UC Davis UC Davis Previously Published Works

Title

Nitrogen Challenges and Opportunities for Agricultural and Environmental Science in India

Permalink

https://escholarship.org/uc/item/3dd4f3tj

Authors

Móring, Andrea Hooda, Sunila Raghuram, Nandula <u>et al.</u>

Publication Date

2021

DOI

10.3389/fsufs.2021.505347

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at https://creativecommons.org/licenses/by/4.0/

Peer reviewed



Nitrogen challenges and opportunities for agricultural and environmental science in India

Andrea Móring^{1*}, Sunila Hooda², N. Raghuram², Tapan K. Adhya³, Altaf Ahmad⁴, Sanjoy K. Bandyopadhyay⁵, Tina Barsby⁶, Gufran Beig⁷, Alison Bentley⁶, Arti Bhatia⁵, Ulrike Dragosits⁸, Julia Drewer⁸, John Foulkes⁹, Sachin D. Ghude⁷, Rajeev Gupta¹⁰, Niveta Jain⁵, Dinesh Kumar⁵, R. M. Kumar¹¹, Jagdish K. Ladha¹², Pranab K. Mandal¹³, C. N. Neeraja¹¹, Renu Pandey⁵, Himanshu Pathak¹⁴, Pooja Pawar^{7, 15}, Till K. Pellny¹⁶, Philip Poole¹⁷, Adam Price¹⁸, D.L.N. Rao¹⁹, David S. Reay¹, N. K. Singh¹³, Subodh K. Sinha¹³, Rakesh K. Srivastava¹⁰, Peter Shewry¹⁶, Jo Smith¹⁸, Claudia E. Steadman^{1*}, Desiraju Subrahmanyam¹¹, Kuchi Surekha¹¹, Karnam Venkatesh²⁰, Varinderpal Singh²¹, Aimable Uwizeye^{22, 23}, Massimo Vieno⁸, Mark A. Sutton⁸

¹University of Edinburgh, United Kingdom, ²Guru Gobind Singh Indraprastha University, India, ³KIIT University, India, ⁴Aligarh Muslim University, India, ⁵Indian Agricultural Research Institute (ICAR), India, ⁶National Institute of Agricultural Botany (NIAB), United Kingdom, ⁷Indian Institute of Tropical Meteorology (IITM), India, ⁸UK Centre for Ecology and Hydrology (UKCEH), United Kingdom, ⁹University of Nottingham, United Kingdom, ¹⁰International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, ¹¹Indian Institute of Rice Research (ICAR), India, ¹²University of California, Davis, United States, ¹³National Research Centre on Plant Biotechnology, Indian Council of Agricultural Research (ICAR), India, ¹⁴National Rice Research Institute (ICAR), India, ¹⁵Shivaji University, India, ¹⁶Rothamsted Research, United Kingdom, ¹⁷University of Oxford, United Kingdom, ¹⁸University of Aberdeen, United Kingdom, ¹⁹Indian Institute of Soil Science (ICAR), India, ²⁰Indian Institute of Wheat and Barley Research (ICAR), India, ²¹Punjab Agricultural University, India, ²²Food and Agriculture Organization of the United Nations (Italy), Italy, ²³Wageningen University and Research, Netherlands

Submitted to Journal: Frontiers in Sustainable Food Systems

Specialty Section: Waste Management in Agroecosystems

Article type: Policy and Practice Reviews Article

Manuscript ID: 505347

Received on: 16 Oct 2019

Revised on: 27 Sep 2020

Frontiers website link: www.frontiersin.org



Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

AM led the formulation, coordination and writing of the manuscript. All authors contributed to manuscript content and revision.

Keywords

Nitrogen, nitrogen use efficiency, Indian agriculture, nitrogen management, fertilizer

Abstract

Word count: 252

In the last six decades, the consumption of reactive nitrogen (Nr) in the form of fertiliser in India has been growing rapidly, whilst the nitrogen use efficiency (NUE) of cropping systems has been decreasing. These trends have led to increasing environmental losses of Nr, threatening the quality of air, soils and fresh waters, and thereby endangering climate-stability, ecosystems and human-health. Since it has been suggested that the fertiliser consumption of India may double by 2050, there is an urgent need for scientific research to support better nitrogen management in Indian agriculture. In order to share knowledge and to develop a joint vision, experts from the UK and India came together for a conference and workshop on "Challenges and Opportunities for Agricultural Nitrogen Science in India". The meeting concluded with three core messages: 1) Soil stewardship is essential and legumes need to be planted in rotation with cereals to increase nitrogen fixation in areas of limited Nr availability. Synthetic symbioses and plastidic nitrogen fixation are possibly disruptive technologies, but their potential and implications must be considered. 2) Genetic diversity of crops and new technologies need to be shared and exploited to reduce N losses and support productive, sustainable agriculture livelihoods. 3) The use of leaf colour sensing shows great potential to reduce nitrogen fertiliser use (by 10 - 15%). This, together with the usage of urease inhibitors in neem-coated urea, and better management of manure, urine and crop residues, could result in a 20 - 25% improvement in NUE of India by 2030.

Contribution to the field

The consumption of reactive nitrogen (Nr) in the form of fertiliser in India has been growing rapidly, whilst the nitrogen use efficiency (NUE) of cropping systems has been decreasing. These trends have led to increasing environmental losses of Nr, threatening the quality of air, soils and fresh waters, and thereby endangering climate-stability, ecosystems and human-health. Fertiliser consumption in India may double by 2050. There is therefore an urgent need for scientific research to support better nitrogen management in Indian agriculture, a topic that is highly relevant to several specialties within Sustainable Food Systems: Crop Biology and Sustainability; Nutrition and Sustainable Diets; Waste Management in Agroecosystems; and Land, Livelihoods and Food Security. We provide an overview of the policy implications and actionable recommendations which emerged from the conference "Challenges and Opportunities for Agricultural Nitrogen Science in India". Soil stewardship is essential and legumes need to be planted in rotation with cereals to increase nitrogen fixation. Genetic diversity of crops and new technologies including leaf colour sensing and urease inhibitors need to be shared to reduce N losses and support productive, sustainable agriculture livelihoods. Together, this could result in a 20 - 25% improvement in NUE of India by 2030.



Nitrogen challenges and opportunities for agricultural and environmental science in India

- 1 Andrea Móring^{1*}, Sunila Hooda², N. Raghuram², Tapan Kumar Adhya³, Altaf Ahmad⁴, Sanjoy
- 2 K. Bandyopadhyay⁵, Tina Barsby⁶, Gufran Beig⁷, Alison R. Bentley⁶, Arti Bhatia⁵, Ulrike
- 3 Dragosits⁸, Julia Drewer⁸, John Foulkes⁹, Sachin D. Ghude⁷, Rajeev Gupta¹⁰, Niveta Jain⁵,
- 4 Dinesh Kumar⁵, R. Mahender Kumar¹¹, Jagdish K. Ladha¹², Pranab Kumar Mandal¹³, C.N.
- 5 Neeraja¹¹, Renu Pandey⁵, Himanshu Pathak¹⁴, Pooja Pawar^{7,15}, Till K. Pellny¹⁶, Philip Poole¹⁷,
- 6 Adam Price¹⁸, D.L.N. Rao¹⁹, David S. Reay¹, N.K. Singh¹³, Subodh Kumar Sinha¹³, Rakesh K.
- 7 Srivastava¹⁰, Peter Shewry¹⁶, Jo Smith¹⁸, Claudia E. Steadman^{1*}, Desiraju Subrahmanyam¹¹,
- 8 Kuchi Surekha¹¹, Karnam Venkatesh²⁰, Varinderpal-Singh²¹, Aimable Uwizeye^{22,23}, Massimo
- 9 Vieno⁸ and Mark A. Sutton⁸
- ¹University of Edinburgh, High School Yards, Edinburgh, EH8 9XP, UK
- ²Guru Gobind Singh Indraprastha University, ARL 112, School of Biotechnology, Dwarka, Sector
- 12 16c, New Delhi, 110078, India
- 13 ³KIIT University, Bhubaneswar, 751024, India
- ⁴Aligarh Muslim University, Department of Botany, Aligarh, 202002, India
- ⁵Indian Agricultural Research Institute, CESRA, Pusa Campus, New Delhi, 110012, India
- ⁶National Institute of Agricultural Botany, Huntington Road, Cambridge, CB3 0LE, UK
- ¹⁷ ⁷Indian Institute of Tropical Meteorology, Dr. Homi Bhabha Road, Pune, 411008, India
- ¹⁸Centre for Ecology and Hydrology, Bush Estate, Penicuik, EH26 0QB, UK
- ⁹University of Nottingham, Division of Plant and Crop Sciences, School of Biosciences,
- 20 Loughborough, LE12 5RD, UK
- ¹⁰International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Hyderabad, 502324,
 India
- ²³ ¹¹Indian Institute for Rice Research, Rajendra Nagar, Hyderabad, 500030, India
- ²⁴ ¹²University of California Davis, 1 Shields Avenue, Davis, 95616, USA
- ¹³National Research Centre on Plant Biotechnology, New Delhi 110012, India
- 26 ¹⁴National Rice Research Institute, Odisha, Cuttack, 753006, India
- 27 ¹⁵Shivaji University, Department of Technology, Vidya Nagar, Kolhapur, 416004, India
- 28 ¹⁶Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK
- ²⁹ ¹⁷University of Oxford, Department of Plant Sciences, South Parks Road. Oxford, OX1 3RB, UK
- ¹⁸University of Aberdeen, School of Biological Science, 23 St Machar Drive, Aberdeen, AB24 3UU,
 UK
- ³² ¹⁹Indian Institute of Soil Science, Nabi Bagh, Berasia Road, Bhopal, 462038, India
- ²⁰Indian Institute of Wheat and Barley Research, Karnal, 132001, India

- ²¹Punjab Agricultural University, Department of Soil Science, Ludhiana, 142022, India
- ²²Food and Agriculture Organization of the United Nations, Animal Production and Health Division,
- 36 Viale delle Terme di Caracalla, Rome 00153, Italy
- ²³Animal Production Systems Group, Wageningen University & Research, PO Box 338, 6700 AH
- 38 Wageningen, the Netherlands

39 *** Correspondence:**

- 40 Andrea Móring
- 41 Andrea.Moring@ed.ac.uk
- 42 Claudia E. Steadman
- 43 Claudia.Steadman@ed.ac.uk

Keywords: Indian agriculture, nitrogen, nitrogen management, nitrogen use efficiency, fertilizer

46 Abstract

47 In the last six decades, the consumption of reactive nitrogen (N_r) in the form of fertiliser in India has 48 been growing rapidly, whilst the nitrogen use efficiency (NUE) of cropping systems has been 49 decreasing. These trends have led to increasing environmental losses of Nr, threatening the quality of 50 air, soils and fresh waters, and thereby endangering climate-stability, ecosystems and human-health. 51 Since it has been suggested that the fertiliser consumption of India may double by 2050, there is an urgent need for scientific research to support better nitrogen management in Indian agriculture. In 52 53 order to share knowledge and to develop a joint vision, experts from the UK and India came together for a conference and workshop on "Challenges and Opportunities for Agricultural Nitrogen Science 54 55 in India". The meeting concluded with three core messages: 1) Soil stewardship is essential and 56 legumes need to be planted in rotation with cereals to increase nitrogen fixation in areas of limited Nr 57 availability. Synthetic symbioses and plastidic nitrogen fixation are possibly disruptive technologies,

- 58 but their potential and implications must be considered. 2) Genetic diversity of crops and new 59 technologies need to be shared and exploited to reduce N losses and support productive, sustainable
- technologies need to be shared and exploited to reduce N losses and support productive, sustainable agriculture livelihoods. 3) The use of leaf colour sensing shows great potential to reduce nitrogen
- fertiliser use (by 10 15%). This, together with the usage of urease inhibitors in neem-coated urea,
- and better management of manure, urine and crop residues, could result in a 20 25% improvement
- 63 in NUE of India by 2030.

