

Chapter 12 Nitrogen and Stress

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[AU1] Nitrogen is an important macroelement for plant growth. However, plant can't use atmospheric nitrogen as such and depend on its availability in more reactive forms such as urea, nitrate, ammonium, amino acids, etc. Even legumes depend on symbiotic N-fixing bacteria to convert N_2 into ammonium ions to meet their N requirements. Therefore, the term nitrogen (N) is used in this review to represent a broad range of reactive species of N compounds. In agricultural soils, N compounds and other nutrients have to be constantly replenished as fertilizers/manures to enable repetitive cropping. As N fertilizers are expensive, N-use efficiency becomes an important determinant of crop productivity.

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Since precision farming techniques to balance the plant nutrient demand with fertilizer supply are not accessible/affordable to farmers in developing countries, the actual availability of N to the crop plant varies from N-deficient state to N-excess state, depending on prevailing fertilizing practices. Both these states can cause nutrient stress to the plant. In addition, loss of reactive N species from the soil-plant system causes widespread environmental stresses, not only through N pollution of ground water and surface water bodies affecting health, biodiversity, and ecosystem services but also through air pollution and climate change (Sutton and Bleeker 2013).

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[AU2] In fact, N_2O as a greenhouse gas is 300 times more potent than CO_2 (Galloway et al. 2008), though carbon dominates the entire climate change discussion. Climate change itself causes/exacerbates abiotic stresses to the plant and its N status (Fig. 12.1).

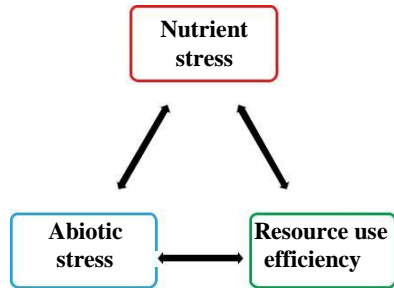
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Nitrogen-use efficiency is therefore not only an economic problem of optimizing input costs to the farmer but also an environmental problem of preventing accumulation of reactive N species outside the agroecosystem. Accordingly, the relationship between plant, nitrogen, and stress is twofold: nutritional stress in terms of plant growth/development/productivity due to variation in N availability or climate and

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Fig. 12.1 Relationship between nutrient stress, abiotic stress, and resource-use efficiency



29 N-induced environmental stress (and climate change that in turn affects the plant),
 30 which can be exacerbated due to N-inefficient cultivars and/or practices. The primary
 31 focus of this book chapter is nutritional stress, but N-use efficiency is a common
 32 concern that links both nutritional and environmental aspects of reactive nitrogen.

33 12.1 Nitrogen Nutrition and N-Use Efficiency

34 Nitrogen-use efficiency has been defined in many different ways by agronomists,
 35 physiologists, and others (Good et al. 2004; Pathak et al. 2008; Hirose 2011). The
 36 simplest among them is total biomass or grain yield per unit N fertilizer added (or
 37 N available in the soil). Improvement in NUE is possible to some extent by non-
 38 plant interventions such as the choice of fertilizer and method/timing of its applica-
 39 tion and other crop management practices. But only biological avenues for crop
 40 improvement are the main focus of this article, whether in relation to NUE or stress
 41 resistance. Plants show improved NUE in N-limiting conditions (Kant et al. 2011).
 42 In other words, a plant that gives the same or higher agronomic output with lesser N
 43 input is considered more N-use efficient than the plant that needs higher N input.

[AU3]44 But what constitutes yield varies from crop to crop, such as grains in cereals or
 45 leaves/fruits/tubers in vegetables, and accordingly what constitutes NUE and how to
 46 improve it in each case (see Chardon et al. 2012 for a recent review). This is also
 47 often true for stress resistance, if it is measured in terms of the impact of stress on
 48 yield, keeping in view the multiplicity of stresses involved. Unfortunately, many of
 49 the high-yielding and/or upmarket varieties of the green revolution era were neither
 50 designed for N-use efficiency nor stress resistance, whereas the farmers selected
 51 traditional cultivars that were more robust to such factors, even if they yielded less.
 52 This means that crop improvement strategies for NUE and stress resistance cannot
 53 be limited to the narrow germplasm of high-yielding varieties and have to include
 54 the wild/traditional varieties.

