APOPLASTIC PH CHANGES IN *VICIA FABA* UNDER DROUGHT

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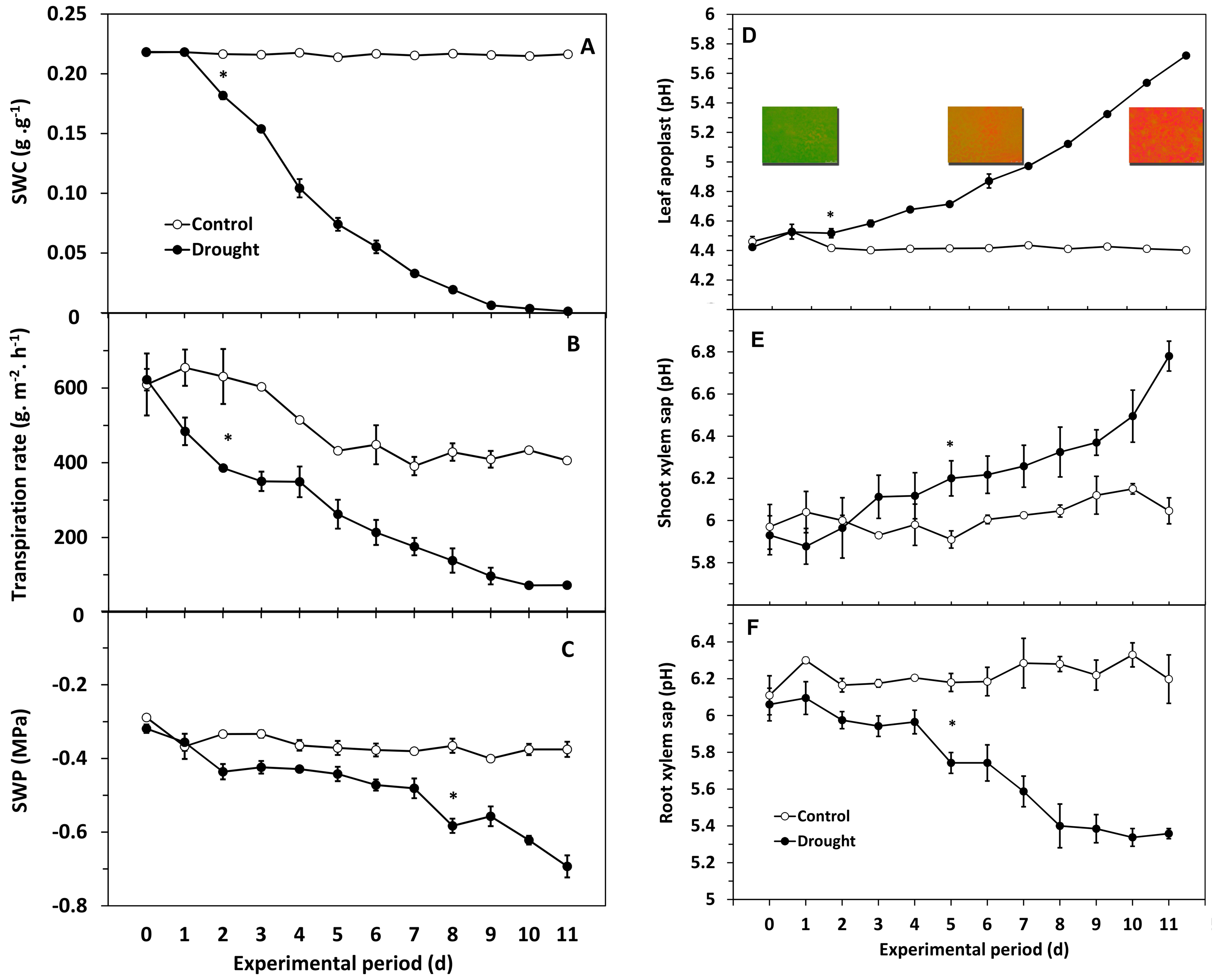
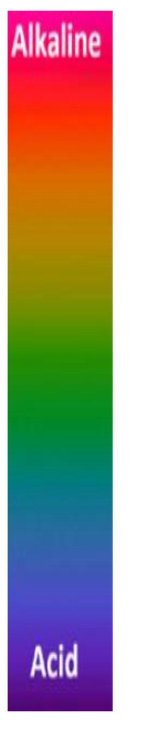
The apoplastic space plays an important role in the response of plants to the environment. Plants experiencing soil water deficits frequently generate a high apoplastic pH and this was proposed as part of sequence in signaling drought and other stresses. Leaf apoplastic pH can have an effect on leaf growth and stomata functioning (Wilkinson and Davies, 1997). However little is known about dynamics of the pH change of leaf apoplast in plants under stress. This is also due to methodological limitations of pH measurements inside of intact plant leaves. Non-invasive optical method of direct *in-situ* pH measurement (Geilfus and Mühling, 2013) can help to clarify role of pH in plant response to drought.