64 **1** Introduction

65 The global nitrogen (N) cycle has been strongly altered by human intervention, leading to rapidly

- 66 growing environmental concerns that increase in parallel with the rise in food demand fuelled by the
- 67 expanding global human population. To feed humanity, N must be in a reactive form (N_r), such as
- 68 ammonium and nitrate, which are essential to support crop production. However, such N_r compounds
- 69 are sparse in the environment. Therefore, to satisfy the global need for N_r, large quantities of
- 70 unreactive atmospheric nitrogen gas (N₂) is converted to N_r as a base of fertilisers. A major step
- 71 toward this goal was the invention of the Haber-Bosch process, producing ammonia (NH₃) from its
- elements (N₂ and hydrogen gas), doubling the world's N_r flows since its invention in the early 20^{th}
- 73 century (Galloway et al., 2003).

- Although the production and usage of fertilisers provides N_r to help feed the population, it poses a
- respectively N_r series of interconnected environmental issues. The root of the problem is that only a proportion of N_r
- content in applied fertilisers is used effectively by crops and grasslands. Therefore, the 'nitrogen use
- efficiency' (NUE) of the global food system integrating crop and livestock production systems is
- very low, being around 15% (Sutton et al., 2017 based on Sutton et al., 2013). This means that 85%
- of N_r applied is lost to air, groundwater or surface runoff, threatening the quality of air, soils and fresh waters, driving climate change, and thereby endangering ecosystems and human-health
- fresh waters, driving climate change, and thereby endangering ecosystems and human-l
 (Galloway et al., 2008b, Aneja et al., 2009, Sutton et al., 2011, Fowler et al., 2013).
- 82 In India, fertiliser consumption shows a continuously growing trend, with a yearly increase of 6%
- since 1970 (Sutton et al., 2017 based on FAO, 2016).. This can be observed on Figure 1, which
- 84 shows the annual fertiliser consumption in India in comparison with population (showing a much
- 85 weaker annual growth rate), and the annual GDP (showing a closer, but much clearer exponential
- 86 growth over the years). This increased fertiliser consumption has resulted in growing losses of N_r, of
- 87 which one of the clearest indicators is the annual emission of ammonia (NH₃) the predominant
- 88 fertiliser-related atmospheric N_r loss with a growing trend in the last six decades (EDGAR, 2016).
- 89 The environmental implications, as well as the health impacts of the increasing environmental losses
- 90 of N_r in India, have been subjects of growing scientific interest (Aneja et al., 2006, Abrol et al., 2007,
- 91 Galloway et al., 2008a, Bijay-Singh and Yadvinder-Singh, 2008).



- 92
- Figure 1. The annual nitrogen fertiliser consumption in India (Mt; Fertilizer Association of India,
 2020) together with the annual GDP (10⁹ current USD; World Bank, 2020a) and population (10⁶
 people, World Bank, 2020b) of India.

96 While the consumption of fertilisers has been increasing in India, the NUE of cropping systems

- 97 (expressed here as N in harvested arable products divided by fertiliser inputs) has been gradually
- decreasing, dropping from about 55% to as low as 35% between 1960 and 2010 (Bijay-Singh, 2017
- 99 based on Lassaletta, 2014). Although Fig. 1 suggests that fertiliser consumption may have stabilized
- 100 since 2010, the latest projections of the Food and Agriculture Organizations of United Nations (FAO,

- 101 2018) suggests that India may double its fertiliser input by 2050. Based on this, it is evident that for
- further sustainable development, a better scientific understanding of N_r flows and losses as well as
- 103 improved technologies to increase NUE are needed.
- 104 A step toward this goal was taken when the BBSRC (Biotechnological and Biological Sciences
- 105 Research Council, UK), DBT (Department of Biotechnology, India) and NERC (Natural
- 106 Environment Research Council, UK) agreed to fund virtual joint centres (VJCs) to investigate the
- 107 ways of managing agricultural N, improving crop production while reducing energy inputs and N_r
- 108 losses to the environment. To achieve these goals, they identified three key options:
- the enhancement of nitrogen-fixation in legumes and introducing its biological nitrogen fixation (BNF) potential to non-leguminous cropping systems,
- 111 2) the improvement of NUE at a plant level,
- 112 3) the improvement of NUE through agronomical practices.
- 113 To support and further implement these alternatives, four VJCs were established in 2016, linking the 114 leading N research communities of India and the UK. These four virtual centres are:
- CINTRIN (Cambridge-India Network for Translational Research in Nitrogen), which aims to reduce the use of agricultural N by exploiting native variation and plant genetics and informing on-farm decision support;
- INEW (Indo-UK Centre for The Improvement of Nitrogen Use Efficiency in Wheat), which investigates the genetic control and biochemical basis of NUE in wheat as well as precision application of N fertilisers;
- IUNFC (India-UK Nitrogen Fixation Centre), which focuses on pigeon pea rhizobial N
 fixation and contribution of endophytes in N nutrition of rice; and
- NEWS India-UK (Newton-Bhabha Virtual Centre on Nitrogen Efficiency of Whole-cropping Systems for improved performance and resilience in agriculture), which explores the options for increasing NUE and better planning of the whole crop rotation, and subsequently aims to apply the new biological and agronomic advances to the farm scale as well as to national scale.
- 128 In October 2017, a joint conference and workshop, entitled "Challenges and Opportunities for
- 129 Agricultural Nitrogen Science in India" was organised at the National Agricultural Science Complex
- 130 (NASC), in New Delhi. At the event, 85 experts came together (see the list of participants in Table
- 131 S1 of the Supplementary Material) from the four virtual joint centres to share findings in order to
- 132 stimulate cooperation and to develop a long-term vision for N research and its policy applications in
- 133 India. The primary aim of this paper is to summarise the outcomes of this conference and workshop.
- 134 The event was organised around three topics: BNF, biological NUE, and agronomic NUE including
- 135 the environmental impacts. In the first part, at the conference, some of the latest results were
- presented. This was followed by a workshop where participants were working in three groups,
- 137 corresponding to the three topics of the event. Each group had to discuss and report their answers to
- 138 the same set of questions:

- What are the key findings?
- 140 What are the common challenges?
- 141 What are the future and science cooperation priorities?
- 142 Are there key messages for the South Asian Nitrogen Assessment?
- What are the 2 or 3 best options emerging for better nitrogen management for your topic?
- How would you formulate a global goal for each option for 2030?
- What could each option realistically achieve (quantitatively) in terms of efficiency
 improvement or nitrogen saving by 2030?
- 147 What are the main messages for current and future policies?
- 148 What would be your group's vision statement?
- 149 The findings of each group were then reported in plenary to the other groups, allowing revision and
- agreement by the conference as a whole. In this paper we firstly provide an overview of the main
- 151 findings drawing on the findings of the groups according to the questions given. Secondly, we
- summarise the recommendations of the working groups for options for better N management in the
- 153 Indian agriculture, and present a long-term vision for action. Each of these sections is organised
- around the three main topics of the conference. Finally, we conclude with the key vision statements
- agreed by the conference, encapsulating the core messages of the meeting.
- 156 NUE can be defined and measured in many different ways depending on the most accepted norms in
- 157 each discipline (Raghuram and Sharma, 2019) and each of these measurements are precise in their
- 158 own way. As such, different projects and their partners approached NUE at different levels using
- 159 different tools based on their expertise and approved within the work packages of their VJCs. While
- 160 harmonizing these different approaches of NUE towards a universally accepted definition or
- 161 measurement is highly desirable, it is beyond the scope of this manuscript. Therefore, where no
- detailed explanation is provided for the calculation of NUE, we use the term NUE here as a general
- 163 concept, in which we consider a change as "increase" if the output N grows (total plant N, grain N;
- biomass yield or grain yield) or N loss decreases (via atmospheric emission or leaching of nitrogen
- 165 compounds) at a given N input level (total N, soil N or N-fertilizer applied). We consider a change in
- 166 NUE as "decrease" if the opposite is true. Biological NUE and agronomic NUE should be understood
- 167 generally, as NUE on plant level and on the level of an agronomic system, respectively.

168 **2** Key findings of the Virtual Joint Centres

169 2.1 Biological nitrogen fixation (BNF)

- 170 The focus of this study was pigeon pea because it is the most important pulse crop in summer in the 171 majority of India's drylands. It constitutes 15% of pulse area grown in 3.9 million ha, with an average grain yield of only 850 kg ha⁻¹ (dry matter), fixing about 0.22 Tg N yr⁻¹ out of a total legume 172 BNF of 0.94 Tg yr⁻¹ (Rao and Balachandar, 2017). Since BNF is limited by drought and poor 173 174 nodulation, improvement strategies were based on identifying tolerant pigeon pea genotypes and 175 Rhizobium strains that have greater tolerance to environmental stresses in the field (Trotman and 176 Weaver 1995). This strategy allowed us to identify the best combinations. Using standard agronomic practices, the principal objective was to improve nodulation and select for sustained nodulation since 177 178 nodulation and N fixation in pigeon pea declines at early stages with little activity at flowering stage 179 (Kumar Rao and Dart 1987). The improvement of soil biology by inoculation with plant growth-180 promoting bacteria and rhizobia, as well as quantitative assessment of BNF rates, are also a priority. 181 Further research is also needed on the benefits of microbial inoculation of rice to increase BNF and
- 182 other nutrient acquisition. Rice harbours several endophytes, including non-N fixing bacteria as well

- as rhizobia, and inoculation of these has been shown to improve rice growth (Ladha et al., 2016 and
- 184 Chalk et al., 2017). Thus, there is a need to improve endophytic BNF and downstream assimilation of
- 185 resulting N_r by rice plants.
- 186 Within IUNFC, a number of rhizobial strains of nodulating pigeon pea in farmers' fields have been
- 187 isolated from three different soil types in India where pigeon pea is widely cultivated: *Inceptisols* of
- 188 North India, *Vertisols* of Central India and *Alfisols* of Peninsular India. A broad range of mainly
- 189 slow-growing and some fast-growing rhizobia were isolated, characterized for their nodulation
- efficiency (Fig. 2) and sequenced for the 16S rRNA gene. Nearly 25 species of rhizobia have been
- detected in three Indian soils belonging to six genera, *Bradyrhizobium*, *Ensifer*, *Rhizobium*,
- 192 Neorhizobium, Burkholderia and Cupriavidus.
- 193 The number of pigeon pea rhizobia in all the soils were counted using plant infection methods and
- 194 the most probable number (MPN) counts were found to range from very low (10^2 cells/g) to low (10^3
- 195 cells/g) with very few exceptions where it was optimum (10^4 cells/g). Rhizobial numbers decrease
- 196 drastically in dry hot summer lasting two months, reducing the numbers of rhizobia in the soil to less
- 197 than 100 cells/g and causing a decline in diversity. Therefore, there is a need to select rhizobia that
- are symbiotically superior under stress (Rao, 2014). Some very high performing rhizobia have
- already been identified and have been put back into the field for inoculation testing. These rhizobia
- are being further tested within the All-India Network of the Indian Council of Agricultural Research
- 201 (ICAR) at multiple locations before their release for mass-scale biofertiliser production and
- subsequent supply to farmers. In addition, rhizobia with greater ability to solubilise phosphorous in
- the rhizosphere through enhanced secretion of 2-keto gluconic acid have been engineered and these will be tasted in pigeon reaching big
- 204 will be tested in pigeon pea rhizobia.