55 It is well known that NUE is an inherited, multigenic, quantitative trait. A study
 56 of natural variation of N uptake and metabolism in 18 accessions of *Arabidopsis*
 57 under high- and low-N conditions showed that while plants may vary in the way
 58 they respond to high- or low-N conditions, their NUE remained similar, indicating
 59 that NUE as a trait is exclusively genetically determined (Chardon et al. 2010,
 60 2012). However, the multiplicity of the definitions of NUE, combined with its poor

biological characterization at the phenotypic or genotypic level makes it difficult to study the impact of stress on NUE. Nevertheless, any discussion on nitrogen and stress has to be understood in terms of the impact of abiotic or nutritional stress on NUE. For example, elevated CO₂ could enhance NUE in some cases (Shimono and Bunce 2009), while heat or water stress could adversely impact NUE (Harrigan et al. 2009), as does wasteful use of N fertilizer. Understanding the various mechanisms underlying these complex interactions will equip us with the means to maintain crop productivity in a changing climate.

12.1.1 Nitrogen Uptake and Metabolism

Plant nitrogen (N) nutrition is a complex and dynamic process, as the plant has to be able to assimilate various forms/amounts of nitrogen in fluctuating micro- and macroenvironments. This can happen even in fertilized soils, depending on the soil N status, nature of the fertilizer used (organic/inorganic), the frequency of their application, and the action of nitrifying bacteria in the soil. Organic N sources (manures/urea) are broken down by nitrifying bacteria to inorganic compounds such as nitrates and ammonium salts, which are the preferred forms of N uptake for most plants, though amino acids can be used under extremely N-poor and cold conditions.

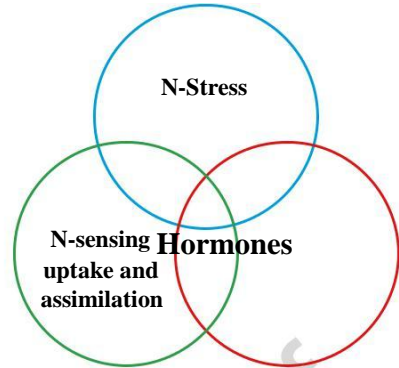
Plants uptake nitrate primarily through the high-/low-affinity transport systems (HATS/LATS) in the roots and are mostly inducible by nitrate and regulated by its downstream metabolites, hormones, stress, etc., except the constitutive HATS (Tsay et al. 2007). The signaling mechanisms involved in the nitrate regulation of NO₃⁻ transporter genes need to be elucidated fully, although few genes in *Arabidopsis* (NRT1.1, NLP7, and CIPK8) have been suggested to play a crucial role (Castaings et al. 2009). NRT1.1 is termed “transceptor” since it is both a transporter as well as a receptor for N signal (Gojon et al. 2011). There are also transporters for ammonium and urea, but their relative contribution to the overall external N acquisition by plants and their regulation are far less understood, despite the growing interest (Vert and Chory 2009; Näsholm et al. 2009; Bouguyon et al. 2012; Wang et al. 2012). Some aspects of N transport under situations of stress have been mentioned later in this chapter, but one can expect a lot more activity in this area in the coming years.

The N compounds taken up by the roots are distributed throughout the plant, where they enter the cellular nitrate assimilatory pathway. Nitrate is reduced in the cytosol by the enzyme nitrate reductase to nitrite, which is transported to the chloroplast, where it is further reduced to ammonium ions by nitrite reductase. The ammonium ions are incorporated into organic acids to form amino acids, through the glutamine synthase and glutamate synthetase (GS-GOGAT cycle) and various transaminases. This is the primary N metabolic pathway in plants, but secondary N remobilization can occur during senescence, in which cytosolic isoforms of GS and GOGAT also play important roles (Xu et al. 2012). Secondary N remobilization to recycle nutrients from senescing leaves could be of critical importance for grain filling and yield in cereal crops (Kichey et al. 2007). Signaling mechanisms play a key role in N metabolic regulation to optimize the N budget under varying situations of plant N demand and supply.

