

Leaf wettability is the main driver for foliar P uptake in P-deficient maize

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ARTICLE INFO

Handling Editor: Shivendra Sahi

Keywords:

Foliar P uptake
Leaf wettability
Maize cultivars
P deficiency
Point of deliquescence
Point of efflorescence
Salt hygroscopicity

ABSTRACT

Foliar fertilisation is an alternative form of nutrient application, which is of particular interest for phosphorus (P), where the efficiency of soil fertilisation is low. However, the uptake of foliar-applied nutrients is insufficiently characterised. The aim of this study was to investigate the individual and combined significance of wettability, foliar fertiliser properties and surfactant on foliar P uptake in P-deficient maize (*Zea mays* L.). Sorption and desorption properties of two P salts used as foliar fertilisers (KH₂PO₄, K₂HPO₄) were determined with dynamic vapor sorption isotherms. Leaf surfaces and foliar spray depositions of two differently wettable maize cultivars were investigated by scanning electron microscopy and contact angle measurement. Phosphorus uptake was then linked to leaf and fertiliser solution properties and its effect on cell ultrastructure was characterised by transmission electron microscopy. Wettability was the key factor for P absorption, as all foliar fertilisers were taken up reaching a tissue-P level of adequately nourished plants. For unwettable leaves, only solutions with surfactant, especially the combination of surfactant and hygroscopic P salt (K₂HPO₄) were taken up. This study provides novel insights into the significance of leaf surface and fertiliser properties, which can thus contribute to an improvement of P fertilisation strategies.

1. Introduction

Phosphorus (P) is considered one of the most limiting plant nutrients worldwide and is required at concentrations of 3–5 mg g⁻¹ plant dry matter for optimal growth (Hawkesford et al., 2023). After plant uptake, P remains in the plant either as inorganic P (Pi), esterifies to esters/diesters, or forms energy-rich pyro- and polyphosphate bonds (Penn and Camberato, 2019; Hawkesford et al., 2023). This macro-element has several functions, e.g. as an ATP and NADPH component, as a structural element of membranes or as a component of glycolysis, respiration and photosynthesis (Carstensen et al., 2019; Hawkesford et al., 2023). However, about 30% of the world's land areas have acidic soils and another 30% are calcareous, resulting in P soil fixation and precipitation and thus low P plant availability (von Uexküll and Mutert, 1995; Oki et al., 2004; Hawkesford et al., 2023). As a result, many arable regions

face P deficiency and thus reduced crop biomass and quality (Lynch et al., 1991), caused by poor tillering, delayed growth or premature senescence as in major cereals such as rice and wheat, or additionally by irregular grain development as in maize (Bergmann, 1983). Furthermore, bound P remaining in the soil could contaminate surface waters through surface runoff, endangering the stability of ecosystems (Heckrath et al., 1995; Jeppesen et al., 1998; Haygarth et al., 1998). Foliar P fertilisation is an alternative form of nutrient application that can complement soil fertilisation, potentially reducing P losses and providing plants with P during periods of high demand. Despite some studies on foliar P fertilisation have been developed in recent years (e.g. Arsic et al., 2020, 2022; Talboys et al., 2020; Görlach et al., 2021a; b; Görlach and Mühling, 2021; Henningsen et al., 2022; Henningsen et al., 2023a) there is still much uncertainty about the efficacy of foliar-applied elements (Fernández et al., 2021).

Abbreviations: DAS, Days after sowing; DRH, Deliquescence relative humidity; ERH, Efflorescence relative humidity; POD, Point of deliquescence; POE, Point of efflorescence.

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<https://doi.org/10.1016/j.plaphy.2023.108170>

Received 25 July 2023; Received in revised form 31 October 2023; Accepted 6 November 2023

Available online 16 November 2023

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The uptake of nutrients into the plant via the leaf depends on numerous factors, which can be roughly grouped into those related to plants (e.g., plant physiology, plant surface composition and structure), environmental conditions (e.g. light, temperature, relative humidity (RH)) and physico-chemical and foliar-spray-formulation-related properties (e.g. chemical properties of nutrient salt, surfactant; [Fernández and Eichert, 2009](#); [Fernández et al., 2020, 2021](#)). The composition and structure of the leaf surface are essential for whether applied droplets of agrochemicals adhere to it or whether they are repelled and thus not absorbed ([Fernández et al., 2021](#)). The structural features are determined by the appearance of trichomes, stomata, papillae, cuticular folds (micro-scale roughness) and epicuticular waxes (nano-scale roughness) ([Barthlott et al., 1998, 2017](#); [Koch and Barthlott, 2009](#); [Almonte et al., 2022](#)). Since most aerial plant parts are covered with a cuticle ([Jeffree, 2006](#); [Shellakkutti et al., 2022](#)), its physico-chemical properties will affect the performance of foliar sprays ([Barlas et al., 2023](#)). After being considered an independent layer from the underlying cell wall material for a long time, recent studies are addressing the cuticle as a specialised part of the primary cell wall consisting mostly of lipophilic components (i.e., cutin and waxes), but also of cell wall polysaccharides ([Guzmán et al., 2014](#); [Philippe et al., 2020](#); [González Moreno et al., 2023](#)). The combined influence of leaf surface structures and chemical composition determines the interaction of the surface with water or aqueous solutes applied to it and results in different contact angles, provided adsorption occurs ([Fernández et al., 2017, 2021](#)). High contact angles reduce the contact area between liquid and surface, while low contact angles increase the contact area and thus potentially enhance foliar absorption, making wettability an important factor to consider for foliar penetration ([Fernández et al., 2021](#)).

Besides the composition and structure of plant surfaces, the chemical properties of the foliar spray formulations are important for uptake, often in relation to environmental conditions ([Fernández and Eichert, 2009](#)). The importance of hygroscopic compounds to be supplied as foliar sprays in response to RH has been emphasised in some investigations ([Schönherr, 2001](#); [Fernández and Eichert, 2009](#)). Foliar-applied solutions are usually not in equilibrium with the water potential of the atmosphere and therefore evaporate quickly from the leaf ([Eichert and Fernández, 2023](#)). Once equilibrium with the atmosphere is reached, the solutions may remain liquid or dry out, depending on ambient RH ([Eichert and Fernández, 2023](#)). Each solute has a specific deliquescence RH (DRH) or point of deliquescence (POD) at which it becomes liquid due to the absorption of water from the atmosphere ([Allan and Mauer, 2016](#); [Fernández et al., 2020](#); [Eichert and Fernández, 2023](#)). Conversely, as the RH decreases, solutions also have an efflorescence RH (ERH) or point of efflorescence (POE) at which the previously saturated solution becomes solidified by crystallisation ([Fernández et al., 2020](#); [Barlas et al., 2023](#)). Thus, when fertiliser drop solutions are deposited onto the leaf, ERH determines its drying and DRH the rehydration due to, for example, increased RH, dew or fog ([Fernández et al., 2020](#); [Barlas et al., 2023](#)). Furthermore, the penetration of foliar-applied agrochemicals depends on the concentration gradient between the solution on the leaf surface and the leaf interior, which works best with the existence of an aqueous diffusion medium, i. e. when the deposited compound is hydrated ([Fernández et al., 2020](#)). Deliquescence RH and ERH may therefore be important parameters for determining the potential of solutes to be taken up by the plant as a result of foliar application ([Eichert and Fernández, 2023](#)). Furthermore, additives (e.g. surfactants, humectants or stickers) can change the properties of the foliar fertiliser solutions such as increasing/decreasing the DRH or reducing surface tension and thereby improving wettability and ultimate foliar uptake ([Fernández and Eichert, 2009](#)).