Does pH change in xylem and leaf apoplast contribute to early regulation of stomatal aperture when soil water availability declines?

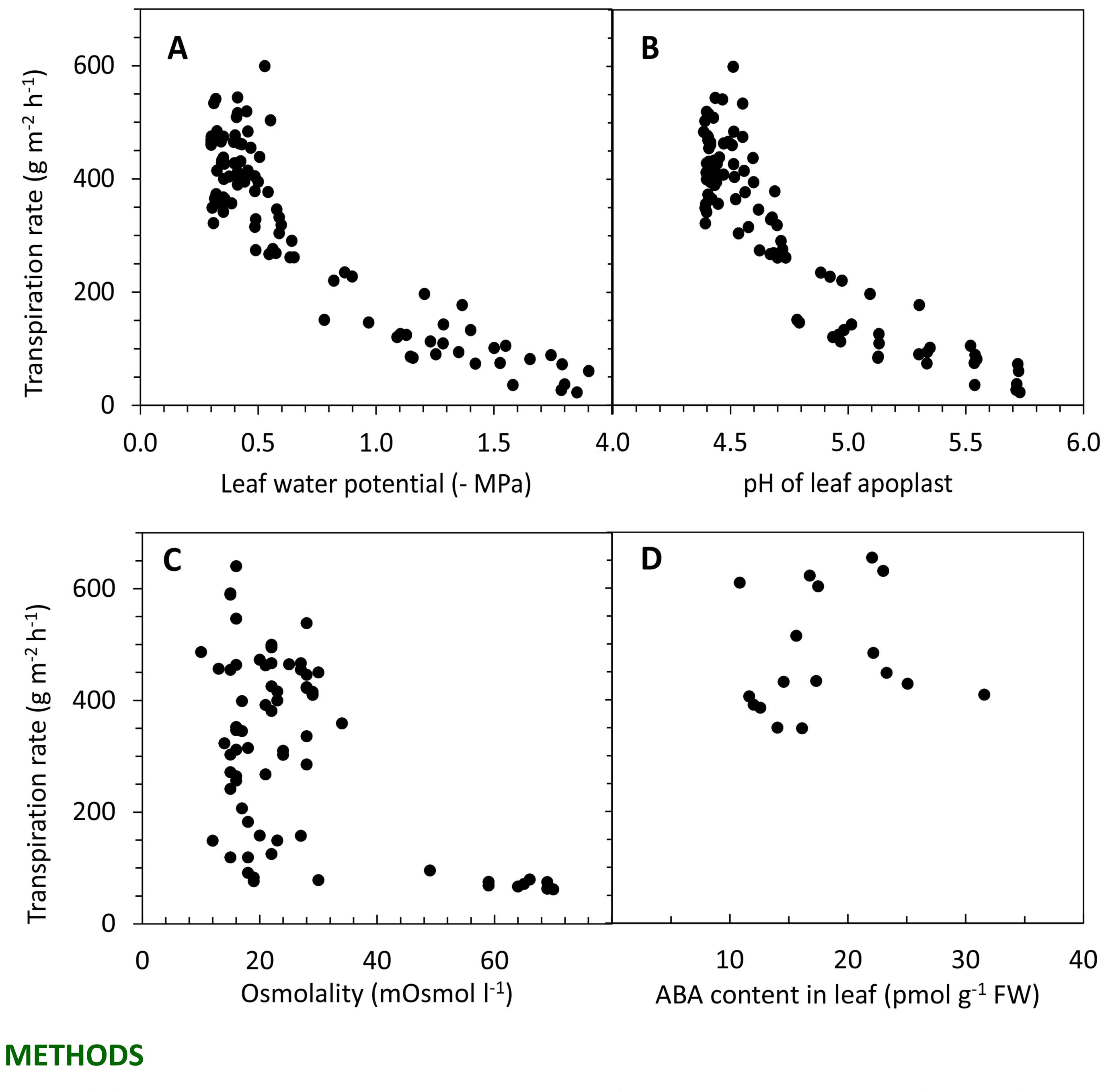
Can pH changes in leaf apoplast be triggered by pH changes in xylem sap?