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Fig. 12.2 Overlap between N stress, hormones, and N sensing, uptake, and assimilation



104 **12.1.2 Signaling in N Metabolic Regulation**

105 Nitrate is not only a nutrient but also a signal for plant metabolic regulation, growth,
 106 and development (Stitt 1999). Other forms of N such as ammonium and glutamine
 107 have also been shown to have signaling roles, but nitrate remains the best-studied
 108 form of N signal, both at the local and systemic levels. Locally, it affects root growth,
 109 seed dormancy, and flowering time, but also has systemic effects on an organism-wide
 110 basis (Ho and Tsay 2010). For example, nitrate has been shown to induce genome-
 111 wide changes in gene expression in model plants such as Arabidopsis (Wang et al.
 112 2003; Scheible et al. 2004), rice (Lian et al. 2006; Cai et al. 2012), maize (Trevisan
 113 et al. 2011), and tomato (Wang et al. 2001), involving hundreds, if not thousands, of
 114 genes. They include various nitrate transporters, enzymes of nitrate assimilation, car-
 115 bon and redox metabolism, several protein kinases, cytochrome families, transcription
 116 factors, etc. The search for nitrate response elements (NREs) in the upstream sequences
 117 of a few nitrate-responsive genes has not yet produced a universally accepted consen-
 118 sus sequence that accounts for most, if not all nitrate-responsive genes found to date
 119 (Das et al. 2007; Konishi and Yanagisawa 2010, 2011; Pathak et al. 2011; Krapp et al.
 120 2014). Similarly, several transcription factors are implicated in mediating nitrate
 121 response, such as ANR1, DOF1/2, LDB37/38/39, NLP6/7, and SPL9 (reviewed in
 122 Krapp et al. 2014), and further research is needed to narrow them down.

123 It is believed that the combinatorial action of local and systemic signaling deter-
 124 mines the ability of a plant to adapt to fluctuating environments (Krouk et al. 2011;
 125 Alvarez et al. 2012; Huang et al. 2012). However, the mechanism of nitrate signal-
 126 ing continues to evade scientists for several decades. Signaling is also involved in
 127 stress response, whether due to N deficiency/excess or due to other abiotic stresses,
 128 impinging on N metabolic regulation. The pathways for stress and nutrient signaling
 129 may either be separate or shared, depending on the specific stress in question, which
 130 will further define whether manipulation of one will impact the response to the other
 131 (Fig. 12.2). This is an issue of crucial agronomic relevance, which can only be
 132 addressed when the signaling mechanisms connecting N metabolic regulation and
 133 stress are better elucidated. Some developments in this regard are elaborated below.

12.2 Nitrogen and Stress

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Nitrogen stress is caused by extreme fluctuations in the soil N level or due to the formation of nitroso compounds in the plant as a consequence of other stresses. The normal intracellular nitrate concentration is in the micromolar range and soil N concentration up to multi-millimolar range fall within the nutritional range (and therefore also the tolerance range) of most plants. N-limitation or N-deficiency stress occurs when soil N levels fall below the sub-millimolar range, eventually leading to N starvation. N-excess conditions require N levels to increase beyond 40 mM, though the precise threshold varies depending on the plant, duration of exposure, soil type, organic content, microbial activity, cropping practices, and climate.

Plants respond in many different ways to changes in N provision (Krouk et al. 2010; Kraiser et al. 2011; Kant et al. 2011). Their responsiveness to N availability depends on both genotype and the interaction of genotype with N fertilization level (Gallais and Hirel 2004; Chardon et al. 2010). They can adjust their molecular machinery in accordance with N nutritional status or abiotic stress, often rapidly and sometimes indefinitely (Krapp and Kaiser 2010). For example, plants respond to N starvation or deficiency by changes such as increase in the root to shoot ratio by enhancing lateral root growth or suppressing shoot growth or early senescence of leaves (Marschner 1995). Characterization of the machinery responsible for N homeostasis in stress helps to identify appropriate sites of intervention for crop improvement. This machinery includes, but is not limited to, the affinity-based N transport systems such as LATS and HATS, which can be reprogrammed to achieve N homeostasis at the local or whole plant level. Several studies have shown that the root uptake capacities for nitrate, ammonia, and urea are strongly downregulated under conditions of N excess and upregulated during N starvation or N limitation (Tsay et al. 2007; Nacry et al. 2013). However, going beyond transporters, it seems that the limiting steps in plant N metabolism are different under high and low N levels (Coque and Gallais 2006). Moreover, while studies that report "high" N often do not make clear distinction between N sufficiency and N excess, studies that report "low" N either deal with N limitation or N starvation but not both, making it necessary to discuss them in their own separate context.