After foliar sprays have been deposited onto the leaf surface, the subsequent uptake into the leaf interior depends on the physico-chemical features of the cuticle and other epidermal structures. Especially stomata ([Eichert and Goldbach, 2008](#); [Eichert et al., 2008](#)), and trichomes ([Li et al., 2018a,b, 2019](#); [2021](#); [Arsic et al., 2022](#)), but also

vascular bundle tissues ([Bahamonde et al., 2018](#); [Arsic et al., 2020, 2022](#); [Henningsen et al., 2023a](#)) have been identified as being involved in the uptake of nutrients via the leaf. Within the cuticle, the embedded polysaccharides are assumed to be key for the cuticle penetration of foliar solutes ([Fernández et al., 2017](#)). Polysaccharides have high polar and hydrogen-bonding components and can therefore adsorb water at high ambient RH, resulting in swelling and shrinking of the cuticle, supposedly enabling uptake ([Chamel et al., 1991](#); [Fernández and Eichert, 2009](#); [Fernández et al., 2016](#)).

Nutrient deficiency can lead to alteration of surface composition and structures ([Rosolem and Leite, 2007](#); [Fernández et al., 2008](#); [Will et al., 2011](#); [Li et al., 2019](#)) and thus to reduced uptake of foliar applied nutrients ([Restrepo-Díaz et al., 2008](#); [Will et al., 2011](#); [Li et al., 2019](#); [Eichert and Fernández, 2023](#)). Phosphorus deficiency was also found to reduce the occurrence of epidermal structures in wheat ([Fernández et al., 2014](#)) while in barley, the cuticle was thicker and the abundance of polysaccharides was diminished, leading to reduced P uptake via the leaf ([Arsic et al., 2022](#)). In maize, on the other hand, it was shown that no change in the surface occurred with different P nutrition. However, different maize varieties had different epicuticular wax topography also during plant ontogeny which involved wettability changes ([Henningsen et al., 2023a,b](#)). Comparison of cultivars of the same crop species with regard to their surface features and influence on the uptake of foliar-applied nutrients have been basically ignored so far, but have the potential to be more accurate and enlightening than the comparison of two species.

Aware that leaf wettability and the characteristics of fertiliser sprays may be crucial for the uptake of foliar-applied nutrients, the objective of this study was to determine the influence of maize leaf wettability (using a wettable versus an unwettable variety) on the uptake of a hygroscopic (K_2HPO_4) and a non-hygroscopic (KH_2PO_4) P salt, supplied as foliar spray with and without surfactant. The hypotheses to be tested were: (i) The cultivars analysed have different leaf wettability rates, with foliar P absorption being higher for wettable versus unwettable leaves, (ii) foliar P fertilisers evaluated have different DRH and ERH values, the salt leading to the highest foliar P absorption rates being the one having the lowest DRH/ERH, (iii) leaf P uptake is higher for foliar fertiliser solution with surfactant regardless of the P form supplied as foliar spray.

2. Materials and methods

2.1. Plant material

Seeds of maize (*Zea mays* L., cv. DKC 3096, Bayer CropScience AG, Monheim, Germany; cv. P7948, Pioneer Hi-Bred International, Inc., Johnston, IA, USA) were placed in an alkaline (pH 8.34) P-deficient (0.35 mg P 100 g⁻¹ soil, determined by Olsen P extraction) sandy loam (S14) at a depth of 30 mm in pots with a volume of 3 L. Eight days after sowing (DAS), plants had germinated and all nutrients required for plant growth were applied to the soil in dissolved form in the following amounts (in g pot⁻¹): 0.5 N (as NH_4NO_3), 0.6 K (as KCl), 0.6 K (as K_2SO_4), 0.25 Ca (as $CaSO_4$), 0.17 Mg (as $MgSO_4$), 0.008 Fe (as Fe-EDDHA), 0.005 Cu (as $CuSO_4$), 0.008 Zn (as $ZnSO_4$), 0.015 Mn (as $MnSO_4$), 0.005 B (as H_3BO_4), 0.001 Mo (as $(NH_4)_6Mo_7O_{24}$). Depending on the treatment, P was applied in an amount of 0.3 or 0.075 g pot⁻¹ in the form of KH_2PO_4 . 14 days after the first nutrient supply, reapplication of the same amounts was carried out.

The plants were cultivated in a greenhouse at the School of Forest Engineering at the Technical University of Madrid (40°27'05.3" N, 3°43'22.5" W) from October to November. The day/night cycle was 16/8 h and the temperature was 16 °C at night and at most 30 °C during the day. The relative humidity (RH) varied between 25% and 100%, with an average of around 85%. Pots were arranged in a completely randomised design and were re-arranged every week.

For comparison of different leaf surface properties, the above-mentioned maize varieties were selected based on the results of

(Henningsen et al., 2023b). Immediately after germination, the plants of both varieties were divided into two P soil-supply levels, namely sufficient (0.3 g pot^{-1}) and deficient (0.075 g pot^{-1}). The deficiently P supplied plants of both cultivars were further subdivided into five treatments, of which four different P application treatments were selected in addition to a control treatment (described in chapter 2.2). Before and after the foliar P application, which was carried out 41 DAS, several plant samples were collected for detailed characterisation of the leaf properties and analysed using the methods described below.

2.2. Foliar P treatment and characterisation of P salt response to humidity

Foliar treatments contained 200 mM P supplied as KH_2PO_4 or K_2HPO_4 , both with and without 0.1% Genapol X-80 (Sigma-Aldrich, St. Louis, MO, USA) surfactant. Foliar P sprays were applied to the leaves of both maize varieties, resulting in four different foliar application treatments. When applying P via the leaf, it was ensured that both the upper (adaxial) and lower (abaxial) leaf surfaces were fully sprayed. Covering the pots and wrapping the stems of the plants prevented contamination of the soil by direct application or stem run-off (Henningsen et al., 2022).

To determine the DRH and ERH of the P salts, a climatic chamber with adjustable relative humidity (RH) and temperature was used (MKF 56, Binder, Tuttlingen, Germany). For assessing the DRH, 1 g of each salt was first placed in a Petri dish and then kept in the climatic chamber at 25°C and initially at a low RH. Subsequently, the RH was increased in small steps until the hydration of salt crystals became visible using an optical microscope. This RH marked the DRH and was then maintained for about an hour to ensure that the salt continued to liquefy due to water sorption and dissolution. The samples were left overnight at the maximum RH level of 95%. In the case of non-hygroscopic KH_2PO_4 , which was not liquefied even at 95% RH, a concentrated solution was prepared close to its solubility value. In a second step, the RH was gradually decreased again and the solutions were observed with an optical microscope to determine the point at which desorption and crystallisation lead to a transition to a solid phase, thus marking the ERH (Fernández et al., 2020).

In order to further characterise the sorption and desorption and thus also the DRH and ERH of KH_2PO_4 and K_2HPO_4 , dynamic vapor sorption (DVS) isotherms were performed at 25°C with a TGA Q5000 instrument (TA instruments, USA; Thermal Analysis and Calorimetry Service IQAC-CSIC, Barcelona, Spain). Approximately 10 mg of the salt was placed in the TGA which was preliminary kept at 20°C and 0% RH for 300 min. Afterwards, the RH was increased at $0.1\% \text{ min}^{-1}$ up to 95% RH to determine the DRH and, after reaching the maximum RH, it was decreased until 0% RH by $0.1\% \text{ min}^{-1}$ to determine the ERH (Fernández et al., 2020).

2.3. Plant tissue sampling and elemental analysis

Maize plants were harvested one (42 DAS; BBCH 14), seven (48 DAS; BBCH 14–15) and 14 (55 DAS; BBCH 15) days after foliar P application for determination of leaf tissue P concentrations. Initially, four plants ($n = 4$) of each treatment were divided into 4th leaf and remaining shoot. This partitioning of the shoot was done due to differences in the wettability of the two selected varieties, which only occur from the 4th leaf upwards as previously discovered (Henningsen et al., 2023b). Subsequently, all plant parts were washed in an acidified (0.1 N HCl) solution added with 0.1% detergent (Fairy, P&G, Cincinnati, OH, USA) before being washed twice with tap water and once with distilled water (Bahamonde et al., 2023b). The samples were then oven-dried at 65°C until constant weight was reached, weight and ground (Cyclotec 1093, Foss Tecator, Höganäs, Sweden). 200 mg of each plant sample was then dissolved with 10 mL of 69% HNO_3 (ROTIPURAN Supra for ICP 69%, Carl Roth GmbH & Co. KG, Karlsruhe, Germany) in a microwave-oven (MARS 6 Xpress; CEM, Matthews, MC, USA) at 1800 W for 55 min.