205

- Figure 2. Screening of rhizobial strains for nodulation of pigeon pea in sterile sand microcosms (left);
 close up of a well-nodulated plant (right) (IUNFC).
- 208 Microbiome selection by plants in soils is highly conserved (i.e, plants select a largely common root
- 209 microbiome) (Sanchez-Canizares et al., 2017). A large number of rice genotypes, including some
- 210 landraces, were selected and characterised for high secretion of dicarboxylic acids into the
- 211 rhizosphere (e.g., malate and citrate secretion), which will support higher levels of fixation by
- 212 diazotrophs (N fixing bacteria). Five promising genotypes are now being studied for changes in
- 213 bacterial community in the rice rhizosphere. Four rice varieties that differ in efficiency in N
- assimilation have been characterised for the endophytic bacterial community that were represented
- 215 by about six genera and nine species. These strains exhibit plant growth promoting (PGP) traits, such
- as N fixation and secretion of auxins, and are now being characterised for their ability to promote
- assimilation of nitrogen fixed as ammonia. Genome sequencing has been done for different
- 218 bradyrhizobial strains isolated from peanut/rice intercropped field. Six bradyrhizobial strains were

- 219 isolated from rice roots (WBOS01, WBOS02, WBOS04, WBOS07, WBOS08 and WBOS16), and
- another six bradyrhizobial strains were isolated from peanut (WBAH10, WBAH23, WBAH30,
- 221 WBAH33, WBAH41 AND WBAH42). Both WBOS or WBAH type strains are able to nodulate
- 222 peanut, however only WBAH strains do it in an effective way. Furthermore, both groups of strains
- promote growth on peanut and rice plants under control conditions (Guha et al., 2016).
- 224 Studies of genomes and phylogenetic structure of a large group of 96 elite rhizobial strains that infect
- pigeon pea are currently being conducted to understand the genetic structure of the bacteria and to
- 226 mine for genetic determinants of competitiveness and effectiveness in nodulation. Alongside this,
- considerable progress has been made in understanding the mechanism of action of rice endophytes
- that are also able to nodulate peanut. These are novel strains whose genomes have been sequenced
- and their mechanisms of action are now being investigated. Considerable progress has also been
- made in screening lines for enhanced secretion of organic acids by rice which is capable of
- 231 supporting elevated N fixation by endophytic diazotrophs.

232 2.2 Biological nitrogen use efficiency

233 While agronomic measures help realize the innate genetic potential of the crop's NUE in the short 234 term (see section 2.3), biological crop improvement is necessary to break the agronomic barrier for 235 further enhancement in the medium to long term (Mandal et al., 2018, Raghuram and Sharma, 2019). 236 The lack of detailed biological understanding of the crop phenotypes and genotypes required for 237 good N response and NUE has been a major limitation in the development of genetic approaches to 238 improve the efficiency of utilisation of fertiliser N. A number of phenotypic characteristics have been 239 reported to be associated with N response and NUE. These include plant height and the timing of the 240 onset of post-anthesis senescence in wheat (Subedi et al., 2007, Gaju et al., 2011), root length, root 241 density and panicle density and form in rice (Morita et al., 1988, Yang et al., 2012, Sun et al., 2014, 242 Peng et al., 2015, Rogers and Benfey, 2015, Steffens and Rasmussen, 2016). N-responsive changes 243 in root system architecture have been the main focus of studies on N response and NUE in rice (Li et 244 al., 2015, Li et al., 2016). More extensive characterization of the phenotypic traits and components 245 conferring efficient NUE is required for different crops.

- 246 It is important to address biological NUE at different levels - morphological, physiological, genetic, genomic, proteomic, or bioinformatics - and using different approaches. Insights from Arabidopsis 247 248 and rice indicate the value of identifying contrasting lines based on their N-sensitivity or 249 responsiveness to the measured growth parameters. Some contrasting lines of Indica rice have 250 already been identified and compared for their N-responsive germination, growth and yield 251 parameters at normal and low N doses using urea and/or nitrate (Sharma et al., 2018). The lifelong 252 evaluation of a few contrasting lines identified from the above study within NEWS enabled the 253 shortlisting of additional traits for NUE in rice. While some of these parameters are commonly 254 measured in relation to many agronomic traits, some are specific to NUE either individually or in 255 combination. Further shortlisting of these parameters is necessary to screen germplasm on a large 256 scale for crop improvement by selection or breeding. Work in CINTRIN is focussed on the branching 257 response to N application which is well defined in model species and could offer new avenues for 258 manipulating crop N response.
- 259 Plants often modulate their root system architecture to compensate for the fluctuations of external N
- 260 concentration in soil and the actual N demand to increase its N-uptake efficiency (Vidal and
- 261 Gutiérrez 2008, Krouk et al., 2010, Alvarez et al., 2012, Sinha et al., 2018). Therefore for precise
- 262 phenotyping of root system architecture traits (under optimum and NO₃⁻ starvation conditions),

263 selected wheat genotypes were evaluated for modulation in root traits in hydroponics and N-free

- solid media (pot) at seedling stage under controlled conditions under the INEW VJC. Furthermore, in
- order to understand the genetic variability in nitrate-uptake capacity (considering both high and low affinity nitrate transporters in selected high and low yielding Indian and UK wheat genotypes),
- nitrate-flux were evaluated using ¹⁵N stable isotope as N source through isotope ratio mass
- 268 spectrometry. The programme also included characterization of the contrasting genotypes (selected
- 269 based on field evaluation) for N-utilization efficiency traits using carbon and N metabolizing
- enzymes and their corresponding genes (Sinha et al., 2015; Gayatri et al., 2018). Results showed
- 271 considerable genotypic variation for both the component traits, both at transcript as well as enzyme
- activity level. The practical relevance of these studies has been evaluated by growing selected
- 273 genotypes in 'precision nutrition' facilities, where fertiliser was applied in direct proximity to the
- roots by fertigation. Training of personnel was also carried out at the precision nutrition facility of
- the Borlaug Institute for South Asia, Ludhiana, India, to promote wider agronomic adoption, field
- validation and use.

277 The establishment of diversity panels of rice, wheat, sorghum and millets, and the availability of

- associated genotyping or genomic information has provided a major resource for identifying
- significant marker-trait associations in available germplasm. Research conducted by NEWS found
- 280 genotypic variation in NUE in 472 rice genotypes of Indian origin from the 3000 sequenced
- accessions from the International Rice Research Institute (Alexandrov et al., 2015) and the 260
- strong Bengal and Assam Aus Panel (Norton et al., 2018). These are being assessed at graded N
- 283 levels at the Indian Institute for Rice Research (IIRR) as part of the NEWS VJC, and the data are 284 being used for genome wide association mapping. Field studies with 10 rice genotypes indicated 50%
- variation in NUE between genotypes. In pearl millet, a total of 25 million whole genome single
- nucleotide polymorphisms (SNPs) are being tested by CINTRIN for association with NUE-related
- traits on the association mapping panel. Similar approaches are being pursued in sorghum and wheat
- in CINTRIN for exploiting natural/synthetic variation for NUE and mapping of the related traits.

289 Using the N-responsive genes identified in rice by microarray analysis and literature sources,

- 290 activation-tagged transgenic rice lines have been identified by NEWS so far for over 50 N-responsive
- 291 genes, including some G-protein regulated genes. Additional lines are expected from the ongoing
- screening of the remaining activation tagged lines. These lines need to be confirmed for the
- 293 overexpression of the N-responsive gene(s) before assessing their impact on N-response and/or NUE.
- The identification of specific N-responsive genes/alleles that contribute to NUE will open
- 295 opportunities for marker development towards marker-assisted selection and/or breeding, or gene-
- editing of the target genes in popular high yielding lines for in situ genetic manipulation of NUE. The
 CINTRIN VJC is modifying a number of candidate NUE ideotype genes by gene-editing and over-
- expression to confirm their role in modulating N-response in both Brachypodium and wheat.

299 Although all plant organs contain the same complement of genome, both the expression of genes and 300 the accumulation of proteins varies widely and therefore, proteomics (a study of proteins) can 301 provide us with a biological snapshot of a tissue, organ or organelle at a particular point in time. At 302 the Aligarh Muslim University, under NEWS, a stage-specific, cultivar-specific and N treatment-303 specific proteomic analysis of rice cultivars was conducted. The leaf proteome analysis reported 34 304 polypeptides that were affected by N treatments. Four polypeptides showed differential expression as 305 a genotypic effect irrespective of N treatments. Expression of some proteins changed consistently at 306 different developmental stages of rice. These proteins are yet to be identified. The identities of 307 proteins that account for variation between treatments, genotypes, and stages of development are of

- 308 crucial value for shortlisting the target genes/proteins for crop improvement, in view of earlier results
- 309 from this group (Hakeem et al., 2012, 2013).
- 310 In INEW, work has focussed on relating the phenological crop growth development of wheat to NUE
- 311 using nested associated mapping (NAM) of lines grown in replicate field trials on three sites in India
- and two sites in the UK. Most of the lines are from populations derived from crosses between
- accessions from the A. E. Watkins collection, a global collection of landraces originating from the
- 1920s and 1930s (to maximise genetic diversity), and a European spring wheat, developed at the
- John Innes Centre, UK. The selection of related lines which are adapted for growth in India and the UK allows the datasets from the two countries to be combined, increasing the power of the analysis.
- 317 In addition, spring types of a composite cross of European elite wheats are being studied in both
- countries, and a diversity panel of Indian genotypes in India only. All these accessions have been
- 319 SNP genotyped and quantitative trait loci (QTLs) are being mapped. These studies are being
- 320 underpinned by laboratory analyses of root phenotypes and gene expression analyses. The
- 321 relationship with the timing of N application and NUE is also being studied using the precision
- 322 agriculture facilities at the Borlaug Institute for South Asia (BISA).

323 2.3 Agronomic nitrogen use efficiency and environmental impacts

324 An important step toward improving agronomic NUE is the development of crop genotypes with

- 325 increased NUE under field conditions. The preliminary results from a major field experiment –
- 326 conducted during the kharif season of 2016 at the research farm of the Indian Agricultural Research
- 327 Institute (IARI), New Delhi, India, as part of the research programme of NEWS tested 10 rice
- 328 genotypes for the most common NUE-related indices. Preliminary results from this analysis showed
- that the lines Nagina-22, Himdhan and Taipe-309, could be considered as the most efficient N users.
- 330 Compared to other genotypes, the values of grain yield were higher in these varieties with higher
- NUE (Fig. 3), while MTU-1010 had the highest harvest index. Furthermore, these efficient N user
- 332 genotypes showed higher agronomic efficiency (AE), physiological efficiency (PE) and partial factor
- productivity (PFP) of applied N in rice (Fig. 4, see the caption for the definition of AE, PE and PFP).
- Further investigations are underway to quantify how much N can potentially be saved nationally by
- using such genotypes.



Figure 3. Grain yield and harvest index of different rice genotypes during the 2016 kharif season (by IARI under NEWS).

■ AE (kg grain increase/kg N) ■ PE (kg grain/ kg N uptake) ■ PFP of Nitrogen (kg grain/kg N) 70 60 50 AE/PE/PFP 40 30 20 10 MIL-1010 BPT-5204 KALA DHAN SWARNA HIMDHAN NAGINA-2 PUSAAA TAPE-309 1^AVA TKM-6 Genotype

339

Figure 4. Agronomic efficiency (AE = [Grain yield (kg/ha) in N treated plot - Grain yield (kg/ha) in control plot] / N rate (kg/ha)), physiological efficiency (PE = [Grain yield (kg/ha) in N treated plot -Grain yield (kg/ha) in control plot] / [N uptake (kg/ha) in N treated plot - N uptake (kg/ha) in control plot]]) and partial factor productivity (PFP = Grain yield (kg/ha) / N rate (kg/ha)) of applied N in different rice genotypes during the 2016 kharif season (by IARI under NEWS).

345 Another significant approach to improve agronomic NUE is to match fertiliser application rates to the 346 actual needs of the plants. This can be identified by monitoring the colour of the leaves (i.e. a proxy 347 for chlorophyll content). For this purpose, one of the simplest and probably the cheapest option 348 (costing about 100 Indian rupees or £1) is to use a leaf colour chart (LCC). An LCC is a plastic 349 colour palette that can help farmers to determine the N needs of the plant based on the appearance of 350 the crop. The concept of deciding fertiliser N application timings based on the greenness of the leaves 351 was initially introduced for rice by the International Rice Research Institute (IRRI), Philippines (IRRI, 1996). Figure 5 shows an LCC refined by the Punjab Agricultural University (PAU) within 352 CINTRIN, referred to as the "PAU-LCC". In case of this LCC, farmers can access the decision 353 354 support system based on the "PAU-LCC' recommendations at their smart phones through "PAU Urea 355 Guide" application freely available both for Android and IOS users. Other versions of the LCC are 356 available from the National Rice Research Institute (NRRI, India) and IIRR as well, where the 357 colours are optimised for different crop contexts. The main advantage of the LCC approach apart 358 from its low price, is that farmers can be easily trained to use the tool so that they can work out 359 themselves the needs-based N fertiliser dose, independently from the crop type, the season or soil 360 type, without any analytical laboratory procedures or use of technology requiring a reliable power 361 supply or calibration.



