The BAHD family acyltransferase encoded by Pun1/AT3 catalyzes the final condensation reaction and is a key factor in pungency formation. Loss-of-function alleles of this gene result in the absence of capsaicin production (Egan et al., 2019). The putative aminotransferase pAMT is responsible for providing vanillylamine and shows strong developmental stage and tissue-specific regulation.

In extremely pungent cultivars, genes such as Pun1, pAMT, KAS, and BCAT are upregulated in both placenta and pericarp tissues, significantly increasing capsaicin content in the whole fruit. The transcription factor MYB31 (Cap1/Pun3) acts as a master regulator that activates genes involved in capsaicin biosynthesis and is an important genetic basis for extreme pungency in C. chinense (Zhu et al., 2019).

4.3 Effects of nitrogen levels on capsaicin accumulation

Nitrogen supply influences the capsaicin biosynthetic pathway by regulating precursor availability, enzyme activity, and gene expression. Moderate nitrogen application can increase total phenolic compounds (precursors), enhance the activity of PAL and capsaicin synthase, and upregulate the expression of genes such as PAL, AT3 (Pun1), 4CL, C4H, COMT, pAMT, and HCT, thereby achieving the highest capsaicin content.

In contrast, both low and excessive nitrogen levels reduce PAL activity, precursor content, and related gene expression, while increasing the activity of competing phenolic pathways and degradation enzymes such as peroxidase (POD) and polyphenol oxidase (PPO).

Similar results have been observed in hydroponically grown C. frutescens. As the nutrient solution (nitrogen) concentration increases, capsaicin content first rises to an optimal range but decreases beyond this point, indicating that excessive nitrogen may cause toxicity or shift metabolism toward basic growth processes (Rahim et al., 2024).

In habanero pepper, capsaicin content is highest under nitrogen stress, decreases significantly after a small nitrogen supply, and increases again at higher nitrogen levels, suggesting a complex nonlinear response to nitrogen (Medina-Lara et al., 2008). Proper NPK or organic nitrogen management can simultaneously improve yield and capsaicin/dihydrocapsaicin content, although the effects depend on cultivar differences (Hammam et al., 2020; Stan et al., 2021) (Table 1).

4.4 Trade-off between yield and capsaicin content

Nitrogen creates a trade-off relationship among vegetative growth, yield, and capsaicin accumulation, rather than a simple opposition. Moderate nitrogen application usually promotes fruit size, placenta development, and yield, while maintaining relatively high capsaicin levels. For example, applying 562.5 kg/ha urea or using a moderate EC level of AB fertilizer can achieve this balance (Rahim et al., 2024).

Excessive nitrogen tends to stimulate vegetative growth and fruit development, but may reduce capsaicin content due to dilution effects, decreased PAL and capsaicin synthase activity, or increased allocation of metabolites to competing phenolic and lignin pathways.

On the other hand, severe nitrogen limitation can induce higher capsaicin accumulation in some genotypes, but this is usually accompanied by reduced yield. By optimizing nitrogen application rates and maintaining balanced nutrient management (including moderate deficit fertilization in some highly pungent cultivars), it is possible to maintain or even increase capsaicin content without significant yield loss.

5 Nitrogen Management Strategies for Optimizing Yield and Quality

5.1 Optimal nitrogen application rate and timing

Chili yield and capsaicinoid content show a curvilinear response to nitrogen (N) application, with the optimal level clearly lower than the excessive rates commonly used in practice. In open-field sweet pepper production, the highest yield was obtained at 153~230 $kg \cdot N \cdot ha^{-1}$. In coastal regions of Bangladesh, 116 $kg \cdot N \cdot ha^{-1}$ was identified as the optimal rate, and no further yield increase was observed at 145 $kg \cdot N \cdot ha^{-1}$ (Nahida et al., 2024).