12.2.1 Nitrogen Starvation/Limitation

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Nitrogen-starved plants show shunted growth; chlorosis; poor yield; poor pigmentation due to carbohydrate accumulation, anthocyanin induction, and phenylpropanoid biosynthesis; ~~and decreased photosynthesis, leaf number, and overall plant development.~~ Studies in which nitrate was supplied to nitrate-starved plants like Arabidopsis and rice (Wang et al. 2000, 2003; Scheible et al. 2004; Lian et al. 2006; Cai et al. 2012) showed the involvement of genes from N/C metabolism, redox metabolism, hormonal response, etc. Many gene families such as cytochrome, protein kinases, and hormone/nutrient transporter were differentially regulated by nitrate in both rice

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174 and Arabidopsis (Cai et al. 2012). Various N transporters such as NRT2.1 and NRT2.2
175 for nitrate; AMT1.1, AMT1.2, and AMT1.3 for ammonia (Tsay et al. 2007); and
176 DUR3 for urea (Kojima et al. 2007) were differentially regulated by N source/avail-
177 ability/concentration. The expression of GLN and GDH genes (Masclaux-Daubresse
178 et al. 2005) were also altered during N starvation. The possible role of NLP7 as a key
179 regulator in N-starved conditions has been suggested recently (Marchive et al. 2013).
180 Studies in Arabidopsis and maize have shown that chronic N limitation elicits a
181 genome-wide response and the genes involved are far more differentially regulated
182 than genes supplied with sufficient N (Bi et al. 2007; Wu et al. 2011).

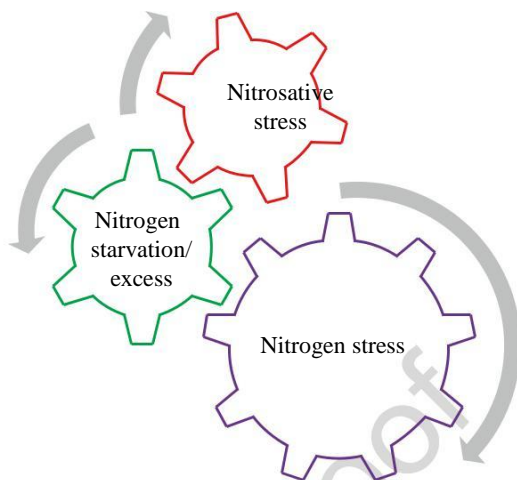
183 In the low concentration range such as 1 μ M, high-affinity transport systems
184 (HATS) are able to scavenge ions from the soil. During N starvation or limitation,
185 NRT1.1 represses lateral root in Arabidopsis by remobilizing auxin, mimicking the
186 role of an auxin transporter (Krouk et al. 2010). Low concentrations of ammonia
187 have also been known to strongly regulate nitrate transport systems. NRT1.1 mutant
188 studies have shown that a protein kinase CIPK23 phosphorylates NRT1.1 during
189 nitrate limitation, thereby influencing primary nitrate response (Ho et al. 2009). The
190 rate of N uptake in roots is determined by ionic concentration (Tsay et al. 2007),
191 which is influenced by various stress conditions (Segonzac et al. 2007). Recently, it
192 was reported that in maize, N-deficiency stress resembled the response of plants to
193 a number of other biotic and abiotic stresses, in terms of transcript, protein, and
194 metabolite accumulation (Amiour et al. 2012). In rice, two proteins, fibrillin and
195 hairpin-binding protein, have been identified previously as N-deficiency stress-
196 responsive proteins (Song et al. 2011; Amiour et al. 2012).

