# Review of Stomatal and Non-stomatal Limitations of Photosynthesis in Plant Leaves: Postprint

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## Abstract

The impacts of environmental stress conditions on plant growth and metabolism are multifaceted, with effects on photosynthesis being particularly pronounced. Photosynthesis in plant leaves constitutes a crucial factor influencing productivity and yield; consequently, plant photosynthesis and its underlying mechanisms remain an active area of research in the field of plant physiological ecology. Nevertheless, under natural or artificially controlled conditions, whether photosynthesis is primarily constrained by stomatal or non-stomatal factors remains unresolved. This article presents a discussion from three aspects: various calculation methods for (relative) stomatal and non-stomatal limitation values are summarized, and the limitations of each method are analyzed to provide a basis for determining the primary limiting factors of photosynthesis under specific conditions; external stress conditions involved in previous studies on stomatal and non-stomatal limitations of photosynthesis are classified and summarized from the perspectives of artificially controlled conditions (water stress, salt-alkali stress, multi-factor combined stress) and natural conditions; and the influence pathways of stomatal and non-stomatal limiting factors on photosynthesis are analyzed. In future research, revealing and exploring the variation characteristics and influence mechanisms of stomatal and non-stomatal limiting factors under conditions such as different growth and development stages of the same species and different habitat types (water-heat-salt-nutrient regimes, artificial control versus natural environment) will be the development trend and key direction.

# Full Text

## Preamble

External stresses affect plant growth and metabolism in numerous ways, particularly photosynthesis (Pn). As Pn represents a crucial factor influencing productivity, the mechanism underlying these effects remains a central topic in plant physiology and ecology. Photosynthesis is primarily constrained by either stomatal or non-stomatal factors, though the relative contribution of each remains to be fully determined. This paper discusses methods for calculating stomatal and non-stomatal limitation values (Ls and Ci/gs), examines these limitations under various stresses, and analyzes their effects on Pn: (1) We summarize methods for calculating (relative) Ls and Ci/gs values, providing a basis for identifying the primary limiting factor. (2) We classify external stresses and review achievements from previous studies conducted under both controlled conditions (water, salt-alkali, and multi-factor stresses) and natural conditions. (3) We analyze how stomatal and non-stomatal factors impact Pn. Exploring the characteristics of Ls and Ci/gs values and their mechanisms across different growth and developmental stages and habitat types (hydrothermal, saline, nutrient, and various manual and natural conditions) will represent a key research direction.

**Keywords:** Photosynthesis; Stomatal limitation; Non-stomatal limitation; Water stress.

## 2 Calculation Methods for Stomatal and Non-Stomatal Limitations

### 2.1 Calculation of Ls Values

The stomatal limitation value (Ls) can be calculated through three primary approaches: (1) The gas exchange method, which derives Ls from the relationship between photosynthetic rate and intercellular CO concentration; (2) The carbon isotope discrimination method, which calculates Ls based on the difference in $^{13}$C between air and plant tissues; and (3) The chlorophyll fluorescence method, which estimates Ls through analysis of the relationship between electron transport rate and CO assimilation rate.

**Table 1 Limits of the Three Methods for Calculating Ls**

| Method | Principle | Advantages | Disadvantages |
|---|---|---|---|
| Gas exchange | Ls = 1 - Ci/Ca | Simple operation, rapid measurement | Affected by mesophyll conductance |
| Carbon isotope | Ls = (Pa - P)/P | High precision, integrated over time | Complex operation, high cost |

| Method | Principle | Advantages | Disadvantages |
| --- | --- | --- | --- |
| Fluorescence | Ls = Ri(R + ΔCi/Δp)RT<= $vP\ CiEU +\ ^{\backprime}u$ | Direct measurement of photosynthetic apparatus | Requires dark adaptation, affected by environmental factors |

Note: P represents photosynthetic rate; Pa and Pi represent atmospheric and intercellular CO partial pressures, respectively; Ri represents the ratio of intercellular to atmospheric CO concentration; R represents the CO compensation point; ΔCi/Δp represents the change in intercellular CO concentration with respect to vapor pressure deficit.

**Table 2 Methods for Calculating the Relative Stomatal (RLs) and Non-Stomatal (RLM) Limitations to Photosynthesis**

| Calculation Method | Formula | Parameters |
| --- | --- | --- |
| RLs = (LT - LM)/LT | LT = (PO - PS)/PO | PO: Potential photosynthetic rate |
| RLM = LM/LT | LM = (PN - PS)/PN | PS: Actual photosynthetic rate under stress |
| RLs = 100 × (rs + r* + rbl)/PO | PN: Photosynthetic rate under non-stress conditions | rs: Stomatal resistance; r*: Mesophyll resistance; rbl: Boundary layer resistance |

### 2.2 Classification of External Stresses

Previous studies have investigated stomatal and non-stomatal limitations under various stress conditions. These can be categorized into: (1) Water stress, including drought and waterlogging; (2) Salt-alkali stress, including NaCl, Na CO , and NaHCO treatments; (3) Nutrient stress, including nitrogen, phosphorus, and iron deficiencies; (4) Heavy metal stress, including Cd, As, and other toxic elements; (5) Combined stresses, such as UV-B radiation combined with NaCl or Cd; and (6) Natural field conditions, including different soil moisture regimes and climatic conditions.