Subsequently, after dilution with double-distilled water to 100 mL, an aliquot of the sample was analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent Technologies 5800 RV, Santa Clara, CA, USA) (Henningsen et al., 2022).

2.4. Contact angle measurements

Equilibrium contact angles were measured with a drop shape analysis system (DSA 100, Krüss, Hamburg, Germany). The adaxial and abaxial side, as well as the midrib of the 4th leaf of both varieties, was mounted on a microscope slide and drops of distilled water, KH_2PO_4 , $\text{KH}_2\text{PO}_4 + 0.1\%$ surfactant, K_2HPO_4 and $\text{K}_2\text{HPO}_4 + 0.1\%$ surfactant were applied. Drops (approximately $1.5 \mu\text{L}$) were deposited with a 1 mL syringe having a 0.5 mm tip and the contact angle was calculated using the tangent method (Bahamonde et al., 2023a).

2.5. Electron microscopy

Adaxial and abaxial surface samples of P-deficient and P-sufficient 4th leaves of both varieties were collected on the day of foliar application, and were examined by scanning electron microscopy (SEM). Immediately after sampling, leaf pieces (10 mm^2) were placed in phosphate buffer (pH 7.2). Subsequently, the samples were dehydrated in a series with pure ethanol (Merck KGaA, Darmstadt, Germany) and distilled water, followed by critical point drying (Leica EM CPD300, Leica Microsystems, Wetzlar, Germany). After gold (Au) sputtering, the samples were observed by SEM (Jeol JSM6400, Jeol Ltd., Akishima, Japan; National Electron Microscopy Center (CNME), Madrid, Spain). In order to characterise leaf depositions of the different foliar P application treatments, also by Energy-dispersive X-ray spectroscopy (EDX), segments of the 4th leaf were additionally investigated in a field emission scanning electron microscope (Sigma 300 VP, Carl Zeiss, Jena, Germany; Miguel Hernández University of Elche, Spain) eight days after foliar application without prior dehydration and sputtering.

Transmission electron microscopy (TEM) was used to assess the influence of P nutritional status and foliar P treatment on cell and surface structures in cross-section. Therefore, pieces (4 mm^2) of the 4th leaf were selected 14 days after foliar application and again immersed in phosphate buffer to be then post-fixed in a 1:1 solution of osmium tetroxide (2%, TAAB Laboratories, Aldermaston, UK) and potassium ferrocyanide (3%, Sigma-Aldrich, St. Louis, MO, USA). The samples were subsequently dehydrated in a series with acetone and distilled water before being immersed in an acetone-Spurr's resin (TAAB Laboratories, Aldermaston, UK) mixture of increasing concentration until pure resin was provided. The leaf pieces were transferred into blocks, embedded in pure Spurr's resin and stored in an oven at 70°C until complete polymerisation. Ultra-thin sections were then cut (Leica Ultracut E, Leica Microsystems, Wetzlar, Germany) and observed with a Jeol JEM 1400plus (Jeol Ltd., Akishima, Japan; CNME, Spain) (Henningsen et al., 2023a).

2.6. Statistical analysis

The statistical software R (version 2022.12.0, R foundation for statistical computing, Vienna, Austria) was used to evaluate the data (R Core Team, 2022). Data evaluation started with the definition of an appropriate statistical model based on generalized least squares (model 1, 2 and 3; Carroll and Ruppert, 1988; Box et al., 2015). Model 1 included the factors variety, treatment and their interaction term. Note that treatment is a pseudo factor, representing the combination of the actual factors leaf part and P supply. This pseudo factor was necessary as the actual factors are not orthogonal (Schaarschmidt and Vaas, 2009). Model 2 included the factors variety, P supply, plant part and harvest, as well as all interaction terms. Also, the correlations of the measurement values due to the several plant parts were taken into account. In model 3, the factors variety, P supply and measurement-time, as well as all

interaction terms were included. Also, the correlations of the measurement values due to the several measurement-times were taken into account. The residuals were assumed to be normally distributed and to be homoscedastic (model 1) or heteroscedastic with respect to the factors variety, P supply and plant part (model 2) or variety, P supply and measurement-time (model 3). These assumptions are based on a graphical residual analysis. Based on these models, a Pseudo R^2 was calculated (Nakagawa and Schielzeth, 2013) and an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests (Hothorn et al., 2008; see also Bretz et al., 2016) in order to compare the several levels of the influence factors, respectively.

3. Results

3.1. Hydration and dehydration characteristics of P salts

Based on DVS isotherms and supplementary climate chamber experiments, the DRH and ERH as key parameters for the characterisation of sorption and desorption processes were obtained for KH_2PO_4 and

K_2HPO_4 (Fig. 1). According to the results of the DVS isotherm, the DRH for K_2HPO_4 is at about 46.9% RH (Fig. 1B). For KH_2PO_4 which is not hygroscopic as derived from the lack of hysteresis of the DVS isotherm which also had a Y axis ranging between 0 and 2% mass change (i.e., the salt had a low water sorption capacity), this point could not be clearly identified, indicating that the DRH is at $> 95\%$ RH (Fig. 1A). The overall increase in mass was significantly greater for K_2HPO_4 (132.9%) than for KH_2PO_4 (−0.04%), also indicating that KH_2PO_4 did not absorb any water and thus did not reach the DRH in the 0–95% RH interval analysed (i.e., the DRH is $> 95\%$; Fig. 1A and B). The increase in weight of K_2HPO_4 corresponds to the molar mass of three H_2O molecules, indicating that the hydration state of the originally anhydrous compound may have changed to three water molecules after desorption.

In climate chamber experiments, the findings from the sorption isotherm of KH_2PO_4 were confirmed, because also in this case no hydration of the salt could be detected at 95% RH (Fig. 1C and D). Additionally, for K_2HPO_4 , the DRH of 46.9% RH (DVS) was verified, as the onset of sorption was observed in a similar RH range (Fig. 1I). When desorbing the saturated KH_2PO_4 solution, the formation of salt crystals

