

in plants, including the transport of miR399 from shoot to root, targeting the transcript *PHOSPHATE2* (*PHO2*) to modulate phosphate homeostasis [9]. A similar translocation pattern was detected in miR827 [10].

Apart from shoot-to-root trafficking of miRNAs, recent evidence suggests that miRNA can be transported into fungal pathogens in plant cells by exosome-like vesicles. In animal cells, membrane-bound vesicles derived from multivesicular bodies (termed exosomes) are generally responsible for intercellular transport of numerous RNAs, including miRNA and other noncoding RNAs. In plant cells, exosome-like vesicles were also observed upon fungal infection, suggesting that miRNA trafficking is potentially mediated by exosomes or other extracellular vesicles, in order to silence fungal virulence-related genes [11]. This result provides a novel perspective of exosome-mediated cross-kingdom RNA interference. Apart from the typical mode of transport through plasmodesmata, a novel polarized gating mechanism was recently identified at particular intercellular connection points, which likely explains intercellular miRNA mobility [12].

Concluding Remarks and Future Research Questions

The studies of Zou *et al.* [2] and Tsikou *et al.* [1] provide new information on the involvement of miR172 and miR2111 in plant infection biology; however, their results also raise questions regarding miRNA-mediated immunity in plants. For example, how are pathogen detection and transcriptional activation/suppression of miRNAs linked mechanistically? In other words, why is miR2111 suppressed in shoots, whereas miRNA172b is activated upon infection? Why is miR2111 synthesized in the shoots and subsequently transported to the roots? Is shoot-to-root trafficking mediated by

exosome-like vesicles? How do plants coordinate dual functions of particular miRNAs during immune response and development, as miRNA172 can also act as a translation repressor during plant flower development. How do plants overcome the suppression of *R* genes by miRNAs and miRNA-phasiRNA cascades during infection? Further in-depth research on miRNA-mediated plant immune responses is necessary to comprehensively understand disease resistance in plants.

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Forum

Iso/Anisohydry: Still a Useful Concept

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The iso/anisohydry concept characterizes plants according to their water status regulation. Coexisting definitions and misconceptions have recently led to considerable criticism. We discuss here reasons for the misconceptions, and propose a robust definition of iso/anisohydry using the leaf turgor loss point to integrate the complex interplay of plant hydraulic traits.

Theoretical concepts can be powerful tools for approaching complex problems in a simplified but informative manner, often by focusing on key elements of the problem. In the field of plant–water relations one of the most widely used concepts to date is the iso-/anisohydry nomenclature. This was introduced by Berger-Landefeldt [1] in 1936 to describe the behavior of leaf water potential (Ψ_L) in response to atmospheric evaporative demand. Since then, the concept has been described by differing definitions. However, the core idea of most of the variants is the same: to typify the stringency of plant water-status regulation. Most definitions rely on the behavior of Ψ_L or stomatal conductance (g_s) in response to a water-related environmental variable,

most often soil water potential (ψ_s), but also air vapor pressure deficit (VPD). Isohydic plants are assumed to maintain their ψ_L relatively constant despite changes in ψ_s or VPD. By contrast, anisohydric plants are assumed to let their ψ_L covary more strongly with ψ_s and VPD.