197 **12.2.2 Nitrogen Excess**

198 In natural and in well-managed agricultural soils, excess N concentrations are rarely
199 found for long, due to microbial conversions, surface runoff, volatilization, or leach-
200 ing, apart from plant uptake. For example, although urea application in excess of
201 100 kg/ha is very common in intensively cultivated areas, its effective concentra-
202 tions are often <70 mM in agricultural soils (Wang et al. 2008). Reaching far higher
203 concentrations that contribute to nonspecific osmotic stress effects is only possible
204 when other solutes are also high, such as in saline soils. This is also true for the con-
205 version products of urea, viz., nitrate/nitrite/ammonium, whose ionic effects saturate
206 in the millimolar range, and they rarely reach 100-fold levels needed to have any
207 osmotic effect on their own. However, they can influence the pH of the soil tempo-
208 rarily, though the extent and duration of that influence on the soil as well as on the
209 plant depend on the soil type/conditions and the plant itself. In any case, the ionic/
210 pH/osmotic effects are indirect and generic effects that are not specific to N and
211 therefore cannot be strictly considered as N stress. Terms such as N "sufficiency" or
212 "excess" or "high N" have to be understood in this context, as they are often used
213 interconvertibly, mainly to contrast with N limitation/starvation (Fig. 12.3).

214 Genes from transporter families have shown altered gene expression during
215 chronic N stress probably because plants need to adjust to the varying levels of N

Fig. 12.3 Nitrogen stress caused by nitrogen starvation/excess and nitrosative stress



available to plants. In the high concentration range, the activity of low-affinity transport systems (LATS) from the large family of transporters for nitrate (NRT1) and peptides (PTR) plays a major role (Tsay et al. 2007). Unlike with the HATS, the LATS-mediated nitrate or ammonia uptake (or influx) does not saturate and shows a generally linear increase with increasing external concentration (Touraine and Class 1997; Nacry et al. 2013). But they could accumulate in plant cells, unless their assimilation can match the uptake. This necessitates a mechanism to regulate cytosolic nitrate concentration, which is provided by a nitrate-inducible efflux system that prevents excessive accumulation of nitrate in the cell (Miller et al. 2007). An efflux transporter, NAXT1, was recently identified belonging to the NRT1/PTR family of transporters (Segonzac et al. 2007; Chapman and Miller 2011).

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Plants also have a capacity to store N in vacuoles as a way of balancing between uptake, assimilation, and translocation to other parts of the plant and minimize losses through efflux/volatilization. Some vegetables grown under excess N conditions have a tendency to accumulate N (Chen et al. 2004; Anjana et al. 2007) that enters our food chain. There is a growing attention toward the adverse health effects of excessive dietary exposure to nitrate and other forms of reactive N, including methemoglobinemia, gastric cancer, respiratory ailments, cardiac disease, etc. (Townsend et al. 2003; Anjana and Iqbal 2007).

12.2.3 Nitrosative Stress

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Reactive nitrogen species (RNS) include NO and related molecules such as S-nitrosothiols (SNOs), S-nitrosoglutathione (GSNO), peroxyntirite (ONOO⁻), dinitrogen trioxide (N₂O₃), and nitrogen dioxide (NO₂). During adverse environmental conditions, these molecules can cause stress to plants, which is designated as nitrosative stress (Fig. 12.3). This can combine with the stress caused by reactive

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241 oxygen species (ROS) to form nitro-oxidative stress in plants. Like ROS, the role of
 242 RNS signaling has been implicated in many abiotic stresses such as salinity, water
 243 stress, temperature stress, and UV radiation. Evidence for RNS signaling in certain
 244 abiotic stresses like salinity and heat stress was strictly species or treatment specific,
 245 and the literature is inconclusive, if not contradictory (Corpas et al. 2011). In other
 246 stresses such as UV-radiation and ozone stress, there is a marked increase in the
 247 activity of RNS species, which leads to cell death (Corpas et al. 2011). The role of
 248 RNS signaling needs to be studied further to understand the changes in plant physi-
 249 ology under stress.

250 12.3 Nitrogen in Abiotic Stresses

251 Nitrogen availability depends on plant–soil–microbe interactions, whereas nitrogen
 252 acquisition is basically driven by transpiration, which is in turn affected by tempera-
 253 ture and levels of CO₂. Climate change models predict that elevated levels of green
 254 house gases (GHGs) like CO₂, CH₄, and N₂O cause increased variability in tempera-
 255 ture, humidity, precipitation, wind velocity, and photosynthetically active radiation,
 256 all leading to major abiotic stresses such as drought, heat/cold stress, waterlogging,
 257 etc. (IPCC 2007; Bloom et al. 2010). All these stresses affect plant phenology and
 258 also alter nitrogen availability and its uptake/retention (Fig. 12.4). For example,
 259 Borner et al. (2008) observed that the snow depth in tundra regions affect N mineral-
 260 ization directly. Volatilization of reactive N from the plant further contributes to GHG
 261 accumulation, completing the vicious cycle. The role of reactive N in plant stress can
 262 be understood in the context of specific stresses as given below or integrated into a
 263 network of multiple interacting stresses, as elaborated later in this chapter.