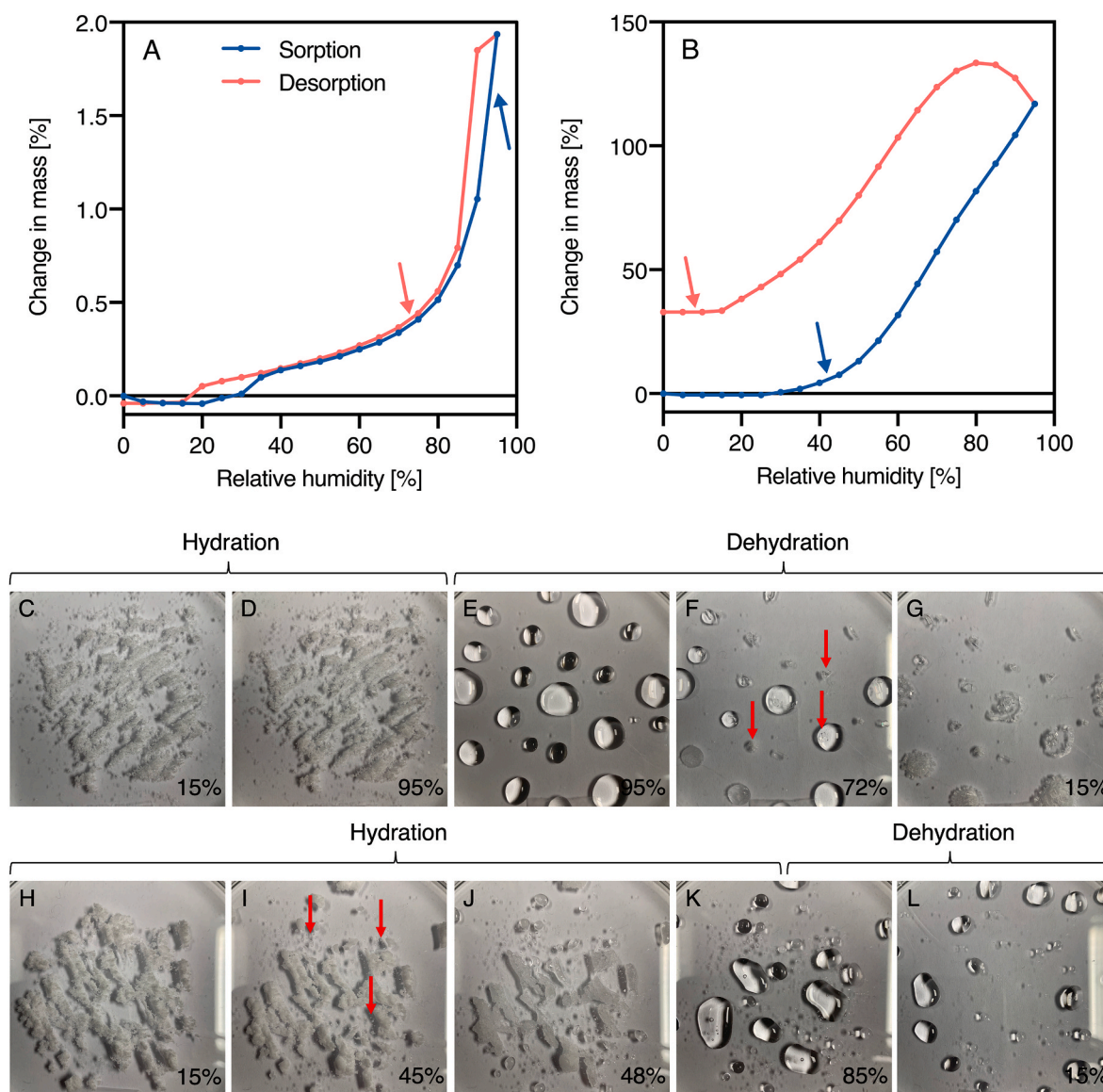


Fig. 1. Dynamic vapor sorption (DVS) isotherms of KH_2PO_4 (A) and K_2HPO_4 (B) at 25 °C and images of the hydration and dehydration processes of KH_2PO_4 (C–G) and K_2HPO_4 (H–L) of climate chamber trials. For DVS isotherms, blue arrows represent the DRH and red ones the ERH. For hydration and dehydration images, red arrows indicate the beginning of crystal formation after reaching the ERH for KH_2PO_4 (F) and the beginning of hydration/change to liquid phase after reaching the DRH for K_2HPO_4 (I). Black numbers indicate the relative humidity [%] when the image was taken.

began at approximately 72% RH, which is the ERH of this compound at 25 °C (Fig. 1F). The ERH of K_2HPO_4 is below 15% RH as visually observed in climate chamber trials (Fig. 1L).

3.2. Leaf surface characteristics

3.2.1. Leaf surface wettability

When drops of pure water were applied onto the surface of the 4th leaf of variety P7948 with a sprayer, they adhered and the entire leaf was covered with small to medium-sized drops (Fig. 2A). The surface of variety DKC 3096, on the other hand, retained only a small amount of fine drops on the leaf lamina. However, more drops adhered to the midrib (Fig. 2B).

These observations can be confirmed by contact angle measurements, which are shown in Fig. 2C–F. When applying solutions of KH_2PO_4 and K_2HPO_4 with pure water (without surfactant), variety P7948 always had lower contact angles compared to DKC 3096, regardless of the plant part (Fig. 2C, E, Supplementary Table S1). However, by adding a surfactant to the spray solution, this relationship was reversed on the abaxial leaf side and on the adaxial side there was no longer any wettability difference between maize varieties (Fig. 2D, F, Supplementary Table S1). Furthermore, the contact angles dropped substantially due to the addition of a surfactant (e.g. from 135° to 42° in DKC 3096; Fig. 2). While the adaxial and abaxial leaf sides basically showed the same wettability, the contact angle of the midrib was significantly lower (Fig. 2C, E, Supplementary Table S1).

3.2.2. Phosphorus salt deposition in drops

Leaf surfaces of all treatments and the KH_2PO_4 and K_2HPO_4 drop deposits formed after foliar spraying are shown in Supplementary Figs. S1.

To provide a closer look at the leaf surface and fertiliser drop deposits formed onto foliar-sprayed leaves, SEM observations were carried out (Fig. 3, Supplementary Fig. S2). First of all, it can be noted that the 4th leaf of all treatments of variety DKC 3096 were covered with epicuticular waxes (Fig. 3A, C, E, G, I, K). These waxes do not appear in variety P7948, which explains its higher wettability (Fig. 3B, D, F, H, J, L). While hardly any deposits were visible to the naked eye as a result of KH_2PO_4 and K_2HPO_4 foliar application in variety DKC 3096 (Supplementary Fig. S1), magnification clearly shows that there were deposits to be found onto leaves (Fig. 3E, I, Supplementary Fig. S2C). Often,

however, fertiliser residues adhered to the epicuticular waxes and did not appear to have extensive contact with the cuticle. The preferential adsorption of foliar spray solutions by the midrib of variety DKC 3096, which was visible to the naked eye, can be confirmed by Supplementary Fig. S2A. In variety P7948, residues were often characterised by their crystalline structure (Fig. 3F, H), some of which were very voluminous and elaborate (Supplementary Figs. S2B and D) and for K_2HPO_4 also by flat, dense patches, as well as dendritic crystals (Fig. 3J, L). To check their chemical composition, SEM-EDX analyses were additionally carried out. From the qualitative atomic percentage of the observed deposits, we noted that there were changes in their potassium (K) and P ratios and thus in their chemical structure (Supplementary Fig. S3, Table S2).

The addition of a surfactant changed the shape of the deposits, so that in variety DKC 3096, irrespective of the type of P salt, predominantly thin, rod-shaped salt crystals were formed, some of which lied on the waxes and some of which were embedded in them (Fig. 3G, K). In the case of variety P7948, the drop deposits were again different, with crusts of varying thickness being formed, lying flat against the leaf surface. These appeared firm and encrusted with KH_2PO_4 + surfactant (Fig. 3H) and less firm and partly somewhat fluid with K_2HPO_4 + surfactant (Fig. 3L).

