies in breeding programs [197].

Selection pressures that vary during the history of crop domestication or with local limiting growth factors [198] provide insights into crop evolution and adaptation. More commonly, studies focus on differences between major subpopulations and how selection drives divergence or convergence between them. For example, divergence between the indica and japonica subpopulations of rice can be accounted by variations in key nitrogen metabolism genes like NPF6.5 [57] or NR2 [72]. Around 8% of the rice genome covering major nitrogen metabolism genes appear to be under selection including AMT1.1, NRT2.3, NAR2.2, NIR1, GS1;2, and GS1;3 [199]. Unfortunately, information is limited about the natural variation in candidate genes that enhance yield and the extent to which they have been under selection (Table 3). For instance, OsNPF6.1, which was identified through GWAS and functions under low nitrogen supply to increase NO\textsubscript{3}\textsuperscript{−} uptake, is considered a rare allele, because it is present in less than 10% of cultivated varieties [55]. The absence or
presence of a functional allele from diverse geographic regions may reflect adaptation to a particular soil nitrogen pool.

Apart from a few studies [83,86], we have limited information on the extent to which natural soil nitrogen availability shapes crop adaptation and, in turn, on subsequent responses to external nitrogen fertilizers in agricultural production systems. For example, *Ghd7* allelic variation also correlates with soil nitrogen deposition rates [86]. Likewise, rice *OsTCP19*, which was identified through a GWAS on tiller responsiveness to nitrogen availability, has a functional allele frequency that is correlated with soil nitrogen concentration, and the nitrogen-responsive genotypes are more common in regions with low nitrogen concentrations [83]. Extensive networks of genes interact to sense and signal perception of nitrogen, especially NO$_3^-$ [106]. Interestingly, expression of *OsTCP19* follows changes in NO$_3^-$, but not NH$_4^+$ [83]. Overall, evidence is insufficient to conclude whether crops like wheat and rice, which have been exposed over the long term to certain nitrogen forms, show adaptation to a particular form. This information is vital for applying robust breeding strategies to improve future crops.

Genetic × environment interactions and expression patterns contingent upon growth conditions influence phenotypic plasticity [200,201], even when the same genes are being modified. Specifically, some genes may only be beneficial in certain environments or may even have detrimental pleiotropic effects in others. Field trials indicate that yield enhancement is highly dependent on growing conditions. For example, overexpression of *ASN1* enhanced rice grain yield in pot experiments under limited nitrogen supply, but had no observable effect under sufficient nitrogen supply in the field [73]. In sites with a longer growing season, *DREB1C* transgenic plants exhibited a much higher yield boost compared to wildtypes [95]. With more advanced molecular breeding and transgenic approaches, promoters inducible in specific tissues or by desirable environmental triggers could perhaps mitigate such issues [12,121]. Precision genome editing methods, like the CRISPR-Cas9 system, facilitate genetic modifications at multiple target tissues, developmental times, and traits all at once without the introduction of foreign genetic materials [202]. Furthermore, advanced GWAS pipelines allow more explicit consideration of environmental variations to quantify plasticity and predict phenotype in a particular environment [203]. Better understanding of crop genetics, yield components, and their responses to the environment should bridge the gap between improved nitrogen metabolism and yield improvement.

Epistatic interactions further complicate breeding for candidate genes in different genetic backgrounds [204]. Gene or trait stacking based on our current understanding of each individual gene, protein, or process have had limited success to date, perhaps because of too little understanding of the complex regulatory network [205]. For example, the introduction of the grain protein content *NAM-B1* transcription factor functional allele, which is generally absent from modern varieties, has only minimal influence on yield, but enhances grain nitrogen and protein content significantly across a wide range of environments [26,206,207]. A meta-analysis across 40 environments showed that 19% of bread wheat genotypes with *NAM-B1* functional alleles exhibit yield enhancement [207], suggesting that the global germplasm still has genetic yield potential. Furthermore, combining multiple nitrogen metabolism genes in the pathway, for example *NR2* and *NPF6.5* [72], or *AMT1;2* with *GOGAT* [69] offers greater chance of yield enhancement than modulating individual genes alone. These observations argue for manipulating either gene networks with multiple genes of relatively small effects or transcription factors that affect several genes and processes at the same time. Further understanding of system biology, especially underlying nitrogen metabolism, should prove useful in guiding such manipulations.