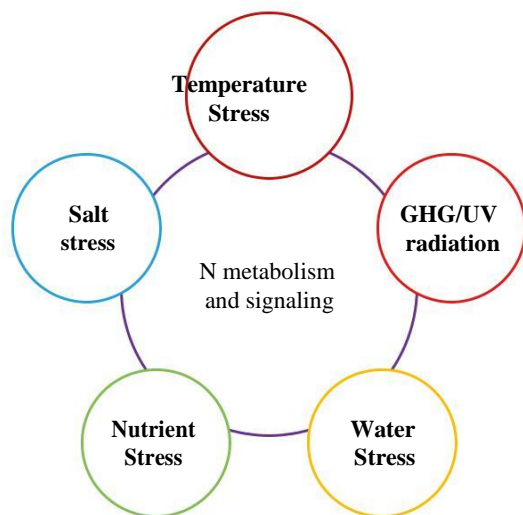


Fig. 12.4 N metabolism and signaling play an integral role in various abiotic stresses

12.3.1 N in Elevated CO₂ and NO₂

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Continuous exposure to elevated atmospheric CO₂ may result in stomatal closure, adversely affecting the rate of transpiration and therefore nutrient uptake of the plants, leading to nutrient N deficiency stress. Decrease in activities of assimilatory enzymes such as nitrate reductase (Ferrario-Méry et al. 1997) and RuBisCO (Bloom et al. 2010) was also observed. However, brief exposure to elevated CO₂ showed enhanced activities of NR and GSA in cucumber and sunflower leaves (Aguera et al. 2006). Inhibition of photorespiration-dependent nitrate assimilation (Rachmilevitch et al. 2004) is also observed at higher levels of CO₂. Elevated CO₂ is known to enhance photosynthesis in C₃ plants and improve NUE (Shimono and Bunce 2009). The form of N used under various CO₂ concentrations affect the nutrients and their distribution in the plant (Natali et al. 2009). For example, wheat plants supplied with ammonium salts as a source of N were more N responsive under elevated CO₂ concentrations, in terms of nutrient accumulation, yield, and yield components, as compared to those supplied with nitrate (Carlisle et al. 2012 and references therein).

Nitrogen dioxide can be absorbed and utilized by the plants in small quantities for assimilation (Mokhele et al. 2012) and therefore elevated NO₂ causes some increase in intracellular nitrate concentration (Qiao and Murray 1998). On the other hand, reduction of ambient NO₂ level has no effect on the organic N content of the plants or on the amount or rate of N uptake in the plants.

12.3.2 N in Water and Salt Stress

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Drought stress alone is projected to double in future, which will lead to loss of yield (IPCC 2007). While photosynthesis can be maintained under fluctuations of water supply (Lightfoot et al. 2007), water deficit can alter the C and N transformations by bringing about changes in the soil–microbe interactions such as reducing the activity of nitrifying bacteria (StClair and Lynch 2010 and references therein). This is evident from the inhibition of nitrogen fixation in legume crops during C and N fluxes under drought (Ladrera et al. 2007; Rogers et al. 2009).

The effect of drought on leaf N status remains uncertain, as it was reported to increase in *Malus domestica* (Jie et al. 2010), decrease in *Prunus persica* (Dichio et al. 2007), and be unaffected in *Quercus* (Li et al. 2013). However, a recent transcriptome study suggests the interactive effects on the genome-wide impact of drought and N limitation in maize (Humbert et al. 2013). It studied 30 conditions involving three major parameters such as organ (leaf, root, or stem), nitrogen supply (optimal or chronic limitation), and water supply (optimal supply, mild water stress by withdrawal for 3 days, severe water stress by withdrawal for 5 days, and recovery from severe stress by rewatering for 2 h or 5 h). The impact of severe stress was more extensive in root and stem than in leaf, in terms of the number of spots/genes affected. The pathways most affected were sucrose and starch metabolism, Calvin cycle, proline, and asparagine biosynthesis. Both photosynthetic assimilation and