3.3. Foliar P absorption and ultrastructural response

3.3.1. Foliar P absorption

One day after foliar application, the wettable 4th leaf of variety P7948 had a higher tissue P concentration than the unwettable one of DKC 3096 for every foliar P fertiliser solution applied (Fig. 4A, D, Supplementary Table S3). Thereby, all foliar treatments in variety P7948 reached the P concentration level of sufficient control plants (Fig. 4D), whereas in DKC 3096, only the application of K_2HPO_4 + surfactant led to significantly increased P concentrations (Fig. 4A). After seven days, an apparently higher amount of P from nearly all foliar sprays was taken up by the 4th leaves of variety DKC 3096 (Fig. 4B). Significantly more leaf tissue P could be determined by applying solutions mixed with 0.1% surfactant, whereby again, DKC 3096 plants supplied with K_2HPO_4 + surfactant clearly absorbed the most P and were thus the only ones to reach the level of P-sufficient control plants (Fig. 4B). In the case of variety P7948, P concentration in the 4th leaves of different treatments remained constant from the first to the second

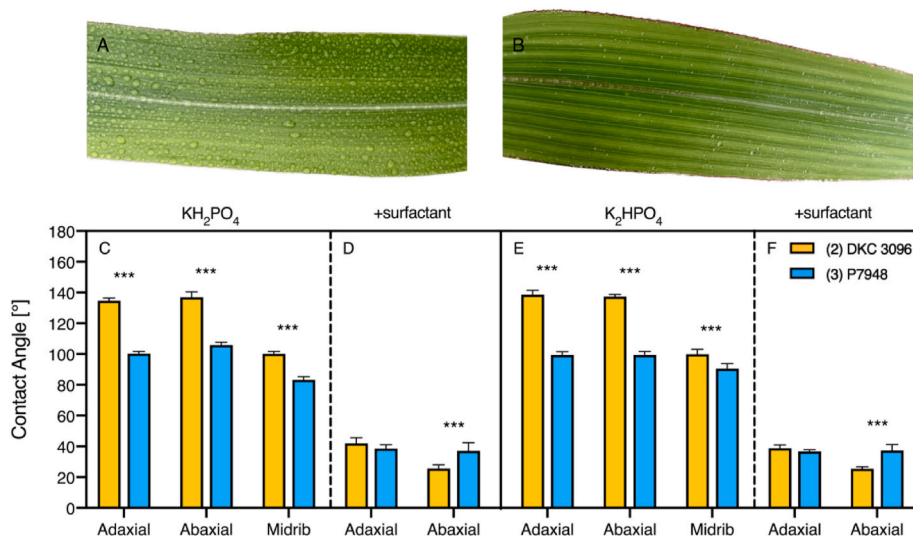


Fig. 2. Differently pronounced droplet adhesion on the well-wettable 4th leaf of maize variety P7948 (A) and on the poorly wettable 4th leaf of maize variety DKC 3096 (B). Contact angles from drops of 200 mM KH_2PO_4 (C), 200 mM KH_2PO_4 + 0.1% surfactant (D) 200 mM K_2HPO_4 (E) and 200 mM K_2HPO_4 + 0.1% surfactant (F) applied to the adaxial and abaxial leaf lamina as well as to the midrib in variety DKC 3096 and P7948. Differences between varieties within the same leaf part and solute are shown according to multiple contrast test. Data are means \pm SD (n = 6). *** = $p \leq 0.001$.

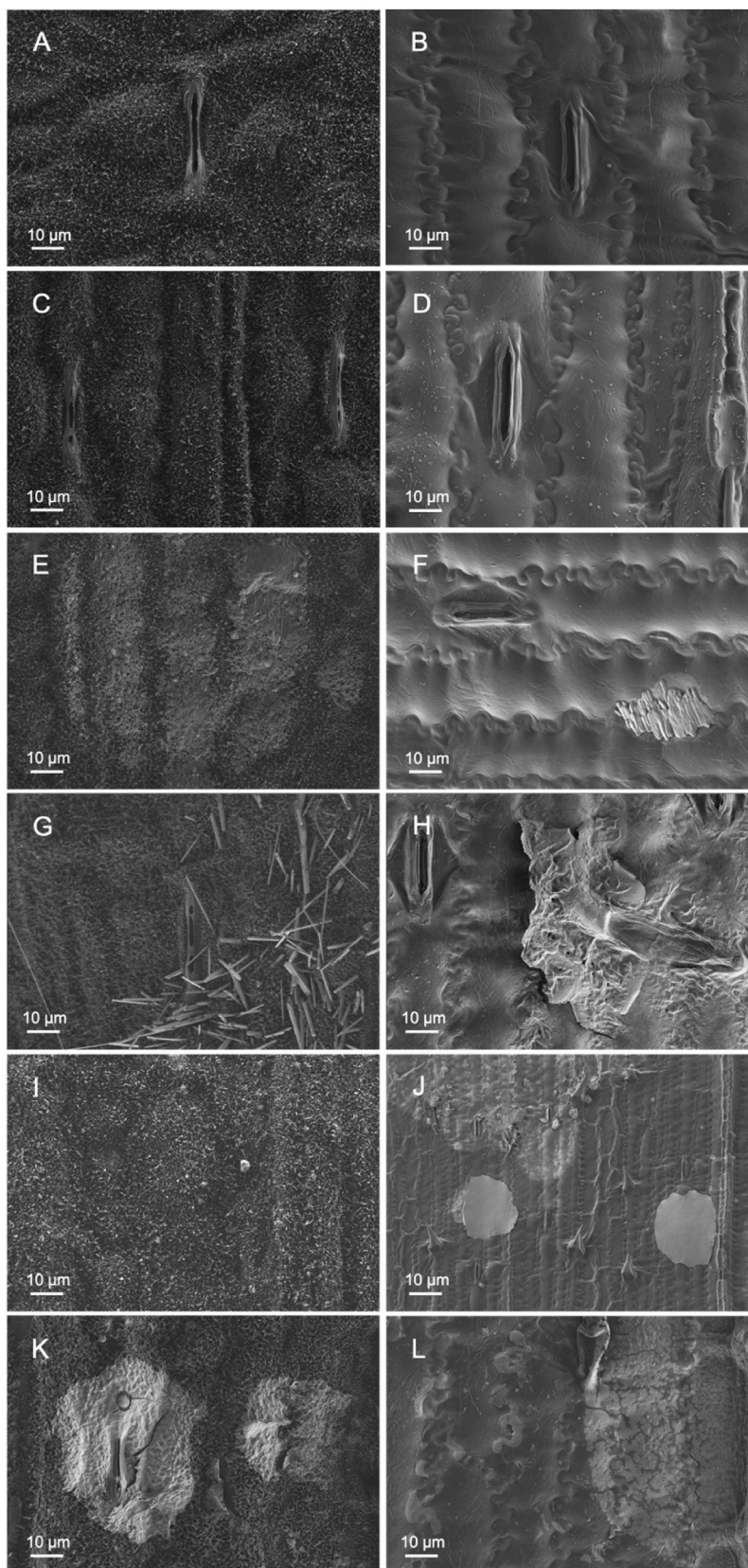


Fig. 3. Scanning electron micrographs with 1.000 \times magnification of the leaf surface of maize cultivar DKC 3096 (A, C, E, G, I, K) and P7948 (B, D, F, H, J, L) showing the P-sufficient (A, B) and P-deficient control (C, D) as well as the deposits formed on the leaf surface as a result of foliar application of 200 mM KH_2PO_4 (E, F), 200 mM KH_2PO_4 + 0.1% surfactant (G, H), 200 mM K_2HPO_4 (I, J) and 200 mM K_2HPO_4 + 0.1% surfactant (K, L).