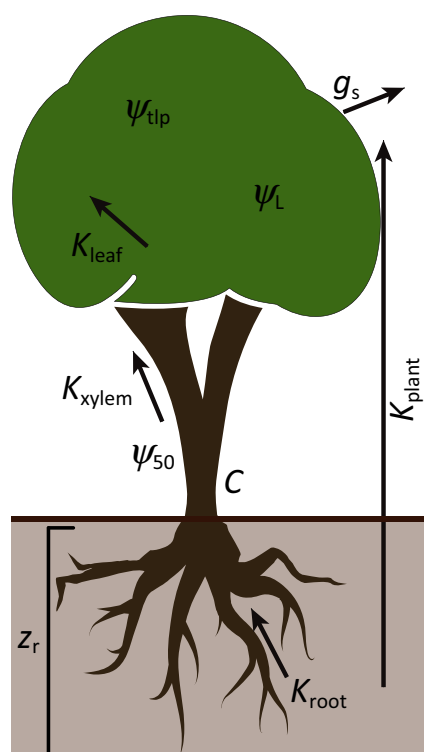
The appeal of the iso/anisohydry concept lies in its simplicity. This has resulted in studies assuming iso/anisohydry to be a simple plant behavioral trait. However, despite the longstanding aim to classify plant species dichotomously as either isohydric or anisohydric, evidence suggests that there is considerable variation in water-status regulation along a continuum between these two extremes within species, genotypes, and even individuals [2]. This variation is most importantly determined by environmental conditions [3] and can thus be apparent for the same species in different environments. The resulting mismatch between its theoretical foundation and how the concept is sometimes applied has led to considerable criticism of the iso/anisohydry nomenclature, and even suggestions to abandon the concept [4]. We identify here sources of the most common misconceptions associated with the iso/anisohydry concept, namely omitting a clear definition and considering iso/anisohydry to be a 'simple' plant trait. We further suggest a potentially promising approach to quantify the stringency of plant water-status regulation.

Clearly Framing and Understanding the Concept

A core element of every concept is its clear definition. Thus, provided that the definition being used is clearly reported, differing definitions of a concept may coexist, each providing valuable information in a different respect. For example, Martínez-Vilalta *et al.* [5] used linear regression slopes to quantify how

strongly daily minimum ψ_L responds to changes in ψ_s , with isohydric plants having slopes closer to 0 and anisohydric plants having slopes closer to 1.0. Another common use of the iso/anisohydry concept is to measure the sensitivity of g_s to ψ_L (e.g., [6]) with high sensitivity (early stomatal closure), indicating isohydric behavior.

A clear definition, however, also requires a sound theoretical foundation. Iso/anisohydry is a relative concept, as are most conceptual and comparative approaches in quantitative ecophysiology, such as drought tolerance and resistance. Consequently, it is not advisable to dichotomously classify species as simply isohydric versus anisohydric.



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Figure 1. Coordinated Traits Involved in Controlling Plant Water Status. Plant water status is controlled by three basic determinants and their interplay: how much water is available, how much is lost to the atmosphere, and how fast it moves through the plant. These factors are the result of a series of coordinated processes and traits that comprise the hydraulic strategy of a plant. Water supply is determined by a series of hydraulic properties: soil water availability and rhizosphere conductance, root system conductance (K_{root}), and rooting pattern (indicated by rooting depth z_r) constrain water uptake from the soil and its transport to the shoot. In stems, xylem conductance (K_{xylem}) determines the axial xylem pressure gradient at a given water flux. Xylem pressure is controlled to avoid catastrophic hydraulic failure (xylem vulnerability to embolism approximated by the water potential causing 50% embolism, ψ_{50}). Particularly in woody plants, stored water derived from stem and root capacitance (C) buffers diel fluctuations in plant water potential. The overall efficiency of water transport through the entire plant can be described by whole-plant conductance (K_{plant}). Water losses to the atmosphere are controlled by stomata, which are turgor-operated valves. Stomatal conductance (g_s) controls transpiration and leaf water potential (ψ_L) in response to leaf turgor at a given combination of atmospheric evaporative demand, canopy water supply, and bulk leaf hydraulic conductance (K_{leaf}). ψ_L is thus regulated within the physiological and structural constraints of a plant before complete stomatal closure around the leaf turgor loss point (ψ_{tip}), which can be regulated via osmotic adjustment.

These two terms represent undefined extremes of a continuum along which some species can exhibit considerable plasticity in their behavior. Thus, in a given environment a species may be characterized as being relatively isohydric compared to another, but anisohydric compared to a further species, with their rankings potentially changing in a different environment.

Each definition of relative iso/anisohydry has its strengths and weaknesses in different respects. Consequently, different definitions of relative iso/anisohydry may coexist for different purposes, provided that they obey the described generic prerequisites. That is, when characterizing a plant or species as relatively iso- or anisohydric, it is important to (i) report the definition of iso/anisohydry this description is based on, (ii) also report the biotic and abiotic growing conditions this characterization refers to, and (iii) acknowledge that relative iso/anisohydry represents a continuum and not a dichotomy.