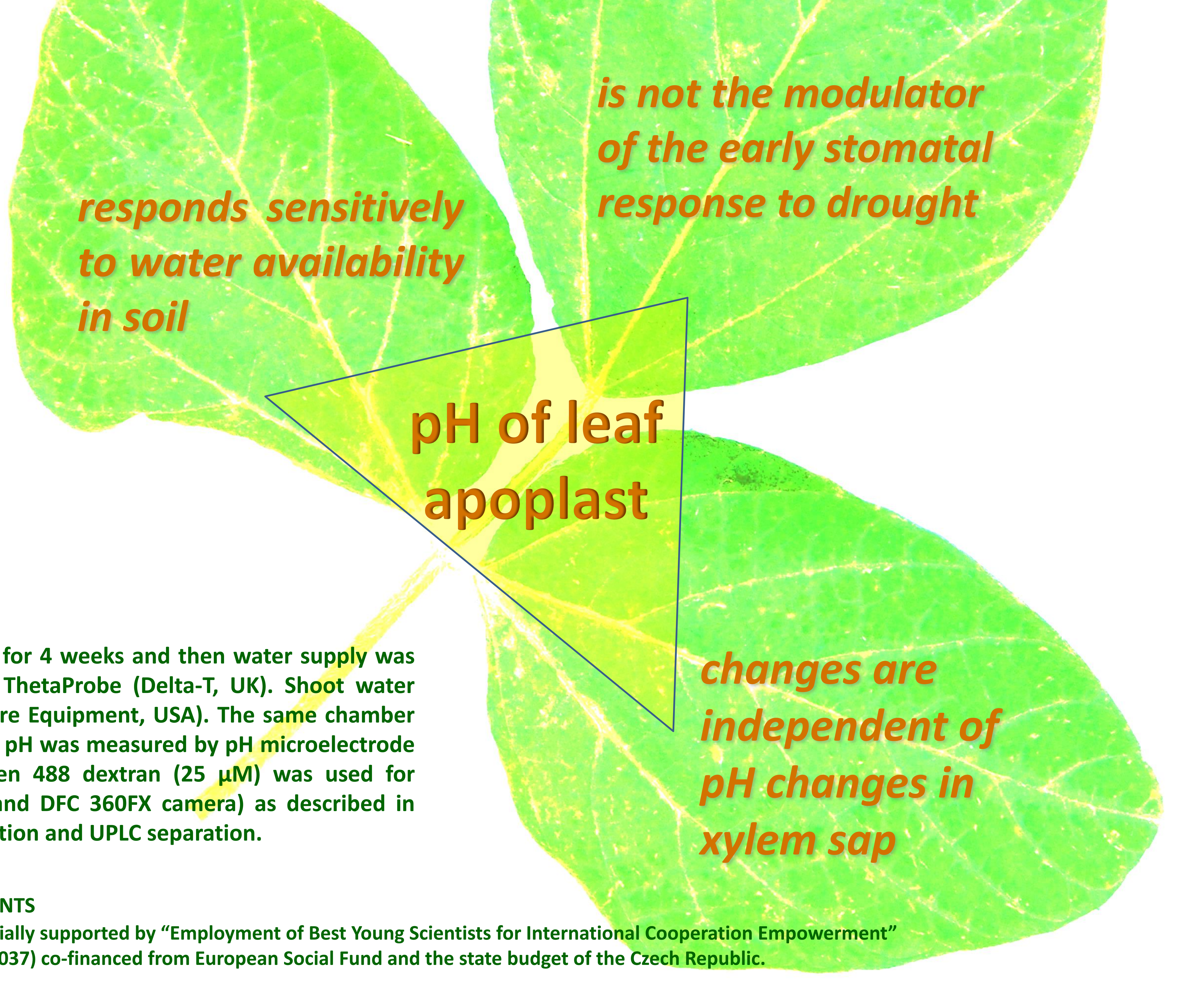


Significant decline of **soil water content (SWC) (A)** was accompanied by decrease of **transpiration rate (B)** of *V. faba*. **Shoot water potential (C)** of plants, however, significantly decreased first on day 8. This indicates fast early response of stomatal regulation to soil drying that does not involve changes in shoot water potential but some other mechanism. Plants were grown and examined under controlled environmental conditions. Shown are means ± SE of 5 replicates.

Significant increase of **pH in leaf apoplast (D)** was already detectable after 2 days of drying. Colour windows show detected signal of indicator in leaf apoplast on days 1, 6 and 11. pH of shoot xylem sap and root xylem sap also significantly changed with progressive drying of soil. **Shoot xylem sap pH (E)** increased significantly after 5 days of drying. In the same time **root xylem sap pH (F)** significantly acidified. This clearly indicates that pH in both leaf apoplast and xylem sap respond sensitively to soil drying but they are independent. Means ± SE of 4 replicates.



Analysis of factors affecting transpiration rate. Massive decrease in transpiration rate in the early phase of drying was not related to the decline of **leaf water potential (A)** or increase of **leaf apoplastic pH (B)**. We also did not find any relationship with **sap osmolality (C)** or **ABA content in leaf biomass (D)** in the first stage of drying. Other signalling mechanism(s) is, therefore, involved in the early sensitive response of stomata to drying soil.