**Table 3 Summary of Stress Conditions and Main Findings**

| Stress Type | Main Findings | Representative Studies |
|---|---|---|
| Water stress | Stomatal limitation dominates under mild-moderate stress; non-stomatal limitation becomes significant under severe stress | Flexas et al. [16], Lawlor [65] |
| Salt stress | Both stomatal and non-stomatal limitations occur; degree depends on salt concentration and duration | Xu et al. [2], Guo & Zhao [30] |
| Nutrient deficiency | Primarily non-stomatal limitation, affecting Rubisco activity and RuBP regeneration | Parry et al. [64], Lawlor [65] |
| Heavy metals | Both limitations observed; stomatal limitation at low concentrations, non-stomatal at high concentrations | Zhang et al. [38], Pereira et al. [42] |
| Combined stresses | Synergistic effects; limitation type depends on dominant stress factor | He et al. [1], Qiang et al. [31] |

## 3 Impact Pathways of Stomatal and Non-Stomatal Factors on Pn

### 3.1 Stomatal Limitation Effects on Pn

Stomatal limitation reduces Pn primarily by decreasing stomatal conductance (gs), which limits CO diffusion into the leaf. Under mild to moderate water stress, stomatal closure represents the primary protective mechanism, reducing water loss while limiting photosynthesis. The relationship between Pn and gs is typically linear under these conditions. However, when stress becomes severe, stomatal conductance approaches zero, and Pn becomes primarily limited by non-stomatal factors. Studies show that under drought conditions, a 50% reduction in gs typically results in a 30-40% decrease in Pn, indicating that stomatal limitation accounts for approximately 60-70% of the total photosynthetic reduction.

### 3.2 Non-Stomatal Limitation Effects on Pn

Non-stomatal limitations affect Pn through multiple pathways, including biochemical, photochemical, and oxidative processes. These limitations become increasingly important under severe stress conditions.

**3.2.1 Water Stress Effects** Under severe water stress, non-stomatal limitations dominate. Reduced water potential affects chloroplast structure and function, decreasing the activity of key enzymes such as Rubisco and limiting RuBP regeneration. Studies on wheat and maize demonstrate that under severe drought, non-stomatal limitations can account for over 60% of photosynthetic reduction. The decline in photosynthetic capacity is associated with damage to photosystem II, reduced electron transport rate, and decreased ATP synthesis.

**3.2.2 Salt Stress Effects** Salt stress induces both stomatal and non-stomatal limitations. High salinity causes ion toxicity and osmotic stress, leading to stomatal closure. Simultaneously, salt accumulation in chloroplasts damages photosynthetic apparatus, reduces Rubisco activity, and disrupts energy transfer. Research on soybean and cotton shows that NaCl concentrations above 100 mM cause significant non-stomatal limitations, with Rubisco activity decreasing by 40-60%.

**3.2.3 Nutrient Deficiency Effects** Nutrient deficiencies primarily cause non-stomatal limitations. Nitrogen deficiency reduces chlorophyll content and Rubisco concentration, directly limiting the photosynthetic capacity. Phosphorus deficiency affects ATP synthesis and RuBP regeneration. Iron deficiency impairs chlorophyll synthesis and electron transport chain function. Studies indicate that nitrogen deficiency can reduce photosynthetic capacity by 50-70% even when stomatal conductance remains unaffected.

**3.2.4 Oxidative Stress Effects** Environmental stresses induce oxidative stress, generating reactive oxygen species (ROS) such as superoxide (O ) and hydrogen peroxide (H O ). These ROS damage thylakoid membranes, degrade chlorophyll, and inactivate enzymes. The antioxidant defense system (SOD, CAT, APX) can mitigate this damage, but under severe stress, ROS accumulation overwhelms these defenses, causing irreversible damage to the photosynthetic apparatus and resulting in non-stomatal limitation.

## 4 Research Perspectives

Future research should focus on: (1) Developing more accurate methods for quantifying stomatal and non-stomatal limitations, particularly under field conditions; (2) Investigating the dynamic changes in limitation types during plant development and stress progression; (3) Exploring the interaction between stomatal and non-stomatal limitations under combined stresses; (4) Identifying genetic and molecular mechanisms underlying non-stomatal limitations to facilitate breeding for stress-tolerant varieties; and (5) Integrating multi-scale approaches from molecular to canopy levels to better understand photosynthetic limitations in natural ecosystems.

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