- 362
- Figure 5. Leaf colour chart as implemented by the Punjab Agricultural University within CINTRIN ("PAU-LCC").
- 365 The LCC technology has been demonstrated to produce potential yields with low N optimum dose in transplanted rice (Varinderpal-Singh et al., 2007, 2014), maize (Varinderpal-Singh et al., 2011, 366 2014), wheat (Varinderpal-Singh et al., 2012, 2017), direct seeded rice, basmati rice and cotton 367 368 (PAU, 2018). Under CINTRIN, a village (Bassian, Ludhiana) has been developed as a role model village in the Indian state of Punjab with the active support of an NGO, the Atam Pargas Social 369 370 Welfare Council. The farmers of the village harvested equivalent yields with the saving an average of 371 75 kg N/ha for rice 2017 and 50 kg N/ha for wheat 2017-18 compared to conventional farmers' 372 practice. The positive effects of the LCC approach were also highlighted by the findings of Bhatia et 373 al. (2012). In this study, when 120 kg/ha urea was applied to rice using the LCC approach, the yield 374 was 12-24% (depending on the LCC scores used) larger than in the case when the same amount of 375 fertiliser was applied in the conventional way, in three equal splits at fixed times.
- Although the LCC approach is easily accessible for farmers, computer-based tools (so-called
 'microcontroller-based' tools) may provide a more accurate solution. These tools are currently
 relatively expensive (about £25); however, work is ongoing within NEWS to make these approaches
 more affordable to farmers, such as in the "Green-Check" crop health meter of IARI. In the case of
- all the mentioned tools, further research should investigate the social barriers of adaptation of such
- 381 methods by farmers.

382 It is important to note that although the crop N supply mainly depends on the N requirement of the crop, climatic and environmental factors can play an important role in influencing NUE (Sharma and 383 Bali, 2018). The amount of applied N that will be taken up by crops or lost from the soil-plant system 384 385 depends not only on the soil (texture, pH, moisture content) and the available water (amount, salinity) 386 but also the atmospheric factors, such as temperature, rainfall, wind speed (Fagodiya et al., 2020, 387 Cameron et al., 2013). Timing of rainfall and irrigation are key factors in determining NUE (Abebe 388 and Feyisa, 2017). If the N application coincides with a rainfall event, most of the applied N is lost as 389 runoff, lowering NUE. In case of a sandy soil, the applied N fertiliser may be leached below the root 390 zone with the rainfall or heavy irrigation, again reducing the NUE. If urea N is applied to dry soil 391 with high pH, then much of the N applied may be lost as ammonia. Thus, for improving NUE, not 392 only the crop (growth stage, variety), but also environmental factors need to be considered. A method 393 to improve agronomic NUE across the whole farm is by reducing losses of N through organic wastes 394 (manures, urine and crop residues), by promoting their uptake as key sources of plant nutrients. 395 Within the NEWS VJC, work in the Mumtajpur village cluster (Pataudi Block in Harvana state) is 396 experimenting to increase the quantity of N in organic wastes that is recycled to the soil, and to

Nitrogen challenges and opportunities

- 397 improve the quality of the organic fertilisers produced. This is being done by using a closed system to
- 398 compost organic wastes instead of the traditional unlined pit or open manure heap. A polytex bag
- 399 (3.7 x 1.2 x 0.6 m, Fig. 6) is staked in the field and covered with a UV-protected transparent plastic
- sheet. The bag is layered with fresh cow dung, urine soaked bedding and soil, followed by singlesuper phosphate, which helps reduce the loss of ammonia during decomposition. This sequence of
- 402 layers is repeated until the bag is full, which usually takes about 30-35 days from three animals.
- 403 Monitoring of greenhouse gas and ammonia emissions indicates lower emissions than in
- 404 conventional practices (Figure 7 ammonia emission not shown). The organic fertiliser is ready to
- 405 use after just three months of storage/preparation, and contains 0.82 to 0.89% N on a dry weight
- 406 basis, compared to 0.35-0.5% N by traditional methods. Dry matter recovery is ~70% of the organic
- 407 wastes, compared to 50-55% by conventional open manure storage. Organic fertilisers made by the
- 408 improved and traditional methods are currently being trialled with vegetables, rice and wheat crops in
- 409 farmers' fields.



410

- 411 Figure 6. The conventional way of manure storage in a heap (on the left), and the improved way of
- 412 farm yard manure making, using polytex bag at farmers' field in Mumtajpur, Pataudi Block, Haryana
- 413 (on the right) (by IARI under NEWS).



414

- 415 Figure 7. Measurement of N2O, CO2 and CH4 fluxes at two villages of Mumtajpur village cluster,
- 416 Lokra and Turkapur, in the case of two different farm yard manure storage methods: composting
- 417 using the improved composting method, in polytex bags ("compost") and storing in a traditional open
- 418 manure heap ("general") (by IARI under NEWS).

- 419 Related to the environmental impacts of agronomic practices, at the conference, regional-scale
- 420 studies using several chemistry transport models were also presented. These demonstrate that the
- $421 \qquad \text{examination of NH_3 related atmospheric processes (emission, dispersion, chemical reactions and \\$
- 422 deposition) over India is crucial. Figure 8 shows the average surface NH_3 concentration in the
- 423 summer derived by three models for three different years as follows:
- Fig. 8a: EMEP model (Simpson et al., 2012) for 2015. The simulation was carried out by the
 global version of the EMEP model (version 4.10) with a resolution of 1°. The input
 meteorological data were derived by the WRF (Weather Research Forecast) weather forecast
 model (Vieno et al., 2016), and the emissions originate from the EDGAR (Emission Database
 for Global Atmospheric Research) database (for 2005).
- Fig. 8b: UKCA model (Bellouin et al., 2011, O'Connor et al., 2014) for 2008. To obtain these results UKCA was run with the aerosol scheme CLASSIC. The meteorology was simulated by the UM climate model and driven by sea surface temperatures and sea ice climatologies.
 Anthropogenic emissions were from HTAP v2.2 year 2008. Fire emissions were from VUA 1.2 Global Biomass Burning Emissions year 2008.
- Fig. 8c: MOZART model (Emmons et al., 2010) for 2010. The input meteorological data set for driving the model was obtained from the MERRA (Modern Era Retrospective-analysis for Research and Applications) database of GEOS DAS (Goddard Earth Observing System Data Assimilation System) for the year 2010. For emissions, the HTAP_v2 (Hemispheric Transport of Air Pollution, Version 2) dataset was used.
- 439 Although in Fig. 8 the different scales suggest very different surface concentration values, it is clear 440 that all three models indicate greatly increased NH₃ concentrations over the Indo-Gangetic Plain 441 during summer, which is in agreement with earlier satellite observations (Clarisse et al., 2009). The 442 difference between the models in the magnitude of concentrations could be caused by various model 443 features, for example, the different heights of the surface layer, different dynamics, different chemistry schemes. The possible sources of differences in the resulting surface ammonia 444 445 concentrations should be the scope of future model comparison studies, where the same emission 446 datasets and the same input meteorological dataset should be used in order to avoid differences 447 originating from the differences between these datasets. This comparison is important as satellite
- observations (Clarisse et al., 2009) indicate that the Indo-Gangetic Plain has the highest regional NH₃
 concentrations on Earth.



450

Figure 8. Average surface NH₃ concentration (ppb) for the summer (June-July-August) of 2015 (a),
2008 (b) and 2010 (c), using the EMEP model, the UKCA model and the MOZART model,

- respectively. For the specifications of these atmospheric chemistry transport models, see the maintext.
- 455

456 **3 Options for better N management**

457 **3.1 Biological nitrogen fixation**

458 The majority of Indian soils are low in soil organic matter (SOM) and soil N available for plants. A 459 key way to increase SOM, and soil N, is to increase legume acreage and to encourage crop rotations 460 with legumes, which also potentially improves soil biodiversity and ultimately, soil health. Planting a 461 deep-rooted legume like pigeon-pea has potential to scavenge nitrate and phosphate and other 462 nutrients from lower soil layers since its roots go as deep as 150 cm (Sheldrake and Narayanan 463 1979). When planted legumes are fixing nitrogen optimally, they reduce the requirements of N 464 fertilisers of the subsequent cereals planted due to the residual effect of the fixed N (Rao, 2014). The 465 amount of nutrients in pigeon pea litter available as residual benefits to succeeding wheat crop have 466 been estimated at about 40 kg N, 2 kg P, 7 kg K, and 2 kg S ha-1 respectively in North India (Rao and Gill 1995) which is known to farmers and the very reason for growing it to improve soil fertility. 467

468 In legumes, improving grain yields in tropical agriculture is best achieved by breeding genotypes 469 under low-N conditions so as to select for high nodulators, employ best-bet agronomic practices and

- 470 intercropping (Vanlauwe et al., 2019). A positive N balance can occur when there are no abiotic
- 471 constraints or nutritional deficiencies that limit legume growth. Due to various constraints N fixation
- 472 can be very low and there is hence a big range in pigeon pea BNF from 7-235 kg/ha/crop (Peoples et
- al., 1995) with the proportion of N fixation ranging from 10-81%. In a study in India, BNF by pigeon
- 474 pea was low, 69 kg/ha and amounted to only 50% of plant N uptake (Kumar Rao and Dart 1987).
- 475 Improvement of N fixation under drought stress depends as much on host genotype selection as on
- 476 rhizobial selection and improving soil organic matter and repeated inoculation. Crop rotation with
- 477 grain legumes, forage and other green manures builds SOM content and protects microbial biomass
- 478 and diversity, populations and their activity (Aparna et al., 2016). Such soil stewardship is essential
- to improve soil health and sustainability under intensive agriculture.
- 480 Currently, there is emerging enthusiasm among some farmers to avoid fertiliser entirely. A major
- 481 regional example is the Zero Budget Natural Farming (ZBNF) system, which represents a system
- based on agroecological principles. One of the key features of the ZBNF movement of Andhra
- 483 Pradesh is its rapid uptake by farmers, where the state policy aims that all 6 million farmers of
- 484 Andhra Pradesh would practice ZBNF by 2024 (RySS 2018).. This movement takes the name ("zero
- 485 budget") from its focus to avoid all purchased inputs, such as pesticides and fertilisers. It aims to
- 486 regenerate soil health by increasing SOM with a full dependence on BNF (and atmospheric inputs)
- 487 for all Nr supply and thus make farming more profitable.

488 At present, the nitrogen performance of many different BNF-based farming systems, including 489 ZBNF, remains to be quantified. Long-term nutrient budget studies are required to quantify nutrient 490 inputs achieved through a mix of nitrogen fixing crops and intercrops in different contexts. For 491 example, recent modelling assessment suggests that available nutrient sources in ZBNF examples 492 maybe sufficient for extensive agricultural production but not sufficient to allow high output 493 agricultural production, risking the potential for soil N depletion (Smith et al., 2020). Farmers may 494 also supplement N inputs on their own or adopt hybrid models (Raghuram, 2020). Conversely, as 495 BNF provides a gradual input of fixed N to the soil, it may be expected to lead to lower levels of 496 surplus mineral N in the soil, suggesting that the fraction of N inputs that are lost to air and water

- 497 pollution will be smaller than in fertiliser-based systems. However, this still needs to be tested, while
- 498 ploughing-in and decomposition of legume based green manures may nevertheless be associated with
- 499 significant N leaching losses. Such knowledge would be useful in better understanding the system
- 500 limitations and could guide further refinement of improved practices.
- 501 Under IUNFC, a strong focus of research has been given to understanding the controls in
- 502 performance of pigeon pea. This crop is selected especially for its importance in dry land systems.
- 503 There is a huge diversity of pigeon pea germplasm to exploit in various regions. Two hundred
- 504 genomes of pigeon pea have been sequenced at the International Crops Research Institute for the
- 505 Semi-arid Tropic (ICRISAT) in Hyderabad, which can be further studied. The translation of the
- 506 findings described in Section 2.1, in terms of transferring the improved elite strains of pigeon pea
- 507 rhizobia to farmers fields and similarly, the endophytes to rice farmers, are under testing in pan-India
- 508 network field trials coordinated by the Indian Council of Agricultural Research (ICAR).
- 509 Subsequently, these strains will be transferred to the inoculant industry for large scale multiplication 510 and deployment.
- 511 India has a robust microbial inoculant industry (FAI, 2018) and the improved strains can easily be
- 512 transferred to farmers for coating legume seeds both as carrier inoculant and liquid inoculants. Post-
- 513 planting inoculation with liquid formulations of rhizobia is also possible and compensates partially
- for yield losses due to not inoculating the seeds at sowing. Similarly, the elite endophyte strains
- 515 isolated in rice will eventually be deployed by the inoculant industry to inoculate and benefit not only
- 516 the rice, but even the subsequent crops grown in rotation.
- 517 Work on other projects funded by the Bill and Melinda Gates Foundation (BMGF) and BBSRC/NSF
- 518 may result in disruptive technologies for N utilisation. BMGF has funded work on the expression of
- 519 nitrogenase complexes in mitochondria and good progress has been made in the successful
- 520 expression of the Fe protein in yeast mitochondria (López-Torrejón et al., 2016). This is still a
- 521 medium-long term project, but it deserves careful consideration as it could be a game changer in the
- N economy of cereals. Likewise, BBSRC/NSF funded work on developing synthetic symbioses has
- 523 made good progress in establishing signalling between plants and microbes, which is a prerequisite
- 524 for controlled interaction between cereals and N fixing endophytes. If nodulation can be established
- 525 in cereals then work on synthetic symbioses and nodulating cereals would come together.