304 nitrate assimilation were shown to be downregulated. The effect of water withdrawal
305 and nitrogen limitation on ammonium assimilation was tissue specific; the tran-
306 scripts for glutamine and glutamate synthases were more in leaf as compared to stem
307 and root. This study also shows that while nitrogen limitation has very little impact
308 on the transcriptome on its own (0.2 % of the spots), even mild water stress makes
309 the plant more vulnerable to N limitation, affecting the expression of a much larger
310 number of genes (Humbert et al. 2013). These observations need to be validated in
311 other plants before wider generalizations could be made. However, studying such
312 interactive effects could be useful to optimize NUE along with water-use efficiency
313 (WUE) (Di Paolo and Rinaldi 2008). On the other hand, heavy use of N fertilizers
314 regardless of the water regime can be detrimental on grain filling and drought toler-
315 ance (Humbert et al. 2013 and references therein).

316 Drought tolerance genes contribute to greater NUE because they improve bio-
317 mass production over an extended range of soil moisture availability and weather
318 conditions (Harrigan et al. 2009). The traits for drought stress include yield poten-
319 tial, WUE, harvest index (HI), improved transpiration efficiency, and deep root pen-
320 etration (to access water and nutrients), all of which are relevant to NUE as well.

321 Flooding is another form of water stress that is expected to increase due to
322 climate change. Waterlogging affects ~10 % of the global land area and an esti-
323 mated 10 million hectares of land in developing countries. It can cause a wide vari-
324 ety of symptoms that can affect yield either directly or indirectly, through affecting
325 leaf senescence, tiller number, and reduced plant height. While N availability could
326 increase under situations in which floods bring silt and nutrients along, it could also
327 decrease in situations where topsoil and fertilizer N are lost or diluted out. Other
328 parameters such as temperature could also result in interactive effects.

329 Salinity is one of the major abiotic stresses that lower the yield and usually is
330 accompanied by water stress. The impact of salt stress is dependent on the cultivar/
331 organ/developmental stage and the degree of salt stress. Salinity is known to alter
332 the activities of various enzymes from the N assimilatory pathway like nitrate
333 reductase in leaves than in roots (Mokhele et al. 2012).

334 ***12.3.3 N in Heat and Cold Stress***

335 Accumulation of greenhouse gases (carbon dioxide, methane, and nitrous oxide) in
336 the earth's atmosphere is expected to warm up the earth's surface by 1.8–4 °C by the
337 end of this century (IPCC 2007). Rising temperatures of both soil and air could alter
338 the rate of water and nitrogen uptake due to their effect on rate of transpiration and
339 soil moisture respectively (Dong et al. 2001). Elevated temperatures also alter N
340 allocation, reduce foliar N concentration and carbohydrate content (Tjoelker et al.
341 1999), damage photosynthetic membranes and cause chlorophyll loss decreasing
342 leaf photosynthetic rate, increase embryo abortion, lower grain number, and
343 decrease grain-filling duration and rates resulting in lower grain yield.

Globally, leaf N concentrations have been found to vary along altitudinal and latitudinal temperature gradients across plant species or functional groups (Reich and Oleksyn 2004). Foliar N content increases from the tropics to the cooler and drier midlatitudes due to “temperature-related plant physiological stoichiometry and biogeographical gradients in soil substrate age, as well as cold temperature effects on biogeochemistry at high latitudes” (Reich and Oleksyn 2004).

Cold stress can produce undesirable responses to nitrogen fertilizers that are often applied in high concentrations to increase yield. For example, high nitrogen supply before/during pollen development aggravates the effect of pollen sterility in rice in extreme cold conditions (Gunawardena et al. 2003). Low temperatures are also known to inactivate RuBisCO carboxylase by *S*-nitrosylation (Corpas et al. 2011).

12.3.4 *N in UV and Other Stresses* 355

Enhanced exposure to UV is one of the consequences of climate change. Plant N uptake and assimilation are significantly inhibited at high levels of UV-B radiation. Excessive UV light along with nutrient deficiencies can lead to photooxidative stress, which is worsened further in other environmental stresses such as metal toxicity (Lynch and StClair 2004 and the references therein). There is a significant increase in the activity of RNS species under UV-radiation stress as well as under ozone stress, which could lead to cell death (Corpas et al. 2011).

