

Risk-taking plants

Anisohydric behavior as a stress-resistance trait

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Water scarcity is a critical limitation for agricultural systems. Two different water management strategies have evolved in plants: an isohydric strategy and an anisohydric strategy. Isohydric plants maintain a constant midday leaf water potential (Ψ_{leaf}) when water is abundant, as well as under drought conditions, by reducing stomatal conductance as necessary to limit transpiration. Anisohydric plants have more variable Ψ_{leaf} and keep their stomata open and photosynthetic rates high for longer periods, even in the presence of decreasing leaf water potential. This risk-taking behavior of anisohydric plants might be beneficial when water is abundant, as well as under moderately stressful conditions. However, under conditions of intense drought, this behavior might endanger the plant. We will discuss the advantages and disadvantages of these two water-usage strategies and their effects on the plant's ability to tolerate abiotic and biotic stress. The involvement of plant tonoplast AQPs in this process will also be discussed.

Isohydric vs. Anisohydric Plant Behavior

Different regions of the world are characterized by different climatic and environmental conditions, which have led to the development of a wide range of plant adaptation mechanisms and survival strategies. Both anisohydric and isohydric behaviors have been observed in numerous plant groups¹ as well as within individual species, such as grapevine (*Vitis vinifera*²) and poplar (*Populus*³), suggesting that the availability of water in the natural environment and dynamic plant-environment relations influence these differences in behavior.^{4–7} A constant midday leaf water potential (Ψ_{leaf}), as a characteristic of isohydric plants, is the result of strict and conservative water-balance management, in which the loss of water is limited by the reduction of stomatal conductance. However, our current understanding of the molecular and cellular factors responsible for these two types of plant behaviors is limited. Evidently, differences in the behavior of isohydric and anisohydric plants are due to differences in the sensitivity of their respective guard cells to a critical Ψ_{leaf} threshold. As a result, under optimal conditions and mild-to-moderate drought conditions, anisohydric plants maintain higher stomatal conductance (g_s) and CO_2 assimilation (A_N) than isohydric plants and, therefore, are more productive under those conditions.^{3,6,8–10}

Recently, we demonstrated that the constitutive expression of a tonoplast aquaporin (TIP AQP), SITIP2;2, in an isohydric tomato line led to an increase of the osmotic water permeability of the tonoplast and extended the capacity of the vacuole for osmotic buffering of the cytoplasm under stress conditions.⁸ This transformation “converted” the isohydric tomato plants, so that they

exhibited anisohydric behavior, which led to greater productivity under optimal and mild-to-moderate-drought conditions. A similar effect has been observed for another TIP in Arabidopsis plants.^{11,12} These observations raise the question of whether anisohydric behavior should be viewed as a valuable agronomic trait.

Effect of Anisohydric Behavior on Abiotic Stress Resistance: A Valuable Agronomic Trait?

From the agronomic point of view, drought resistance is defined as enhanced productivity under the examined conditions. Thus, any innate factor that leads to an increase in crop productivity under stressful conditions may be viewed as a valuable agronomic trait. Due to their higher g_s and A_N , anisohydric crops will most likely improve their yield under conditions of optimal to moderate water availability.^{8,10} Nonetheless, there is a need to determine the soil moisture threshold below which anisohydric plants lose their agronomic advantage. To answer this question, one must take in consideration the plant's ability to recover from the stressful period (i.e., its embolism and desiccation resistance) and return to its pre-stress productivity rate. Several studies have reported that anisohydric plants are resistant to cavitation,^{13–18} suggesting that these plants may recover rapidly following exposure to drought. These findings support the hypothesis that anisohydric behavior contributes to agronomic drought resistance. Observations of our converted TIP2;2 tomato plants also support this hypothesis.

TIP2;2 plants maintained significantly higher performance (i.e., harvest index) even when received 50% deficit irrigation, as long as they were watered frequently. Interestingly, these plants

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out-performed those treated with the full irrigation regime, as long as they were not irrigated very frequently (i.e., irrigation once a week, which exposes the plants to longer periods of drought) eliminate their advantage and emphasizing the vacuole “reservoir” role in the short-term.⁸

A similar trend was reported in a comparison of the drought resistance of isohydric and anisohydric grass species (*Miscanthus sinensis* and *Eragrostis spectabilis* respectively), in which better performance of *Eragrostis spectabilis* was observed under favorable to moderate moisture conditions, but little difference was noted when the plants were subjected to severe drought stress.¹⁰ This demonstrates that the anisohydric plant is an “opportunistic risk-taker” whose behavior is beneficial under conditions of minimal to moderate stress, but will confer no benefit under conditions of prolonged stress.