526 **3.2 Biological nitrogen use efficiency**

527 There is evidence that excessive application of N fertilisers harms yield, and attracts pests and 528 diseases (Huber et al., 2012). This requires increased attention of future research programmes. There

- are also major differences between crop types. For example it has been suggested that millets need
- relatively low levels of N input to fix carbon due to their physiological characteristics (Wang et al.,
- 531 2018) compared with other cereals, and are also healthier on other accounts (Anuradha et al., 2017).
- 532 Recognising that it would require a re-design of supply chains, further investigation of the case to
- 533 promote consumption of millets instead of rice and wheat is warranted to examine how this could
- contribute to increased NUE in the food chain. This is a major objective of the UK-India TIGR2ESS
- 535 GCRF GROW project (TIGR2RESS, 2019).
- 536 In order to exploit better the biological potential for improving NUE in crops, genetic diversity from
- 537 outside the standard breeders' germplasm should be further explored. This is particularly true for
- those crops (like rice and wheat in India) that are generally bred/selected under high N input
- 539 conditions (Mandal et al., 2018, Raghuram and Sharma, 2019). Although significant steps have been
- 540 made towards finding alleles that contribute to NUE (Section 2.2), some cultivars containing such

- 541 alleles may either have been lost or screened out unintentionally in yield-centric screening
- 542 programmes that emphasized N-response over NUE (Mandal et al., 2018, Raghuram and Sharma,
- 543 2019). Landraces, such as the A.E. Watkins collection utilised in the INEW VJC, and wild varieties
- 544 may bring in the missing alleles and give important clues towards finding new genetic avenues for
- 545 improving NUE, thereby providing agronomists with better genotypes on which they can further
- 546 optimise N management.
- 547 Efficient procedures for timely access to promising crop germplasm with reduced N demand could be
- 548 a critical driver in this regard, as sometimes it may take a year or more to obtain seeds, especially
- 549 from overseas. Establishment of low N-input breeding programmes for all major N-fertiliser-
- 550 consuming cereal crops and a system for efficiently sharing information and plant material are
- 551 important policy aspects for better N management and increased NUE. Further investments are also
- needed in research towards phenotype and genotype characterization, and identification of
- 553 contrasting genotypes for NUE. Field phenomics, and remote sensing, gene-editing and other 554 emerging technologies can improve research efficiencies in phenotyping and early breeding.
- 534 emerging technologies can improve research enficiencies in phenotyping and early breedin

555 **3.3** Agronomic nitrogen use efficiency and environmental impacts

- 556 Based on the key findings mentioned in Section 2.3, one of the most promising options to improve
- agronomic NUE is to monitor crop colour, and to decide on the amount and timing of N application
- according to the appearance of the crop, using for example, LCCs or electronic sensors. There is an
- 559 opportunity and a need to compare the performance and limitations of a variety of LCCs such as
- those used by several research institutes (like PAU, IIRR, NRRI... etc.).
- 561 The preliminary national-scale model results clearly highlight the role of NH₃ emission in
- 562 atmospheric N pollution in India, especially in the context of worsening air quality across the Indo-
- 563 Gangetic Plain. A possible option to reduce NH₃ emission from urea application, could be to
- 564 combine neem-coated (i.e. urea coated with neem oil, a natural nitrification inhibitor) with a urease
- inhibitor ("Neem Plus"), which could potentially increase NUE and decrease N losses. Currently,
 only artificial urease inhibitors are available in India, such as N-(n-Butyl) thiophosphoric triamide
- 567 (NBPT), phenylphosphorodiamidate (PPD/PPDA) and hydroquinone. However, a plant-based urease
- 568 inhibitor, similar to the neem oil used as nitrification inhibitor, would be expected to increase social
- acceptance. Thus, the great challenge is to find molecules that are of natural origin, have low toxicity,
- 570 are chemically stable, efficient at low concentrations, and are competitively priced. Some natural 571 plant phenolic products, such as protocatechuic aldehyde and vanillin derivatives, have been reported
- 571 prant phenonic products, such as protocatechnic aldenyde and vanifin derivatives, nave been reporte 572 as having soil urease inhibition properties (Horta et al., 2016); however, this requires further
- 572 as naving son urease innotition properties (florta et al., 2010); nowever, this requires further 573 investigation. Furthermore, future research should also examine the effectivness of the usage of a
- 575 investigation. Furthermore, future research should also examine the effectivness of the usage 574 urease inhibitor along with the neem coating of urea
- urease inhibitor along with the neem coating of urea.
- 575 There are potential opportunities in integrating agronomic strategies (i.e. practice changes) with 576 opportunities for increased biological NUE (i.e. plant genotypes with increased N efficiency). The 577 extent of possible synergies between improved genetics and improved agronomy, and the extent to 578 which agronomic changes need to be made to exploit the genetic potential are not yet known. In 579 principle, there is a major opportunity to reduce the costs of agricultural production and levels of 580 environmental pollution at the same time (Fageria and Santos, 2014). Future research should focus on 581 integrating outcomes from breeding for higher NUE into field studies in relation to agronomic 582 practices that demonstrate how environmental sustainability can go hand in hand with reduced and 583 more efficient use of fertilisers (Ladha et al., 1998, Cheng et al., 2011).

- 584 Further work is also vital to improve recycling of organic wastes to the soil. This needs to consider
- 585 crop residue, animal urine and human excreta. The movement of produce to markets results in a year-
- 586 on-year loss of N from the agricultural system, and leaves an excess of organic wastes in urban areas.
- 587 Drying and pelletizing of composts, slurries from anaerobic digestion and biochar from pyrolysis of
- 588 market wastes have a great potential to facilitate the return of N to agricultural areas. Development of 589
- technologies is also needed to avoid loss of NH₃ during drying of these products. There is ongoing 590 work in upscaling nutrient recovery, such as by ammonia stripping from the liquid remaining
- 591 following anaerobic digestion of organic material (Buckwell and Nadeu, 2016), which may provide a
- 592 major opportunity to include recycled N in new fertiliser products.
- 593 Finally, substantial improvements could be achieved in NUE by combining agronomic research with 594 research on the environmental and health impacts of N pollution from agriculture in order to develop 595 a more holistic perspective. Scaling up this approach from farm level to national and food system
- 596 level, in tandem with modelling the economic impacts could demonstrate the opportunities for India, 597
- with potential policy benefit. For example, the interventions to improve NUE at crop level and the
- 598 recycling of organic wastes can result in the overall increase of NUE for the overall food chain.
- 599 These interventions must be coupled with other solutions to limit N losses from other stages of the
- 600 food production systems, such as avoidance of post-harvest losses or reduction of food wastes and
- 601 losses (HLPE, 2014, Uwizeye et al., 2016).

602 4 Actionable recommendations: global goals for 2030

603 Based on the latest research results and the possible options for better N management in Indian 604 agriculture, the participants of the workshop identified a set of global goals related to all three topics 605 for 2030.

- 606 For BNF, one of the most important goals is to extend knowledge on how legumes can be used to 607 improve NUE. Firstly, the research has identified the need to establish a pan-Indian, virtual or
- 608 physical centre for legume research. As indicated in Section 2.1 pigeon pea has a large potential to
- 609 enhance NUE. Currently, this crop has an average yield of 850 kg/ha. Secondly, the workshop agreed
- 610 a goal for 2030 is to double or even triple this yield by 2030. Along with these aims, it is reasonable
- 611 to set a third, more general goal, namely, by 2030, to provide a substantial amount of cereal N via
- 612 synthetic N symbioses and/or plastidic N fixation (Section 3.1).
- 613 As mentioned in Section 2.2, numerous traits have been identified for NUE in rice, wheat, sorghum
- 614 and millets. These promising results represent clear steps for the future. Based on this, one of the
- 615 goals set in terms of improving biological NUE is to better define the biological signatures of crop N
- 616 status, including some that are farmer-friendly. The findings presented at the conference clearly
- 617 support the biological potential for NUE improvement and encourage information and germplasm
- 618 exchange. As such, the goal set for 2030 is to increase biological NUE by at least 20-30%.
- 619 The latest research clearly shows the success of leaf colour-based fertiliser application methods,
- 620 especially that of the LCC approach (Section 2.3), in improving NUE on arable farms. Therefore, one
- 621 of the main goals for 2030 is to ensure that all arable farmers use LCC or other sensor technologies to
- 622 decide on N applications. In the first instance, the focus is on conventional farmers optimising their
- 623 use of fertiliser to avoid excessive and wasteful inputs. However, there may also be interest in
- 624 considering the use of leaf colour sensing technology in relation to organic farming. Such an
- 625 approach would require a plant available source of organic fertiliser N that could provide rapid plant

- response following application, such as is provided by bioslurry. Conversely, a LCC could be used to
- test for N deficiency in BNF based cropping systems.
- To meet the goal for leaf colour sensing by 2030 such tools should be provided to every farmer in
- 629 India, along with documented success stories. With these technologies, based on expert judgement,
- 630 20-25% N could be saved (Lassaletta et al., 2014, Zhang et al., 2015). The positive outcomes of the
- 631 presented research (Section 2.3) also allow a goal to be set for farms with livestock production. An
- 632 important goal would be to re-use all manure and urine to maximize their fertiliser value, allowing
- reduced fertiliser inputs and subsidy savings. Future research should explore the social barriers of the
- application of these measures, building on the ongoing work of the South Asian Nitrogen Hub and
 the International Nitrogen Management System. The expected increase in NUE using these
- the International Nitrogen Management System. The expected increase in NUE using these
 approaches could be 5-10%, which could also lead to a proportional reduction in N fertiliser
- approaches could be 5-10%, which could also lead to a proportional reduction in N fertiliser
 requirement. Large-scale social movements that focus on BNF rather than mineral fertilisers, may
- have a very important part to play in achieving the necessary transition to an India characterised by
- 639 high NUE, low N pollution and improved livelihoods.
- 640 Finally, the results (Section 2.3) highlight the role of NH₃ in the N pollution of India. In order to
- 641 manage the high levels of NH₃ emissions it is recommended that, by 2030, all urea products should
- be both neem-coated and include a urease inhibitor ("Neem Plus"). This option is expected to offer a
- 5% N saving over neem-coated urea (which already achieves a 5 10% savings above uncoated
- 644 urea). Since natural additives are culturally better accepted in India, the ultimate goal should be the
- 645 development of viable plant-based urease inhibitors.
- 646 While we identified specific measures of key importance, we recognize for a wider nitrogen cycle
- 647 perspective that there is a need to reduce overall nitrogen losses from agriculture, in addition to NH3
- loss, including N2O, NO, and N2 emissions, as well as nitrate and other forms of nitrogen leaching.
- Each of these losses is driven by different processes. For example, ammonia loss is driven by
- 650 immediate excess ammonium levels after fertilisation with urea or organic sources (urine, manure
- etc.). Conversely, losses of N2O, NO and N2 tend to be more driven by excess nitrogen availability
- in the soil (beyond plant needs), while nitrate leaching is substantially exacerbated during periods of
 bare soil. This means that there is a need for further research to develop coherent 'packages of
- measures' that seek to reduce multiple forms of nitrogen loss (and their impacts) simultaneously,
- 655 thereby also providing opportunities to improve NUE (TFRN, 2020).
- Altogether, this suite of changes would be expected to make a major contribution to the recently
- agreed commitment of the International Nitrogen Initiative "to support a global goal to halve nitrogen waste by 2030" (NEWS, 2018). How the different elements fit together towards a coherent holistic N
- 658 waste by 2030" (NEWS, 2018). How the different elements fit together towards a coherent holistic N 659 approach should be the subject of future research in interaction with farmers, policy makers and
- approach should be the subject of future research in interaction with farmers, policy makers and
 wider society. This should include identification of the barriers to the widespread adoption of the
- above solutions/measures at the social, economic and policy levels, as well as options for their
- 662 mitigation. While the Indian Nitrogen Assessment has laid the ground for this work (Abrol et al.,
- 663 2017, Bhattacharya et al., 2017), further work can build on the ongoing work of the South Asian
- Nitrogen Hub, the International Nitrogen Management System and the Global Partnership on
- 665 Nutrient Management.