12.3.5 *N in Multiple Interacting Stresses* 363

Most of the plant stress studies were done changing or observing a single variable factor, i.e., changing CO₂, temperature, or water concentrations, but very few studies considered the combinatorial effect of these stresses. Studies show increased concentrations of CO₂ can increase the demand for nutrient, but increase in temperatures can influence the length of growing season and in turn reduce the demand for nutrients (Nord and Lynch 2009; Mittler and Blumwald 2010). Rice plants exposed to increases levels of CO₂ have shown increased sensitivity to cold stress (Shimono and Bunce 2009). Both drought and heat stresses together affect N availability storage and remobilization in pine trees (Rennenberg et al. 2009; Huang et al. 2012). The activity of many nitrate-regulated genes is hypothesized to be regulated by light, and evidence shows that both NRT2 and NR activity is dependent on light as well as nitrogen (Lillo 2004, 2008; Chapman and Miller 2011). Thus, mounting evidence suggests that the effect of various abiotic stresses can lead to unanticipated changes in plant growth and development.

378 **12.4 Conclusions and Prospects**

379 There is a growing demand for developing crops with resilience to climate change,
380 abiotic stress, and N-use efficiency for global food security and environmental sus-
381 tainability. While studies on individual stresses and the signaling mechanisms
382 involved in the plant's response to them have made impressive progress, integrative
383 studies are needed that can model the complex interactions between various abiotic
384 stresses and the signaling and/or regulatory events involved in them. Similarly, in
385 the area of N-response and N-use efficiency, integration of crop genetics and func-
386 tional genomics approaches have begun to make rapid strides, but the links between
387 nitrogen and stress remain peripheral, especially at the level of signaling and regula-
388 tory interface of stress and N, except in the area of nitrosative stress. This calls for
389 new synergies to be forged between research on abiotic stress and resource-use
390 efficiency in general and N-use efficiency in particular, to identify some common
391 signaling aspects or regulatory targets for developing not only stress-resistant and
392 climate-resilient crops but also N-use-efficient or resource-use-efficient crops.

393 Crop improvement through QTL mapping and marker-assisted selection/breeding
394 seems to be a promising route in this regard. Extensive studies in various plants have
395 lead to the mapping of many agronomic traits such NUE, yield, biomass, N uptake,
396 and remobilization (Habash et al. 2007; Fontaine et al. 2009). The possibility of co-
397 localization of multiple agronomically important QTLs is an increasingly attractive
398 avenue to explore in this regard. For example, in tropical maize, QTLs for grain yield
399 and secondary traits were identified under varying N and water supply, some of
400 which were found to be co-localized (Ribaut et al. 2007). More efforts in this direc-
401 tion can be enabled by suitable national policies and intergovernmental cooperation
402 for germplasm exchange and collaboration. The role of public sector may prove to be
403 at least as crucial as that of the private sector in facilitating affordable access to such
404 technologies for the farmers, breeders, and consumers alike.

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Author Queries

Chapter No.: 12

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Queries	Details Required	Author's Response
AU1	Please check if edit to sentence starting "Nitrogen and its..." is okay. [metadata content]	
AU2	Please check if edit to sentence starting "In fact, N ₂ O..." is okay.	
AU3	Please check latter part of sentence starting "But what constitutes..." for clarity.	
AU4	Following references "Gojon et al. 2011; Song et al. 2011; Li et al. 2013; Rennenberg et al. 2009; Habash et al. 2007; Fontaine et al. 2009" are cited in text but not given in the reference list. Please provide details in the list or delete the citation from the text.	
AU5	Please check if "glutamine synthase" should be changed to "glutamine synthetase" throughout the text.	
AU6	Please check if edit to sentence starting "Nitrogen-starved plants..." is okay.	
AU7	Please check if " <i>Querus</i> " should be changed to " <i>Quercus</i> ".	
AU8	Following references "Chen et al. (2013); Masclaux-Daubresse et al. (2010)" are not cited in text. Please cite in the text or delete from the list.	
AU9	Please check and confirm the inserted information in the reference "Chapman and Miller (2011)".	
AU10	Please provide complete details for reference "Krapp and Kaiser (2010)."	