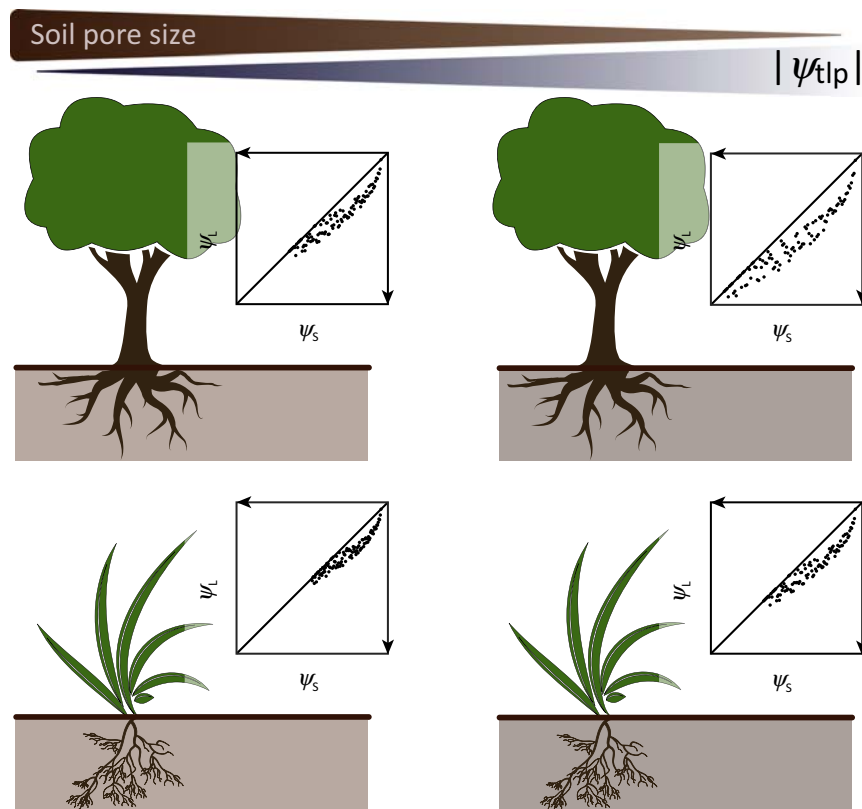
A Way Forward

A key question associated with the iso/anisohydry nomenclature still remains: which plant traits and which concept definition accurately and quantitatively describe the stringency of water-status regulation? A prerequisite for any such approach is to depict interspecific differences in isohydry (and thus 'hydraulic behavior') in a given environment while allowing for intraspecific plasticity across different environments and accounting for all relevant processes of plant hydraulic functioning.

In a search for this approach, a good starting point is to acknowledge that iso/anisohydry is not a simple functional trait nor a strategy employed by autonomous stomata (Figure 1). Stomata are the most important means of a plant to control water loss to the atmosphere. Stomatal opening and thus conductance is in

turn directly controlled by leaf turgor [7]. Therefore, maintaining leaf turgor is of primary importance for maintaining the ability of the plant to actively regulate water loss while still being able to take up CO_2 by avoiding complete stomatal closure approximately at the point of turgor loss. Moreover, cell expansion and subsequent division (and therefore growth) are directly dependent on turgor. Leaf turgor depends on leaf osmotic

potential, soil water potential, transpiration rate, and the efficiency of water supply to the canopy. The latter, in turn, depends on other plant hydraulic variables such as whole-plant leaf-specific hydraulic conductance and rooting patterns. Similarly, stomatal responses to VPD will partly depend on plant and leaf hydraulic architecture, which affect the impact of a VPD-induced increase in transpiration on leaf and stomatal guard-cell