666 **5 Conclusion**

This paper brings together the main outcomes of the conference and workshop, "Challenges and
Opportunities for Agricultural Nitrogen Science in India" organised in October 2017, in New Delhi,

669 India. After sharing knowledge at the conference and working toward developing a joint vision on

- 670 how agricultural nitrogen management could be made better in India at the workshop, the event
- 671 concluded with three vision statements:
- Stewardship is essential and legumes need to be planted in rotation with cereals. Synthetic
 symbioses and plastidic nitrogen fixation are possibly disruptive technologies; we must
 consider both their potential and implications.
- 675
 676
 676
 676
 677
 677
 2. Genetic diversity and new technologies need to be shared and exploited to reduce nitrogen fertiliser use for productive, sustainable agriculture livelihoods and reducing the associated losses.
- 6783. The use of leaf colour sensing shows great potential to reduce nitrogen fertiliser use on-farm679by 10 15%. This tool, together with the usage of urease inhibitors when using urea-based680fertilisers, and better management of manure, urine and crop residues, could result in a 20 25% improvement in NUE of India by 2030.

682 6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financialrelationships that could be construed as a potential conflict of interest.

685 7 Author Contributions

686 AM led the formulation, coordination and writing of the manuscript. All authors contributed to 687 manuscript content and revision.

688 8 Funding

689 Funding was provided by NEWS India-UK (BBSRC BB/N013492/1), with contributions from

690 CINTRIN, INEW and IUNFC, as well as the UKRI GCRF South Asian Nitrogen Hub, RySS and

691 SUNRISE (Sustainable Use of Natural Resources to Improve Human Health and Support Economic

692 Development).

693 9 Acknowledgments

This paper is an outcome of the joint conference and workshop, "Challenges and Opportunities for

695 Agricultural Nitrogen Science in India" supported by the UK Biotechnology and Biological Sciences

696 Research Council (BBSRC), the UK Natural Environment Research Council (NERC), the Indian

- 697Department of Biotechnology (DBT), and INMS (Towards the Establishment of an International
- 698 Nitrogen Management System). The authors are grateful for all the support they received for this

699 overview paper from the 4 VJCs, under the lead of NEWS India-UK (BBSRC BB/N013492/1), with

- contributions from CINTRIN, INEW and IUNFC, as well as the UKRI GCRF South Asian Nitrogen
- Hub, RySS and SUNRISE (Sustainable Use of Natural Resources to Improve Human Health and
 Support Economic Development). The paper is a contribution to the work of the International
- 702 Support Economic Development). The paper is a contribution to the v
- 703 Nitrogen Initiative (INI).

704 10 References

- Abebe, Z. and Feyisa, H., 2017. Effects of Nitrogen Rates and Time of Application on Yield of
- Maize: Rainfall Variability Influenced Time of N Application. Int J of Agron, 2017, 1545280.
 https://doi.org/10.1155/2017/1545280
- Abrol, Y.P., Raghuram, N., Sachdev, M.S., 2007. Agricultural Nitrogen Use & Its Environmental
 Implications. IK International, New Delhi.
- Abrol, Y. P., Adhya, T. K., Aneja, V. P., Raghuram, N., Pathak, H., Kulshrestha, U., Sharma, C. and
- 711 Singh, B. (Eds.), 2017. Abrol, Y. P., Adhya, T.K., Aneja, V.P., Raghuram, N., Pathak, H.,
- 712 Kulshrestha, U., Sharma, C., Singh, B. (Eds.), The Indian Nitrogen Assessment: Sources of
- Reactive Nitrogen, Environmental and Climate Effects, Management Options, and Policies.Elsevier.
- Alexandrov, N., Tai, S., Wang, W., Mansueto, L., Palis, K., Fuentes, R.R., Ulat, Victor J.,
 Chebotarov, D., Zhang, G., Li, Z., Mauleon, R., Hamilton, Ruaraidh S., McNally, K.L., 2015.
 SNP-Seek database of SNPs derived from 3000 rice genomes. Nucleic Acids Res, 43, D1023D1027. https://doi.org/10.1093/nar/gku1039.
- Alvarez, J.M., Vidal, E.A., Gutiérrez, R.A., 2012. Integration of local and systemic signaling
 pathways for plant N responses. Curr Opin Plant Biol, 15, 185-191.
 https://doi.org/10.1016/j.pbi.2012.03.009.
- Aneja, V.P., Schlesinger, W.H., Nyogi, D., Jennings, G., Gilliam, W., Knighton, R.E., Duke, C.S.,
 Blunden, J., Krishnan, S., 2006. Emerging national research needs for agricultural air quality. Eos,
 Trans Am Geophys Union, 87, 25-29. https://doi.org/10.1029/2006EO030001.
- Aneja, V.P., Schlesinger, W.H., Erisman, J.W., 2009. Effects of Agriculture upon the Air Quality and
 Climate: Research, Policy, and Regulations. Environ Sci Tech, 43, 4234-4240.
 https://doi.org/10.1021/es8024403.
- Anuradha, N., Satyavathi, C.T., Bharadwaj, C., Nepolean, T., Sankar, S.M., Singh, S.P., Meena,
 M.C., Singhal, T., Srivastava, R.K., 2017. Deciphering Genomic Regions for High Grain Iron and
 Zinc Content Using Association Mapping in Pearl Millet. Front Plant Sci, 8, 412.
 https://doi.org/10.3389/fpls.2017.00412.
- Aparna, K., Rao, D.L.N., Balachandar, D., 2016. Microbial Populations, Activity and Gene
 Abundance in Tropical Vertisols Under Intensive Chemical Farming. Pedosphere, 26, 725-732.
 https://doi.org/10.1016/S1002-0160(15)60079-0.
- Bhattacharya, S., Adhya, T.K., Pathak, H., Raghuram, N. and Sharma C., 2017. Issues and Policies
 for Reactive Nitrogen Management, in: Abrol, Y. P., Adhya, T.K., Aneja, V.P., Raghuram, N.,
 Pathak, H., Kulshrestha, U., Sharma, C., Singh, B. (Eds.), The Indian Nitrogen Assessment:
 Sources of Reactive Nitrogen, Environmental and Climate Effects, Management Options, and
- 739 Policies. Elsevier, pp. 491-512.
- Bellouin, N., Rae, J., Jones, A., Johnson, C., Haywood, J., Boucher, O., 2011. Aerosol forcing in the
 Climate Model Intercomparison Project (CMIP5) simulations by HadGEM2-ES and the role of
 ammonium nitrate. J Geophys Res Atmos, 116, D20206. https://doi.org/10.1029/2011JD016074.
- Bhatia, A., Pathak, H., Jain, N., Singh, P.K., Tomer, R., 2012. Greenhouse gas mitigation in rice–
 wheat system with leaf color chart-based urea application. Environ Monit Assess, 184, 3095-3107.
 https://doi.org/10.1007/s10661-011-2174-8.
- Bijay-Singh, 2017. Management and Use Efficiency of Fertilizer Nitrogen in Production of Cereals
 in Indiad Issues and Strategies, in: Abrol, Y. P., Adhya, T.K., Aneja, V.P., Raghuram, N., Pathak,

- H., Kulshrestha, U., Sharma, C., Singh, B. (Eds.), The Indian Nitrogen Assessment: Sources of
- Reactive Nitrogen, Environmental and Climate Effects, Management Options, and Policies.Elsevier, pp. 9-28.
- Bijay-Singh, Yadvinder-Singh, 2008. Reactive nitrogen in Indian agriculture: Inputs, use efficiency
 and leakages. Curr Sci, 94, 1382-1393.
- Buckwell, A., Nadeu, E., 2016. Nutrient Recovery and Reuse (NRR) in European agriculture. A
 review of the issues, opportunities, and actions. RISE Foundation, Brussels, Belgium.
- Cameron, K., Di, H. and Moir, J., 2013. Nitrogen losses from the soil/plant system: a review. Ann
 Appl Biol, 162, 145-173. https://doi.org/10.1111/aab.12014
- Chalk, P.M., He, J.-Z., Peoples, M.B., Chen, D., 2017. 15N2 as a tracer of biological N2 fixation: A
 759 75-year retrospective. Soil Biol Biochem, 106, 36-50.
 https://doi.org/10.1016/j.soilbio.2016.12.010.
- Cheng, J.-f., Jiang, H.-y., Liu, Y.-b., Dai, T.-b., Cao, W.-x., 2011. Methods on Identification and
 Screening of Rice Genotypes with High Nitrogen Efficiency. Rice Sci, 18, 127-135.
 https://doi.org/10.1016/S1672-6308(11)60018-8.
- Clarisse, L., Clerbaux, C., Dentener, F., Hurtmans, D., Coheur, P.-F., 2009. Global ammonia
 distribution derived from infrared satellite observations. Nat Geosci, 2, 479-483.
 https://doi.org/10.1038/ngeo551.
- EDGAR, 2016. Emissions Database for Global Atmospheric Research v4.3.1.
 http://edgar.jrc.ec.europa.eu/overview.php?v=431 (accessed 15 February 2018).
- Emmons, L.K., Walters, S., Hess, P.G., Lamarque, J.F., Pfister, G.G., Fillmore, D., Granier, C.,
 Guenther, A., Kinnison, D., Laepple, T., Orlando, J., Tie, X., Tyndall, G., Wiedinmyer, C.,
 Baughcum, S.L., Kloster, S., 2010. Description and evaluation of the Model for Ozone and
- Related chemical Tracers, version 4 (MOZART-4). Geosci Model Dev, 3, 43-67.
 https://doi.org/10.5194/gmd-3-43-2010.
- Fageria, N.K., Santos, A.B., 2014. Lowland rice genotypes evaluation for nitrogen use efficiency. J
 Plant Nutr, 37, 1410-1423. https://doi.org/10.1080/01904167.2013.868482.
- Fagodiya, R.K., Kumar, A., Kumari, S., Medhi, K. and Shabnam, A.A., 2020. Role of Nitrogen and
- 776 Its Agricultural Management in Changing Environment, in: Naeem, M., Ansari, A., Gill S. (Eds.),
- 777 Contaminants in Agriculture. Springer, Cham. https://doi.org/10.1007/978-3-030-41552-5_12
- FAI 2018. Biofertiliser Statistics 2017-18, 10th Edn. New Delhi, India.
- FAI, 2020. Statistical Database: All-India Consumption of Fertiliser Nutrients 1950-51 to 2018-19.
 https://www.faidelhi.org/statistics/statistical-database (accessed 10 September 2020).
- 781 FAO, 2016. United Nations Food and Agriculture Organization, Statistics.
- 782 http://www.fao.org/faostat/ (accessed 15 October 2016).
- FAO, 2018. The future of food and agriculture Alternative pathways to 2050. United Nations Food
 and Agriculture Organization. Rome, Italy.
- Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A.,
- 786 Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K.,
- 787 Dentener, F., Stevenson, D., Amann, M., Voss, M., 2013. The global nitrogen cycle in the twenty-
- first century. Phil Trans Roy Soc Lond B Biol Sci, 368. https://doi.org/10.1098/rstb.2013.0164.