This perception can explain the disadvantage of some anisohydric grapevines (*Vitis vinifera*), such as cv Chardonnay and cv Shiraz, under drought conditions.^{18,19} In general, anisohydric grapevines were reported to have lower midday water potentials under stressful conditions, but did not have any advantage over isohydric grapevines in terms of g_s or water use efficiency.²⁰ This suggests that isohydric grapevines may have an advantage in dry ecosystems. Typically, grapevines are grown without any irrigation, which leaves them exposed to a wide variety of soil moisture levels. This, might explain the contradictory reports of isohydric and anisohydric behavior among plants of the same genotype.²¹ In addition, recent studies have shown that grapevines could regulate their isohydricity during the growth season and switch from isohydric to anisohydric with varying soil moisture. The authors contribute the changes to hydraulic/hormone signaling.²²⁻²⁴ This new mechanism could be an interesting view of how to examine the isohydric/anisohydric behavior.

Interestingly, the anisohydric strategy has been reported to be beneficial for survival during long periods of drought. In recent studies, about 75% of the examined juniper trees (*Juniperus monosperma*), which are anisohydric, survived 24 mo of drought, as compared with 5% of the isohydric pinyon trees (*Pinus edulis*).^{25,26} The fact that the juniper trees could maintain gas exchange at a significantly lower leaf water potential than the pinyon trees^{9,13} suggests that, at least for this species under these conditions, the risk of hydraulic failure and desiccation is worthwhile. There are additional examples of tree species that seem to benefit from their anisohydric behavior. The anisohydric white oak (*Quercus alba* L.) has been shown to survive drought better than the isohydric black walnut (*Juglans nigra* L.).²⁷ In another study, the anisohydric *Eperua falcata* was found to be less sensitive to soil drought and atmospheric drought than the isohydric *Diploptropis purpurea*.²⁸

Nevertheless, we find that defining plant water-balance regulation solely in terms of Ψ_{leaf} regulation is incomplete. After all, a plant’s primary necessity is to maintain its water content so as to maintain its biochemical functionality. For this reason, definitions of isohydric vs. anisohydric behavior should include the regulation of plant relative water content (RWC). We suggest that the stable Ψ_{leaf} that is typical of isohydric plants is a symptom of the maintenance of high RWC. For this reason, we

expect isohydric plants to regulate RWC more strictly than Ψ_{leaf} . To test this assumption, we monitored the RWC and Ψ_{leaf} of our anisohydric tomato TIP2;2 plants and a corresponding isohydric control as the plants were exposed to drought stress. We observed that the isohydric plants maintained their RWC more strictly than their Ψ_{leaf} (Fig. 1). The mechanism regulating this hierarchy is not clear. We suggest that cell-wall elasticity (ϵ) might play a role. Plants with high ϵ are more sensitive to water loss and translate minimal RWC loss into maximal Ψ_{leaf} change.²⁹ ϵ may play a central role in the sensing of the water-loss signal and its conversion into a water-potential signal that can be sensed by the stomata, leading to their closure (i.e., cell walls with higher ϵ will be more sensitive to small changes in water-content differences, resulting in the more rapid closure of the stomata). Furthermore, ϵ could act to maintain relative water content at the turgor-loss point and prevent cell dehydration.³⁰

Isohydric and Anisohydric Effect on Biotic Stress Resistance

The role of anisohydric behavior in the response of plants to biotic stress is not well understood. Similar to responses to abiotic stress, biotic stress response is a complex trait involving multiple mechanisms, including changes in phytohormones and protein

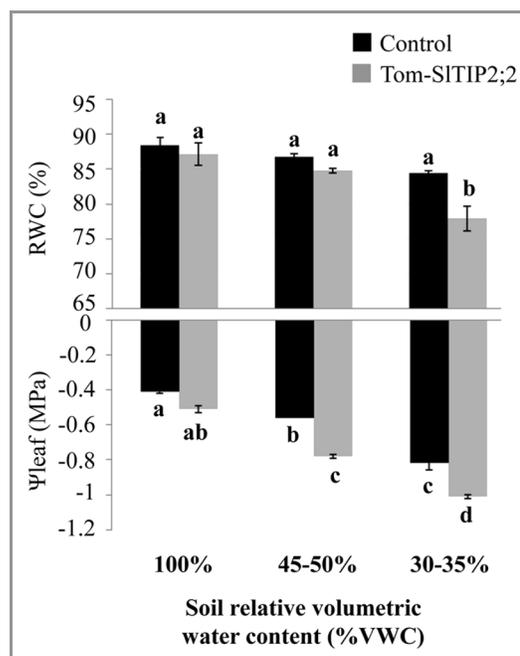


Figure 1. Comparison of midday leaf water potential (Ψ_{leaf}) and leaf relative water content (RWC) of Tom-SITIP2;2 and control plants at different levels of relative soil volumetric water content (VWC). Two independent Tom-SITIP2;2 lines (Sade et al., 2009)⁸ and control plants were subjected to drought and the decreasing soil VWC was monitored using a soil moisture probe (10HS, Decagon). (A) Midday leaf Ψ_{leaf} and (B) leaf RWC were measured at three different VWC levels (100%, ~50%, ~30%) using a pressure chamber (ARIMAD3000, MRC). Different letters above the columns represent significant differences (Tukey-Kramer test, $p < 0.05$). Data points are means \pm SE ($n = 6$).