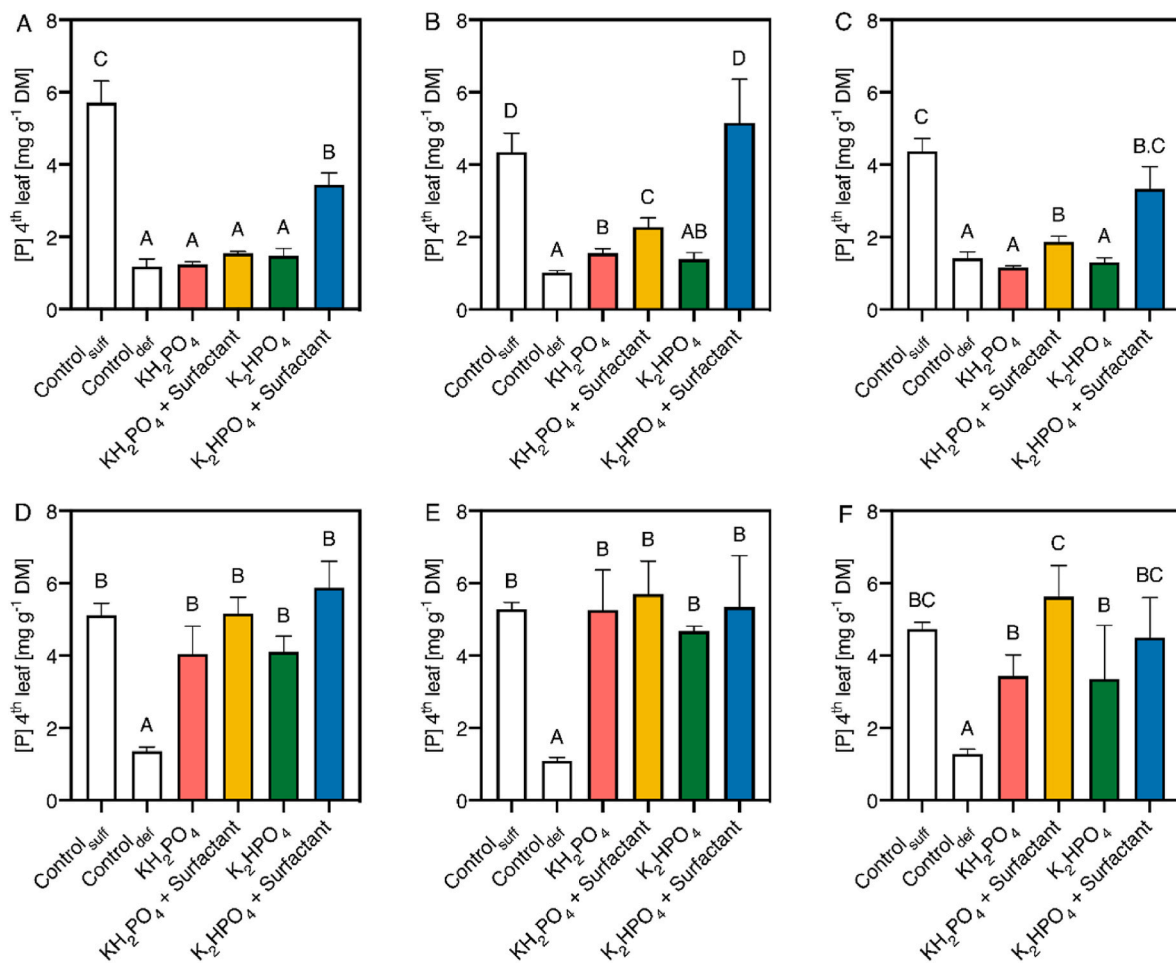


Fig. 4. Plant tissue P concentrations [P] of the 4th leaf from maize variety DKC 3096 (A–C) and P7948 (D–F) harvested one day (A, D), seven days (B, E) and 14 days (C, F) after foliar application of 200 mM KH₂PO₄, 200 mM K₂HPO₄, 200 mM KH₂PO₄ + 0.1% surfactant and 200 mM K₂HPO₄ + 0.1% surfactant. Letters indicate differences between the treatments within each variety and time of harvest according to multiple contrast test ($P \leq 0.05$). Data are means \pm SEM ($n = 4$).

measurement date (Fig. 4E). However, after the third harvest, 14 days after foliar spraying, the previously only slightly indicated trend towards higher P concentrations in the +surfactant treatments was now clearly recognisable and even significant. Nevertheless, all foliar P applied plants still had significantly higher P concentrations than P-deficient control plants (Fig. 4F). In DKC 3096, tissue P concentrations of the 4th leaves also decreased after 14 days and only the +surfactant treatments remained significantly higher than the P-deficient control plants (Fig. 4C).

The remaining shoot (whole shoot except for 4th leaf) had a generally higher P concentration level than the 4th leaf (Fig. 5, Supplementary Table S3). The differences between the P treatments in variety DKC 3096 were similar to those in the 4th leaf (Fig. 5A–C). Also for this part of the plant, the K₂HPO₄ + surfactant foliar spray resulted in the highest P concentrations at all harvest dates, but the level of sufficiently P supplied plants could only be reached seven days after the foliar treatment (Fig. 5B). While in the 4th leaf, the tissue P concentrations following foliar application were mostly significantly higher in variety P7948 than in DKC 3096, for the remaining shoot this was only the case in the KH₂PO₄ + surfactant treatment on day 1 and 14 and in the KH₂PO₄ treatment on day 14 after foliar spray (Fig. 5B, C, E, F, Supplementary Table S3). The highest P uptake was again determined by the application of solutions with surfactants (Fig. 5D–F). In contrast to variety DKC 3096, however, also the application of pure nutrient solutions (without surfactant) in variety P7948 resulted in a significantly higher tissue P concentration compared to the P-deficient control plants on the 7th and 14th day (Fig. 5E and F).

3.3.2. Leaf ultrastructure

The cross-section ultrastructure of the 4th leaf of maize plants is shown with variety P7948 as an example (Fig. 5). P deficiency has led to a significantly reduced number and size as well as to the malformation of chloroplasts (Fig. 6B). Phosphorus foliar application (Fig. 6C), in this case with the well-absorbed KH₂PO₄ + surfactant, improved the number and structure of chloroplasts compared to P-deficient plants (Fig. 6B), but could not achieve the ultrastructure of P-sufficient plants.

4. Discussion

In this study, we examined the influence of a wettable and an unwettable maize variety on the foliar uptake of a hygroscopic and a non-hygroscopic P salt supplied as a foliar spray with and without surfactant to P-deficient plants. In addition, the influence of foliar P application on the ultrastructure of mesophyll and bundle sheath cells was examined. An array of variations was recorded regarding the effect of P supply, variety and response to foliar P application as described in the following paragraphs.

4.1. P salts differ in DRH and ERH and thus in P-salt deposition

According to Schönher (2002), nutrient salts used for foliar fertilisation should have a low DRH. The reason for this is the fact that nutrients are absorbed by leaves/isolated cuticles at higher rates when supplied as solutes (i.e., dissolved in water) (Fernández et al., 2020). Therefore, a compound with a high DRH value means that after solution