- Gaju, O., Allard, V., Martre, P., W. Snape, J., Heumez, E., Legouis, J., Moreau, D., Bogard, M.,
 Griffiths, S., Orford, S., Hubbart-Edwards, S., Foulkes, M., 2011. Identification of traits to
 improve the nitrogen-use efficiency of wheat genotypes. Field Crop Res, 123, 139-152.
 https://doi.org/10.1016/j.fcr.2011.05.010.
- Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby,
 B.J., 2003. The Nitrogen Cascade. BioScience, 53, 341-356. https://doi.org/10.1641/00063568(2003)053[0341:TNC]2.0.CO;2.
- Galloway, J., Raghuram, N., Abrol, Y.P., 2008a. A perspective on reactive nitrogen in a global,
 Asian and Indian context. Curr Sci, 94, 1375-1381.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A.,
 Seitzinger, S.P., Sutton, M.A., 2008b. Transformation of the Nitrogen Cycle: Recent Trends,
 Questions, and Potential Solutions. Science, 320, 889-892.
 https://doi.org/10.1126/science.1136674.
- Gayatri, Rani, M., Mahato, A.K., Sinha, S.K., Dalal, M., Singh, N.K., Mandal, P.K., 2018.
- Homeologue Specific Gene Expression Analysis of Two Vital Carbon Metabolizing Enzymes—
 Citrate Synthase and NADP-Isocitrate Dehydrogenase—from Wheat (Triticum aestivum L.)
- 805 Under Nitrogen Stress. Appl Biochem Biotechnol Enzym Eng Biotechnol.
- 806 https://doi.org/10.1007/s12010-018-2912-2.
- Guha, S., Sarkar, M., Ganguly, P., Uddin, M.R., Mandal, S., DasGupta, M., 2016. Segregation of
 nod-containing and nod-deficient bradyrhizobia as endosymbionts of Arachis hypogaea and as
 endophytes of Oryza sativa in intercropped fields of Bengal Basin, India. Environ Microbiol, 18,
 2575-2590. https://doi.org/10.1111/1462-2920.13348.
- Hakeem, K.R., Chandna, R., Ahmad, A., Qureshi, M.I. and Iqbal, M., 2012. Proteomic analysis for
 low and high nitrogen-responsive proteins in the leaves of rice genotypes grown at three nitrogen
 levels. Appl Biochem Biotechnol, 168, 834-850.
- Hakeem, K.R., Mir, B.A., Qureshi, M.I., Ahmad, A. Iqbal, M., 2013. Physiological studies and
 proteomic analysis for differentially expressed proteins and their possible role in the root of Nefficient rice (Oryza sativa L.). Mol Breed, 32, 785–798.
- HLPE 2014. Food losses and waste in the context of sustainable food systems. A report by the High
 Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security.
 Rome, Italy.
- Horta, L.P., Mota, Y.C.C., Barbosa, G.M., Braga, T.C., Marriel, I.E., de Fatima, A., Modolo, L.V.,
 2016. Urease Inhibitors of Agricultural Interest Inspired by Structures of Plant Phenolic
 Aldehydes. J Braz Chem Soc, 27, 1512-1519. https://doi.org/10.21577/0103-5053.20160208.
- Huber, D., Römheld, V., Weinmann, M., 2012. Relationship between nutrition, plant diseases and
 pests, in: Marschner, H. (Ed.), Mineral Nutrition of Higher Plants 3rd Edition. Science Press,
 Beijing, China, pp. 283-298.
- IRRI 1996. Use of leaf colour chart (LCC) for N management in rice. Crop Resource. Manage
 Network Technol Brief 2. Manila, Philippines.
- Krouk, G., Crawford, N.M., Coruzzi, G.M., Tsay, Y.-F., 2010. Nitrate signaling: adaptation to
 fluctuating environments. Curr Opin Plant Biol, 13, 265-272.
- 830 https://doi.org/10.1016/j.pbi.2009.12.003.

- Kumar Rao, J.V.D.K. and Dart, P. J., 1987. Nodulation, nitrogen fixation and nitrogen uptake in
 pigeon pea (Cajanus cajan (L.) Millsp) of different maturity groups. Plant Soil 99, 255-266.
- 833 https://doi.org/10.1007/BF02370872
- Ladha, J.K., Kirk, G.J.D., Bennett, J., Peng, S., Reddy, C.K., Reddy, P.M., Singh, U., 1998.
 Opportunities for increased nitrogen-use efficiency from improved lowland rice germplasm. Field
 Crop Res, 56, 41-71. https://doi.org/10.1016/s0378-4290(97)00123-8.
- Ladha, J.K., Tirol-Padre, A., Reddy, C.K., Cassman, K.G., Verma, S., Powlson, D.S., van Kessel, C.,
 de B. Richter, D., Chakraborty, D., Pathak, H., 2016. Global nitrogen budgets in cereals: A 50year assessment for maize, rice, and wheat production systems. Sci Rep, 6, 19355.
 https://doi.org/10.1038/srep19355.
- Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J., Garnier, J., 2014. 50 year trends in nitrogen use
 efficiency of world cropping systems: the relationship between yield and nitrogen input to
 cropland. Environ Res Lett, 9, 105011. https://doi.org/10.1088/1748-9326/9/10/105011.
- Li, P., Chen, F., Cai, H., Liu, J., Pan, Q., Liu, Z., Gu, R., Mi, G., Zhang, F., Yuan, L., 2015. A
 genetic relationship between nitrogen use efficiency and seedling root traits in maize as revealed
 by QTL analysis. J Exp Bot, 66, 3175-3188. https://doi.org/10.1093/jxb/erv127.
- Li, X., Zeng, R., Liao, H., 2016. Improving crop nutrient efficiency through root architecture
 modifications. J Integr Plant Biol, 58, 193-202. https://doi.org/10.1111/jipb.12434.
- López-Torrejón, G., Jiménez-Vicente, E., Buesa, J.M., Hernandez, J.A., Verma, H.K., Rubio, L.M.,
 2016. Expression of a functional oxygen-labile nitrogenase component in the mitochondrial matrix
 of aerobically grown yeast. Nat Comm, 7, 11426. https://doi.org/10.1038/ncomms11426.
- Mandal, V., Sharma, N., Raghuram, N., 2018. Molecular targets for improvement of crop nitrogenuse efficiency: Current and emerging options, in: Shrawat, A., Zayed, A. & Lightfoot, D. A.
 (Eds.), Engineering nitrogen utilization in crop plants. Springer, Cham, Switzerland, pp. 77-93.
- Morita, S., Suge, T., Yamazaki, K., 1988. The relationship between root length density and yield in
 rice plants. Jpn J Crop Sci, 57, 438-443. https://doi.org/10.1626/jcs.57.438.
- NEWS, 2018. INI commits to support a global goal to halve nitrogen waste by 2030. http://news india-uk.international/latest_news/our_ocean_conference_2018 (accessed 11 February 2019).
- Norton, G.J., Travis, A.J., Douglas, A., Fairley, S., Alves, E.D.P., Ruang-areerate, P., Naredo,
- 860 M.E.B., McNally, K.L., Hossain, M., Islam, M.R., Price, A.H., 2018. Genome Wide Association
- 861 Mapping of Grain and Straw Biomass Traits in the Rice Bengal and Assam Aus Panel (BAAP)
- Grown Under Alternate Wetting and Drying and Permanently Flooded Irrigation. Front Plant Sci,
 9, 1223. https://doi.org/10.3389/fpls.2018.01223.
- O'Connor, F.M., Johnson, C.E., Morgenstern, O., Abraham, N.L., Braesicke, P., Dalvi, M., Folberth,
 G.A., Sanderson, M.G., Telford, P.J., Voulgarakis, A., Young, P.J., Zeng, G., Collins, W.J., Pyle,
 J.A., 2014. Evaluation of the new UKCA climate-composition model Part 2: The Troposphere.
 Geosci Model Dev, 7, 41-91. https://doi.org/10.5194/gmd-7-41-2014.
- 868 PAU 2018. Package of practices for crops of Punjab. Ludhiana, India.
- 869 Peng, X., Yang, Y., Yu, C., Chen, L., Zhang, M., Liu, Z., Sun, Y., Luo, S., Liu, Y., 2015. Crop
- Management for Increasing Rice Yield and Nitrogen Use Efficiency in Northeast China. Agron J,
 107, 1682-1690. https://doi.org/10.2134/agronj15.0013.

- Peoples, M.B., Herridge, D.F. and Ladha, J.K., 1995. Biological nitrogen fixation: an efficient source
 of nitrogen for sustainable agricultural production? Plant and Soil, 174, 3-28.
- 874 https://doi.org/10.1007/BF00032239
- Raghuram, N., 2020. Zeroing in on farm budgets or zero budget natural farming? A perspective from
 India. Perspectives 37, UN Environment Programme.
- 877 https://www.unenvironment.org/resources/perspective-series/zeroing-farm-budgets-or-zero-
- budget-natural-farming-unep-perspective (accessed 10 September 2020).
- Raghuram, N. and Sharma, N., 2019. Improving Crop Nitrogen Use Efficiency, in: Moo-Young, M.,
 (Ed.), Comprehensive Biotechnology, Vol. 4. Elsevier: Pergamon, pp. 211–220.
 https://dx.doi.org/10.1016/B978-0-444-64046-8.00222-6
- Rao, D.L.N., 2014. Recent Advances in Biological Nitrogen Fixation in Agricultural Systems. Proc
 Indian Natn Sci Acad, 80, 359-378. https://doi.org/10.16943/ptinsa/2014/v80i2/55114.
- Rao, D.L.N. and Gill, H.S., 1995. Biomass production and nutrient recycling through litter from
 pigeonpea (Cajanus cajan L. Millsp.). Bioresour Technol, 54(2), 123-128.
 https://doi.org/10.1016/0960-8524(95)00102-6
- Rao, D.L.N., Balachandar, D., 2017. Nitrogen inputs from biological nitrogen fixation in Indian
 agriculture, in: Abrol, Y. P., Adhya, T.K., Aneja, V.P., Raghuram, N., Pathak, H., Kulshrestha, U.,
 Sharma, C., Singh, B. (Eds.), The Indian Nitrogen Assessment: Sources of Reactive Nitrogen,
 Environmental and Climate Effects, Management Options, and Policies. Elsevier, pp. 117-132.
- Rogers, E.D., Benfey, P.N., 2015. Regulation of plant root system architecture: implications for crop
 advancement. Curr Opin Biotechnol, 32, 93-98. http://doi.org/10.1016/j.copbio.2014.11.015.
- RySS, 2018. Andhra Pradesh: India's 1st Natural Farming State. Zero-Budget Natural Farming,
 Guntur, India.
- Sanchez-Canizares, C., Jorrin, B., Poole, P., Tkacz, A., 2017. Understanding the holobiont: the
 interdependence of plants and their microbiome. Curr Opin Microbiol, 38, 188-196.
 https://doi.org/10.1016/j.mib.2017.07.001.
- Sharma, L.K. and Bali, S.K., 2018. A Review of Methods to Improve Nitrogen Use Efficiency in
 Agriculture. Sustain 10, 51. https://doi.org/10.3390/su10010051
- 900 Sharma, N., Sinha, V.B., Gupta, N., Rajpal, S., Kuchi, S., Sitaramam, V., Parsad, R., Raghuram, N.,
- 2018. Phenotyping for Nitrogen Use Efficiency: Rice Genotypes Differ in N-Responsive
- Germination, Oxygen Consumption, Seed Urease Activities, Root Growth, Crop Duration, and
 Yield at Low N. Front Plant Sci, 9. https://doi.org/10.3389/fpls.2018.01452.
- Sheldrake, A.R. and Narayanan, A., 1979. Growth, development and nutrient uptake in pigeonpeas
 (Cajanus cajan). J Agric Sci, 92, 513-526. https://doi.org/10.1017/S0021859600053752
- Simpson, D., Benedictow, A., Berge, H., Bergström, R., Emberson, L.D., Fagerli, H., Flechard, C.R.,
 Hayman, G.D., Gauss, M., Jonson, J.E., Jenkin, M.E., Nyíri, A., Richter, C., Semeena, V.S.,
 Tsyro, S., Tuovinen, J.P., Valdebenito, Á., Wind, P., 2012. The EMEP MSC-W chemical
- transport model; technical description. Atmos Chem Phys, 12, 7825-7865.
- 910 https://doi.org/10.5194/acp-12-7825-2012.
- 911 Sinha, S.K., Rani, M., Bansal, N., Gayatri, Venkatesh, K., Mandal, P.K., 2015. Nitrate Starvation
- 912 Induced Changes in Root System Architecture, Carbon:Nitrogen Metabolism, and miRNA
- 913 Expression in Nitrogen-Responsive Wheat Genotypes. Appl Biochem Biotechnol Enzym Eng
- Biotechnol, 177, 1299-1312. https://doi.org/10.1007/s12010-015-1815-8.