interactions.³¹ Moreover, both types of stress lead to similar physiological responses, such as decreased g_s and decreased A_N ,^{32,33} suggesting that anisohydric plants might be more tolerant of biotic stress as a result of their greater carbon surpluses.⁵ Another example of an association between anisohydric behavior and biotic stress resistance may be seen in the ABA-deficient tomato mutant *sitiens* (*Lycopersicon esculentum* Mill. cv MoneyMaker³⁴). This mutant could be considered to be the ultimate anisohydric plant as it maintains high stomatal conductance at all times.³⁵ Indeed, these plants were reported to be highly resistant to the necrotrophic fungus *Botrytis cinerea*.³⁶ Yet, it was suggested that this resistance might not be related to carbohydrate balance, but rather to the relatively high levels of salicylic acid present in these mutants (probably as a feedback response to the low levels of ABA, since salicylic acid is an antagonist of ABA³⁷ and known to play a role in plant resistance to biotic stress³⁸).

When our anisohydric TIP2;2 plants were inoculated with *Botrytis cinerea*, they exhibited a higher level of disease resistance than the isohydric control (Fig. 2). Moreover, the TIP2;2 plants were also more tolerant to tomato yellow leaf curly virus (TYLCV; unpublished data, D. Sade and N. Sade) than the control. These results support the suggestion that anisohydric plants may be more tolerant of biotic stress, but we still do not understand the mechanism of this tolerance. It has been suggested that isohydric species have higher levels of ABA than anisohydric species.^{1,27,39,40} This suggests that anisohydric resistance to biotic stress may be related to an ABA-salicylic acid-regulated plant defense mechanism.

We would like to suggest an additional explanation for the resistance of anisohydric plants to biotic stress. We suggest that the lower leaf RWC that is characteristic of anisohydric plants⁸ (and Fig. 1) inhibits the replication and movement of biotic agents such as bacteria and fungus through the apoplast,⁴¹ as well as the movement of virions through the plasmodesmata.⁴² The identification of the origin of this observed resistance to biotic stress is a matter for further research.

Concluding Remarks

Additional research is needed to increase our understanding of the molecular basis for the different strategies that plants use to regulate their water balance. The identification of specific AQP genes with defined roles in the plant's water-budgeting activities

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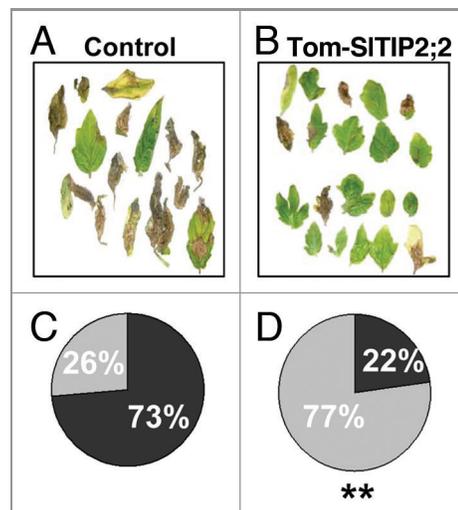


Figure 2. Inoculation of detached leaves with the necrotrophic fungus *Botrytis cinerea*. (A) Leaves from Control plants (n = 19) and (B) leaves from two independent transgenic Tom-SITIP2;2 lines (n = 22) were inoculated with 5 μ l of a solution containing 1,500 spores/ μ l. After inoculation, plants were sealed in plastic bags and transferred to a growth chamber for 4 d. Disease was evaluated as > 50% of leaf surface (infected) or < 50% of leaf surface (uninfected) and these data are presented (C and D) as relative incidence of infected (black) and uninfected (gray) leaves among the total number of inoculated leaves. **Significant difference (comparisons of two ratios binomial, $p < 0.01$).

will enhance our understanding of stomatal regulation and provide novel molecular tools for improving plant resistance to many other types of abiotic and perhaps even biotic stress, thereby contributing to our future food, feed and fiber security. It is important that any claim for behavior-related resistance or tolerance of a crop to stress take into consideration the stress level, the duration of the exposure to the stress and the rate at which the plant recovers from this exposure.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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