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Figure 2. Hydraulic Adjustment of Two Hypothetical Species Growing in Different Environments. With decreasing soil grain and pore size, plants operate at lower leaf water potentials (ψ_L) [12] because water is held more tightly by smaller pores (insets showing a potential response of daily minimum ψ_L to soil water potential, ψ_S). As the soil dries and ψ_S declines, ψ_L also declines until $\psi_L \approx \psi_S$ (1:1 diagonal line), the point at which ψ_L has reached the turgor loss point (ψ_{tlp}), stomata are entirely closed, and the plant is severely water stressed. Further declines in ψ_L are beyond the control of the plant, and thus of limited value in determining the degree of iso/anisohydry. Operating at low ψ_L in fine-textured soils will also require lower ψ_{tlp} to maintain CO_2 uptake at lower ψ_S . Using ψ_{tlp} as proxy for relative iso/anisohydry, plants thus become more anisohydric in fine-grain soils. They adjust their relative isohydry to their growing environment as well as other hydraulic traits (Figure 1). The extent to which plants acclimate to environments differing in water availability and evaporative demand is species-specific, indicated by a hypothetical tree species (top row) and herbaceous species (bottom row). Comparisons of relative iso/anisohydry are thus only meaningful for one species across different environments (within rows) or across species in comparable environments (within columns). Note that water potentials in soils and plants are typically negative.

water status. Other means of maintaining leaf turgor when the air or the soil becomes drier are, for example, to reduce leaf area or to increase leaf-level osmolyte supply. Thus, leaf turgor is the result of a suite of plant hydraulic traits and processes. This makes Ψ_L at turgor loss (Ψ_{tip}), the point a plant must avoid to maintain control over water loss, a promising trait to quantify the relative isohydry of a plant [8]. Although we expect Ψ_{tip} to be a good approximation of full stomatal closure, in rare cases stomata may remain partly open at leaf water potentials below Ψ_{tip} . Those exceptions, however, do not make Ψ_{tip} a less-promising predictor of plant hydraulic behavior and thus of relative iso/anisohydry. The Ψ_{tip} value of a given plant can vary in response to changing Ψ_S and VPD within the physiological (e.g., osmotic adjustment) and structural (e.g., hydraulic architecture) boundaries of the plant [9]. This, in turn, will result in intraspecific variation of apparent iso/anisohydry with environmental conditions (Figure 2), and may provide valuable insights concerning the physiological and structural ability of a species to acclimate to different moisture regimes.

In general, the environment plays a major role in determining the degree of iso/anisohydry of a plant, and direct comparison of the stringency of water-status regulation across individuals and species is only meaningful under comparable environmental conditions. Thus, applying the iso/anisohydry concept should be constrained to either comparing the responses of different species under similar environmental conditions or the response of a single species across environments (Figure 2). In the former case, interspecific differences in relative isohydry as measured by Ψ_{tip} may well be apparent [8], underlining the strength of the iso/anisohydry concept for typifying species in a defined framework.

Concluding Remarks

The way plants regulate their water status as measured by water potential is highly complex. Any concept holistically describing this regulation should thus account for the interplay of plant hydraulic traits/processes and their dependence on environmental conditions. The iso/anisohydry concept has long been applied to describe plant water-status regulation, but with varying success, mainly because of persistent misconceptions. It is now clear that a continuum of coordination and trade-offs among coevolved traits leads to a continuum of stringency of plant water-status regulation [10]. This stringency, in turn, can be characterized as spanning the continuum of relative isohydry to anisohydry [11], and not as a dichotomy, which any approach using this nomenclature should account for. A promising approach is based on the turgor loss point Ψ_{tip} as a proxy for iso/anisohydry, because Ψ_{tip} is coordinated with a suite of plant hydraulic traits, whereas differences in Ψ_{tip} , and thus in relative iso/anisohydry, for a given species reflect the ability of that species to adjust to different environments [11]. We therefore believe that, provided the concept being used is clearly defined and the relevant environmental conditions reported, assessments of iso/anisohydry may contain considerable information and should not be abandoned.

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Forum

Microbiome Applications from Lab to Field: Facing Complexity

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Plant microbiota are the subject of new product developments, primarily aimed at improving plant health, nutrition, and stress resilience. However, current application of microbes in the field faces multiple