- 915 Sinha, S.K., Rani, M., Kumar, A., Kumar, S., Venkatesh, K., Mandal, P.K., 2018. Natural variation
- 916 in root system architecture in diverse wheat genotypes grown under different nitrate conditions
- 917 and root growth media. Theor Exp Plant Physiol, 30, 223-234. https://doi.org/10.1007/s40626918 018-0117-2.
- Smith, J., Yeluripati, J., Smith, P. and Nayay, D.R., 2020. Potential yield challenges to scale-up of
 zero budget natural farming. Nat Sustain, 3, 247–252. https://doi.org/10.1038/s41893-019-0469-x
- Steffens, B., Rasmussen, A., 2016. The Physiology of Adventitious Roots. Plant Physiol, 170, 603.
 https://doi.org/10.1104/pp.15.01360.
- Subedi, K.D., Ma, B.L., Xue, A.G., 2007. Planting Date and Nitrogen Effects on Grain Yield and
 Protein Content of Spring Wheat. Crop Sci, 47, 36-44.
 https://doi.org/10.2135/cropsci2006.02.0099.
- Sun, H., Qian, Q., Wu, K., Luo, J., Wang, S., Zhang, C., Ma, Y., Liu, Q., Huang, X., Yuan, Q., Han,
 R., Zhao, M., Dong, G., Guo, L., Zhu, X., Gou, Z., Wang, W., Wu, Y., Lin, H., Fu, X., 2014.
 Heterotrimeric G proteins regulate nitrogen-use efficiency in rice. Nat Genet, 46, 652.
 https://doi.org/10.1038/ng.2958.
- Sutton, M.A., Howard, C.M., Erisman, J.W., Bealey, W.J., Billen, G., Bleeker, A., Bouwman, A.F.,
 Grennfelt, P., van Grinsven, H., Grizzetti, B., 2011. The challenge to integrate nitrogen science
 and policies: the European Nitrogen Assessment approach, in: Sutton, M. A., Howard, C. M.,
 Erisman, J. W., Billen, G., Bleeker, A., Grennfelt, P., Van Grinsven, H. & Grizzetti, B. (Eds.), The
 European Nitrogen Assessment: Sources, Effects and Policy Perspectives. Cambridge University
- 935 Press, Cambridge, UK, pp. 82-96.
- 936 Sutton, M.A., Bleeker, A., Howard, C.M., Bekunda, M., Grizzetti, B., deVries, W., vanGrinsven,
- 937 H.J.M., Abrol, Y.P., Adhya, T.K., Billen, G., Davidson, E.A., Datta, A., Diaz, R., Erisman, J.W.,
- 238 Liu, X.J., Oenema, O., Palm, C., Raghuram, N., Reis, S., Scholz, R.W., Sims, T., Westhoek, H.,
- 239 Zhang, F.S., with contributions from Ayyappan, S., Bouwman, A.F., Bustamante, M., Fowler, D.,
- 940 Galloway, J.N., Gavito, M.E., Garnier, J., Greenwood, S., Hellums, D.T., Holland, M., Hoysall, 941 C. Jaramillo, V.L. Klimont, Z. Ornetto, J.D. Bethel, H. Pless, E. J. Let, W. D. J.
- 941 C., Jaramillo, V.J., Klimont, Z., Ometto, J.P., Pathak, H., Plocq Fichelet, V., Powlson, D., 942 Bergelwicker K. Berg, A. Standard, K. Sharma, C. Si, J. D. Si, J. W. X. Zi, J. J. Standard, K. Sharma, C. Si, J. B. Si, J. Standard, K. Sharma, C. Si, J. B. Si, J. Standard, K. Sharma, C. Si, J. Standard, K. Sharma, C. Si, J. Standard, K. Sharma, C. Si, J. Standard, K. Sharma, S. Standard, S. Sharma, Sharma, S. Sharma, S. Sharma, Sharma, Sharma, Sharma, Sharm
- Ramakrishna, K., Roy, A., Sanders, K., Sharma, C., Singh, B., Singh, U., Yan, X.Y., Zhang, Y.,
- 943 2013. Our Nutrient World: The Challenge to Produce More Food and Energy With Less Pollution.
 944 Global Overview of Nutrient Management. Centre for Ecology and Hydrology on behalf of the
- Global Partnership on Nutrient Management and the International Nitrogen Initiative, Edinburgh,
 United Kingdom.
- Sutton, M.A., Drewer, J., Moring, A., Adhya, T.K., Ahmed, A., Bhatia, A., Brownlie, W., Dragosits,
 U., Ghude, S.D., Hillier, J., Hooda, S., Howard, C.M., Jain, N., Kumar, D., Kumar, R.M., Nayak,
 D.R., Neeraja, C.N., Prasanna, R., Price, A., Ramakrishnan, B., Reay, D.S., Singh, R., Skiba, U.,
- 950 Smith, J.U., Sohi, S., Subrahmanyan, D., Surekha, K., van Grinsven, H.J.M., Vieno, M., Voleti,
- 951 S.R., Pathak, H., N., R., 2017. The Indian Nitrogen Challenge in a Global Perspective, in: Abrol,
- 952 Y. P., Adhya, T.K., Aneja, V.P., Raghuram, N., Pathak, H., Kulshrestha, U., Sharma, C., Singh,
- 953 B. (Ed.), The Indian Nitrogen Assessment: Sources of Reactive Nitrogen, Environmental and
- 954 Climate Effects, Management Options, and Policies. Elsevier, pp. 9-28.
- 955 TFRN, 2020. Guidance Document on Integrated Sustainable Nitrogen Management. UNECE
- 956 Convention on Long-range Transboundary Air Pollution, Geneva.
- 957 http://www.unece.org/fileadmin/DAM/env/documents/2020/AIR/WGSR/Final_For_submission_
- 958 Draft_guidance_23_June.pdf (accessed 10 September 2020).

- 959 TIGR2RESS, 2019. TIGR2ESS: Transforming India's Green Revolution by Research and
- 960 Empowerment for Sustainable food Supplies. https://tigr2ess.globalfood.cam.ac.uk/ (accessed 25
 961 April 2019).
- Trotman, A.P. and Weaver, R.W., 1995. Tolerance of clover rhizobia to heat and desiccation stresses
 in soil. Soil Sci Soc Am J, 59, 466-470.
- 964 https://doi.org/10.2136/sssaj1995.03615995005900020028x
- 965 Uwizeye, U.A., Gerber, P.J., Schulte, R.P.O., Boer, I.J.M.d., 2016. A comprehensive framework to
 966 assess the sustainability of nutrient use in global livestock supply chains. J Clean Prod, 129, 647967 658. https://doi.org/10.1016/j.jclepro.2016.03.108.
- Vanlauwe, B., Hungria, M., Kanampiu, F. and Giller, K.E., 2019. The role of legumes in the
 sustainable intensification of African smallholder agriculture: Lessons learnt and challenges for
 the future. Agric, Ecosyst & Environ, 284, 106583. https://doi.org/10.1016/j.agee.2019.106583
- Varinderpal-Singh, Yadvinder-Singh., Bijay-Singh, Baldev, S., Gupta, R.K., Jagmohan, S., Ladha,
 J.K., Balasubramanian, V., 2007. Performance of site-specific nitrogen management for irrigated
 transplanted rice in northwestern India. Arch Agron Soil Sci, 53, 567-579.
 10.1080/03650340701568971.
- Varinderpal-Singh, Yadvinder-Singh., Bijay-Singh, Thind, H.S., Kumar, A., Vashistha, M., 2011.
 Calibrating the leaf colour chart for need based fertilizer nitrogen management in different maize
 (Zea mays L.) genotypes. Field Crop Res, 120, 276-282. https://doi.org/10.1016/j.fcr.2010.10.014.
- Varinderpal-Singh, Bijay-Singh, Yadvinder-Singh, Thind, H.S., Gobinder, S., Satwinderjit, K.,
 Kumar, A., Vashistha, M., 2012. Establishment of threshold leaf colour greenness for need-based
 fertilizer nitrogen management in irrigated wheat (Triticum aestivum L.) using leaf colour chart.
 Field Crop Res, 130, 109-119. https://doi.org/10.1016/j.fcr.2012.02.005.
- Varinderpal-Singh, Bijay-Singh, Thind, H.S., Yadvinder-Singh, Gupta, R.K., Satinderpal-Singh,
 Meharban-Singh, Kaur, S., Manmohanjit-Singh, Brar, J.S., Angrez-Singh, Jagmohan-Singh, AjayKumar, Sukhwinder-Singh, Kaur, A., Balasubramanian, 2014. Evaluation of leaf colour chart for
 need-based nitrogen management in rice, maize and wheat in north-western India. J Res Punjab
 Agr Univ, 51, 239-245.
- Varinderpal-Singh, Bijay-Singh, Yadvinder-Singh, Thind, H.S., Buttar, G.S., Kaur, S., Meharban, S.,
 Kaur, S., Bhowmik, A., 2017. Site-specific fertilizer nitrogen management for timely sown
 irrigated wheat (Triticum aestivum L. and Triticum turgidum L. ssp durum) genotypes. Nutr Cycl
 Agroecosys, 109, 1-16. https://doi.org/10.1007/s10705-017-9860-z.
- Vidal, E.A., Gutiérrez, R.A., 2008. A systems view of nitrogen nutrient and metabolite responses in
 Arabidopsis. Curr Opin Plant Biol, 11, 521-529. https://doi.org/10.1016/j.pbi.2008.07.003.
- Vieno, M., Heal, M.R., Williams, M.L., Carnell, E.J., Nemitz, E., Stedman, J.R., and Reis, S., 2016.
 The sensitivities of emissions reductions for the mitigation of UK PM_{2.5}, Atmos Chem Phys, 16, 265–276. https://doi.org/10.5194/acp-16-265-2016.
- Wang, J., Vanga, K.S., Saxena, R., Orsat, V., Raghavan, V., 2018. Effect of Climate Change on the
 Yield of Cereal Crops: A Review. Climate, 6. https://doi.org/10.3390/cli6020041.
- 998 World Bank, 2020a. Data: GDP (current US\$).
- 999 https://data.worldbank.org/indicator/NY.GDP.MKTP.CD?locations=IN (accessed 3 September 2020).

- 1001 World Bank, 2020b. Data: Population, total.
- 1002 https://data.worldbank.org/indicator/SP.POP.TOTL?locations=IN (accessed 3 September 2020).
- Yang, J.-c., Zhang, H., Zhang, J.-h., 2012. Root Morphology and Physiology in Relation to the Yield
 Formation of Rice. J Integr Agr, 11, 920-926. <u>https://doi.org/10.1016/S2095-3119(12)60082-3</u>.
- 1005 Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y., 2015.
- 1006 Managing nitrogen for sustainable development. Nature, 528, pp. 51–59.
- 1007 https://doi.org/10.1038/nature15743





Figure 2.JPEG









Figure 6.JPEG





Figure 8.JPEG