METHODS

Vicia faba L., minor cv. Fuego grown in 2L plastic containers in soil in a climate chamber for 4 weeks and then water supply was completely stopped for following 11d. Soil water content (SWC) was measured using ThetaProbe (Delta-T, UK). Shoot water potential (SWP) was estimated by pressure chamber technique (Model 3005, Soil Moisture Equipment, USA). The same chamber was used for xylem sap sampling from shoot and also from intact root systems in soil. Sap pH was measured by pH microelectrode (Microelectrodes Inc., Bedford, MA, USA). H^+ sensitive fluorescent probe Oregon green 488 dextran (25 μM) was used for apoplastic pH measurement in leaves with the Leica inverted microscope (DMI6000B and DFC 360FX camera) as described in Geilfus and Muhling (2013). ABA content was estimated by mass spektrometry after extraction and UPLC separation.

REFERENCES

Geilfus G-M, Mühling K-H (2013) New Phytol. 197: 1117-1129
Wilkinson S, Davies WJ (1997) Plant Physiol. 113: 559-573

ACKNOWLEDGEMENTS

This work was financially supported by “Employment of Best Young Scientists for International Cooperation Empowerment” (CZ.1.07/2.3.00/30.0037) co-financed from European Social Fund and the state budget of the Czech Republic.