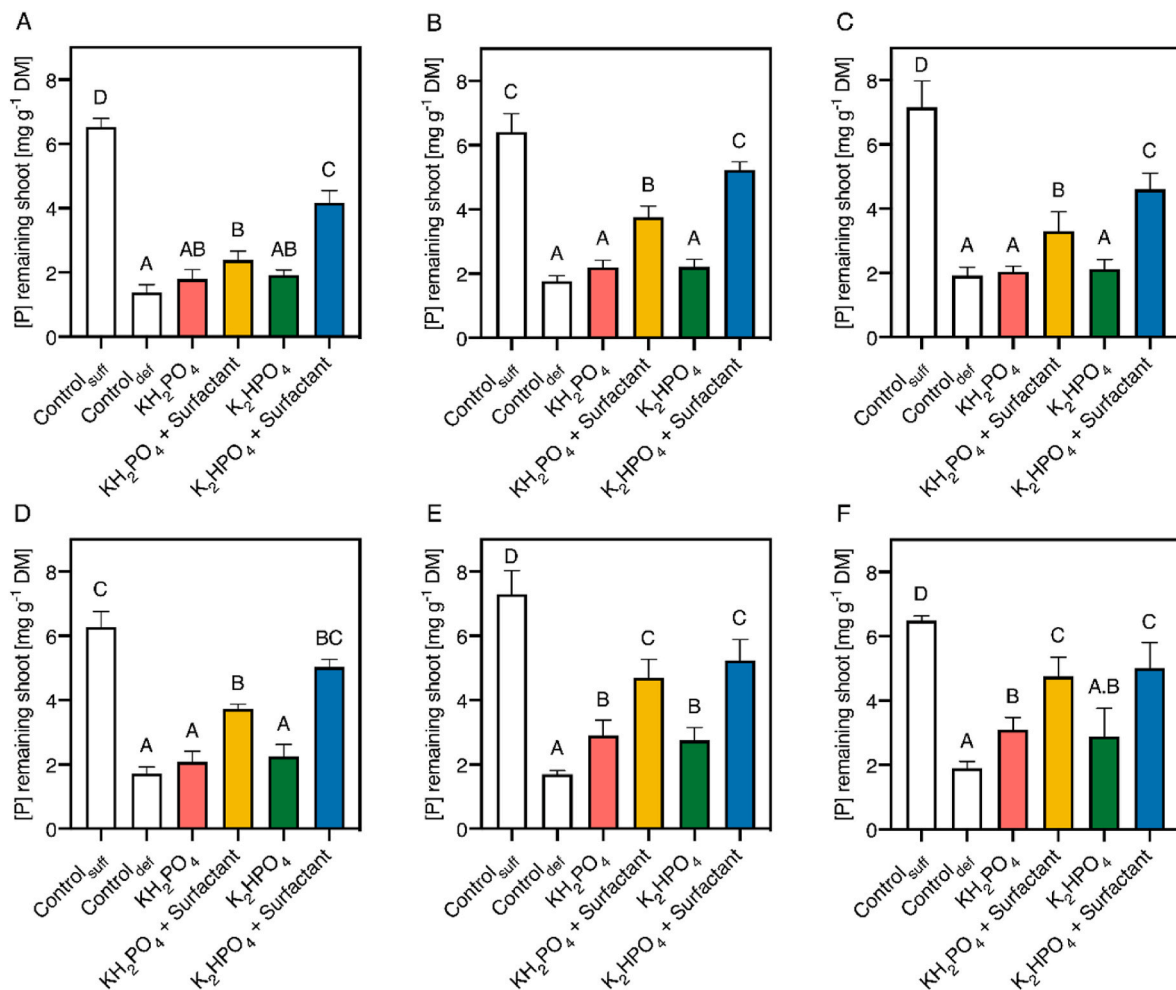


Fig. 5. Plant tissue P concentrations [P] of the whole shoot (except of the 4th leaf) from maize variety DKC 3096 (A–C) and P7948 (D–F) harvested one day (A, D), seven days (B, E) and 14 days (C, F) after foliar application of 200 mM KH₂PO₄, 200 mM K₂HPO₄, 200 mM KH₂PO₄ + 0.1% surfactant and 200 mM K₂HPO₄ + 0.1% surfactant. Letters indicate differences between the treatments within each variety and time of harvest according to multiple contrast test (P ≤ 0.05). Data are means ± SEM (n = 4).

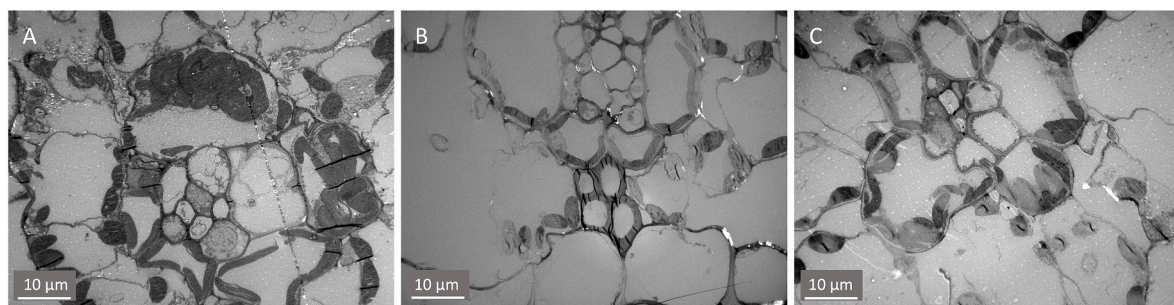


Fig. 6. Transmission electron micrographs of the leaf mesophyll and bundle sheath cells of variety P7948 for P-sufficient 4th leaf (A), P-deficient 4th leaf (B), and P-deficient 4th leaf with foliar P application of KH₂PO₄ + surfactant (C) 14 days after foliar application.

drying, it can only be absorbed if plant leaves are exposed to a high RH, i.e. only in a narrow RH range. Foliar sprays from compounds with a low DRH, on the other hand, may potentially penetrate leaves at a wider RH range. Efflorescence RH, which is an important indicator of the dehydration properties of solutions, has mostly been neglected in studies dealing with salt hygroscopicity with few exceptions (Fernández et al., 2020; Barlas et al., 2023; Bahamonde et al., 2023b). The P salts used as foliar fertilisers in this study have a DRH of 47% (K₂HPO₄) and > 95% (KH₂PO₄) and an ERH of < 15% (K₂HPO₄) and 72% (KH₂PO₄; Fig. 1).

The varying hydration and dehydration properties of the P salts analysed, as indicated by their respective DRH and ERH values, are helpful for characterising their different response to humidity (i.e., their hydrating and drying cycle) as a result of the environmental RH changes measured in the greenhouse during the experiment. In the study by Burkhardt et al. (2012) on the properties of NaCl and NaClO₃, it was shown that these cycles led to a change in the distribution of the applied compound. This was particularly noticeable after several wetting and drying cycles, when the dendritic growth of crystals occurred

(Burkhardt et al., 2012). This could also be observed with K_2HPO_4 in variety P7948 (Fig. 3J, L) and could indicate that K_2HPO_4 has undergone several cycles of crystallisation-dissolution which may lead to structural changes in crystals also associated with different salt hydration states. However, RH mostly fluctuated in the range from 65 to 100%. Thus, K_2HPO_4 was mostly dissolved, which may favour foliar uptake (Eichert and Fernández, 2023). On the other hand, KH_2PO_4 was always close to its DRH/ERH values. Just prior to reaching the DRH/ERH threshold, the salt solution is at its highest concentration, which may enhance the foliar penetration process that is driven by concentration gradients (Eichert and Fernández, 2023). Relative humidities above the DRH, on the other hand, will dilute the solution, potentially influencing the process of foliar absorption. However, it seems that not only the distribution and P concentration of deposits, but also the composition of the initially applied compounds was subject to changes following fertiliser drop drying and rehydration. Leaf SEM-EDX analyses indicate from the atomic percentage that some deposits experienced changes in their K and P ratios and thus, in their chemical structure. For example, the atomic composition of the crystal shown in Supplementary Fig. S2D is more likely to be KH_2PO_4 , even though K_2HPO_4 was initially supplied to the foliage (Supplementary Fig. S3, Table S2). Furthermore, Burkhardt et al. (2012) reported that in the course of hydrating and drying cycles, the initial DRH of compounds changed, which may additionally support the observed change in chemical structure. Alike the water sorption and desorption performance of K_2HPO_4 as observed in the DVS diagram (Fig. 1B), Fernández et al. (2020) reported molecular hydration state changes for $CaCl_2$ after subjecting it to a hydration-drying cycle. The resulting potential modifications in structural water molecules that occur during the days after foliar fertiliser spraying may lead to physico-chemical changes in parameters such as solubility or DRH, also including the patterns of salt re-crystallisation onto the foliage. The increasingly broad distribution due to dendritic growth of crystals as reported by Burkhardt et al. (2012) may facilitate the process of hydration and dissolution of salt particles, but the chemical nature of the resulting compounds (i.e., potential changes in structural water molecules) will determine the dynamics of concentrated salt solutions and crystallised drop deposits onto leaves. No such change was determined for KH_2PO_4 (Fig. 1A).