6. Concluding Remarks

Both yield and nitrogen metabolism pathways are complex traits with multiple layers of genetic control. While actual farm yield has increased in some regions of the world, increases in cereal potential yield—the scenario with no limitation on crop growth—have
fallen down to below 1% annually [7]. We urgently need to apply new breeding strategies that accelerate genetic gains to meet the demands of our growing human population.

Here, we considered NUE on the basis of grain and total biomass production per unit of nitrogen applied or assimilated. Improvement of nitrogen acquisition, however, does not always translate into higher yields. For example, overexpression of transporter \( \text{NPF7.4} \) resulted in higher \( \text{NO}_3^- \) uptake, lower \( \text{NO}_3^- \) accumulation, but higher tissue amino acid concentration, indicating improved nitrogen assimilation; nevertheless, such enhanced nitrogen acquisition decreased biomass and grain production [58]. Knocking out \( \text{Lysine-Histidine-type Transporter 1 (LHT1)} \), which transports amino acid, helped improve grain nutritional quality at maturity, but at the expense of vegetative biomass, grain weight, and germination rate [208,209]. Henceforth, defining and setting NUE as breeding targets to lower agricultural nitrogen inputs must take into account grain protein content [210,211]. These efforts are prime candidates for improving grain nutritional values. Therefore, if we define NUE as the amount of organic nitrogen that ends up in the consumable grains per nitrogen applied, these genes are worthy of consideration.

Breeding strategies that focus concurrently on both carbon and nitrogen assimilation also offer an opportunity to break the longstanding antagonistic relationship between grain biomass and protein concentration [212] that hampers genetic gains in yield over time. Genetic solutions are needed because management practices like applications of nitrogen fertilizers at booting stage to meet grain nitrogen demand can only partially alleviate this negative relationship at the field level [213]. Control of \( \text{NGR5} \) that uncouples yield components from nitrogen-dependent responses [82], or \( \text{GRF4} \) that breaks the tie between dwarfism-induced yield improvement and reduced nitrogen assimilation [42], establish the possibility of maximizing both yield and NUE at the same time. Genomic selection is theoretically feasible and genomic breeding tools are becoming readily available for breeders to target both sets of traits simultaneously [197,214].

7. Conclusions

This review highlights achievements in manipulating the genetics underlying nitrogen metabolism pathways to enhance yield of rice and wheat, focusing on relationships between yield components and crop nitrogen use during growth. Further fundamental understanding of ortholog genes between species, how different forms of nitrogen influence growth and development, and natural variation of desirable traits responsive to nitrogen should prove useful in achieving higher crop yields. Hopefully, continuous, albeit slow, progress on genetic gain in crop nitrogen assimilation and yield over time can fulfill the yield gap needed to feed our global community.

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References


2. Ellis, R.J. The Most Abundant Protein in the World. Trends Biochem. Sci. 1979, 4, 241–244. [CrossRef]


27. Teng, W.; He, X.; Tong, Y. Genetic Control of Efficient Nitrogen Use for High Yield and Grain Protein Concentration in Wheat: A Review. Plants 2022, 11, 492. [CrossRef] [PubMed]


49. Lee, S. Recent Advances on Nitrogen Use Efficiency in Rice. *Agronomy* 2021, 11, 753. [CrossRef]


63. Yan, M.; Fan, X.; Feng, H.; Miller, A.J.; Shen, Q.; Xu, G. Rice OsNAR2.1 Interacts with OsNRT2.1, OsNRT2.2 and OsNRT2.3a Nitrate Transporters to Provide Uptake over High and Low Concentration Ranges: Rice Two Component Nitrate Transport. *Plant Cell Environ.* 2011, 34, 1360–1372. [CrossRef]


76. Fang, Z.; Bu, B.; Ji, Y. The Amino Acid Transporter OsAAP4 Contributes to Rice Tilling and Grain Yield by Regulating Neutral Amino Acid Allocation through Two Splicing Variants. *Rice* 2021, 14, 2. [CrossRef]


202. Gao, C. Genome Engineering for Crop Improvement and Future Agriculture. *Cell* 2021, 184, 1621–1635. [CrossRef]


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