4.2. Variety, leaf part and P salt influence the contact angles of foliar spray solutions

The occurrence of epicuticular wax crystals in the form of membranous platelets (Barthlott et al., 1998) on the 4th leaf of cultivar DKC 3096 resulted in significantly higher contact angles for pure water foliar fertiliser solutions (i.e., without surfactant) compared to cultivar P7948, where not such nano-structured wax crystals were formed (Fig. 2C, E, 4) (Henningsen et al., 2023b). This relationship between the occurrence of wax crystals and wettability has been shown in several studies (e.g., Koch and Barthlott, 2009; Barthlott et al., 2016, 2017; Henningsen et al., 2023b). Differences in wettability resulted in the 4th leaf of P7948 adsorbing foliar sprays to a large extent even without the addition of a surfactant, whereas the 4th leaf of DKC 3096 was mostly repellent and was only fully wetted by fertiliser solutions containing a surfactant (Fig. 2A and B). However, in both varieties, the midrib had a lower contact angle than the leaf laminae, so even in the actually unwettable 4th leaf of variety DKC 3096 the midrib was wettable, as confirmed by depositions (Supplementary Figs. S1C and S2A). Thereby, the absence of crystallised epicuticular waxes (as seen in Supplementary Fig. S2A) cannot be the only reason for lower contact angles, as even in variety P7948, where there were no wax crystals on the surface anyway, the contact angle of the midrib was smaller compared to the leaf lamina (Fig. 2C, E, Supplementary Table S1). Another reason may be a chemically and structurally different surface of veins and bundle sheath extensions (Fernández et al., 2021). Bahamonde et al. (2018) also observed lower contact angles on veins of beech, thus increasing the

uptake of foliar-applied nutrients, which can also be confirmed by recent studies on barley and tomato (Arsic et al., 2020, 2022; Henningsen et al., 2023a).

Foliar spray residues were not only influenced by their chemical properties but also by different leaf wettability. While a broad distribution was achieved on the wettable leaf surface, especially with pure K_2HPO_4 and both P-salt-solutions with surfactant (Fig. 3H, J, L), a pronounced, spatially delimited crystal formation was more evident on the unwettable surface and with K_2HPO_4 on the wettable one (Fig. 3F, G, K, Supplementary Fig. S2B). An exception for unwettable surfaces regarding deposit distribution was K_2HPO_4 + surfactant, where also broadly distributed deposits were present (Fig. 3K). The distribution of the deposited solutes, which will also be influenced by hydration and dehydration cycles (Burkhardt et al., 2012), thus seems to be enabled only by certain foliar solute characteristics and leaf surface properties, in this case, the lack of epicuticular wax crystals and thus wettability, whereby surfactants only seem to have a major influence on this process in combination with specific hygroscopic salts, in our case K_2HPO_4 (Fig. 3G, K).

4.3. Foliar P absorption is largely influenced by wettability rather than P salt hygroscopicity

Leaf wettability, chemical properties of foliar fertilisers and the use of surfactants are factors that have been identified as important parameters for the uptake of foliar-applied nutrients (Schönherr, 2001, 2002; Fernández and Eichert, 2009; Peirce et al., 2019; Fernández et al., 2021). However, the individual significance in the combination of these factors remained unclear to date. Factors influencing foliar absorption have often been investigated in isolation and under artificial conditions (e.g. isolated cuticles; Fernández et al., 2021). In this study, the comparison of two maize cultivars under greenhouse conditions showed that wettability is the major factor influencing the uptake of foliar applied P (Fig. 4). Irrespective of the foliar P treatment ($KH_2PO_4 \pm$ surfactant, $K_2HPO_4 \pm$ surfactant), P concentrations of adequately nourished plants ($3.5\text{--}6\text{ g P kg}^{-1}$ dry matter according to Bergmann (1983)) were obtained in wettable leaves after foliar spraying of P-deficient plants (Fig. 4D and E). This finding contradicts the statement by Schönherr (2002), that salts with high DRH (e.g. KH_2PO_4) are generally not suitable as foliar fertilisers. Bearing in mind that hydration and dehydration cycles can even alter DRH (Burkhardt et al., 2012; Fernández et al., 2020), this statement is weakened even more.

However, if the leaf surface was unwettable, the chemical properties of the hygroscopic/non-hygroscopic P salt in combination with the use of a surfactant became a major factor (Fig. 4A–C). Even though both P salts were absorbed significantly more by the addition of a surfactant, the absorption of K_2HPO_4 + surfactant was again significantly higher and even reached the level of P-sufficient control plants, seven days after foliar application (Fig. 4B). The significant increase in P concentration one day after foliar application showed that the duration of the liquid phase of the sprayed P solution, due to a low ERH, in combination with a broad distribution by surfactant, were key drivers for foliar P uptake (Fig. 4A). After a few days, the importance of hydrating-drying cycles also became apparent, with treatments containing a surfactant having significantly higher uptake (Fig. 4B). However, it should be noted that low DRH/ERH alone had little effect on P uptake in the unwettable 4th leaf of DKC 3096, because only in combination with a surfactant a high P uptake rate may be achieved (Fig. 4A–C). Higher P absorption rates were further confirmed by the occurrence of light leaf burn, which is a consequence of, for example, excessive nutrient concentrations or the addition of surfactants that may alter the leaf surface (Fernández and Eichert, 2009; Burkhardt, 2010).

Previously it was revealed, that only the 4th and 5th leaves of P7948 were more wettable than those of DKC 3096 (Henningsen et al., 2023b). Therefore, when comparing both varieties, the uptake rates of the remaining shoot without 4th leaf were more similar than those of the 4th

leaf only (Figs. 4 and 5). The most distinct differences between the varieties were that significantly higher P concentrations were also measured in the remaining shoot of wettable P7948 as a result of the application of foliar fertilisers without surfactant, showing again the significance of leaf wettability for foliar P uptake (Fig. 5).

As a result of high foliar P uptake rates, chloroplasts were more abundant and had a better appearance in mesophyll and bundle sheath cells compared to P-deficient plants (Fig. 6). Henningsen et al. (2022) were able to demonstrate that foliar P application temporarily increased the photosynthetic rate compared to maize control plants. Due to the significantly higher P uptake as a result of good wettability and the apparent positive influence on cell ultrastructure, it is to be expected that physiological parameters in this study are thus improved even more. These relationships should be investigated in further studies.

5. Conclusions

Focussing on (i) the influence of leaf surface wettability, (ii) the hydration-dehydration responses of P salts with different hygroscopicity supplied as foliar sprays, and (iii) the effect of adding or not a surfactant to the foliar P treatments, the relative importance of such factors for the uptake of foliar-applied P in P-deficient maize was assessed. Wettability proved to be the predominant factor for the uptake of foliar-applied P, so if the plant surface was wettable, other influencing factors were of minor importance. However, if the surface was unwettable, the highest foliar P uptake was achieved by a hygroscopic P-salt with a low DRH/ERH and the addition of a surfactant. Phosphorus foliar uptake triggered the development of chloroplasts in leaf mesophyll and bundle sheath cells. A better understanding of the interaction between leaf surfaces and the foliar nutrient sprays applied to them is essential to develop efficient foliar fertilisation strategies and to integrate them into fertilisation planning. Furthermore, the gain of further knowledge on the uptake of foliar-applied nutrients will be important for eventually being able to develop models including also machine learning, that may help us predict the effectiveness of foliar treatments and increase their efficacy when supplied to different crop plants under variable conditions. This study provides insights into the importance of wettability, nutrient fertiliser properties and the influence of adding a surfactant. It can be assumed that the obtained results will also apply to plants grown under field conditions, but field trials will also be necessary for improving plant responses to foliar treatments in the future.

Author contributions

Conceptualisation, B.M.G., V.F., J.N.H.; methodology, V.F., J.N.H.; formal analysis, J.N.H.; investigation, V.F., J.N.H.; resources, R.R.G., J.M.Q., V.F.; writing—original draft preparation, V.F., J.N.H.; writing—review and editing, B.M.G., R.R.G., J.M.Q., K.H.M., V.F., J.N.H.; visualisation, V.F., J.N.H.; supervision, V.F.; project administration, K.H.M., V.F., J.N.H. All authors have read and agreed to the published version of the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Jon Niklas Henningsen receives a grant from the Konrad-Adenauer-Foundation e.V., which is gratefully acknowledged. We would also like to gratefully acknowledge the help of Prof. Mario Hasler in carrying out the statistical analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2023.108170>.

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