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## Association of carbon isotope discrimination with leaf gas exchange and water use efficiency in maize following soil amendment with superabsorbent hydrogel

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### ABSTRACT

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The correlation of carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) with photosynthetic gas exchange and water use efficiency (WUE) in maize was investigated under low rainfall conditions with or without superabsorbent polymer (SAP). SAP (45 kg/ha) was mixed into the top 10 cm soil layer at sowing in lysimeters. Compared with the control plants not treated with SAP, the application of SAP increased net photosynthesis rate; stomatal conductance ( $g_s$ ); transpiration rate; chlorophyll content (*Chl*) and intrinsic water use efficiency at leaf level ( $\text{WUE}_l$ ), but decreased intercellular  $\text{CO}_2$  concentration ( $C_i$ ) and leaf  $\Delta^{13}\text{C}$ . In plants supplied with SAP, leaf  $\Delta^{13}\text{C}$  was positively correlated with  $C_i$  ( $r = 0.864$ ,  $P < 0.01$ ) and negatively correlated with  $g_s$  and  $\text{WUE}_l$  ( $r = -0.860$  and  $-0.626$ ,  $P < 0.01$ , respectively). Leaf  $\Delta^{13}\text{C}$  was not correlated with *Chl* with or without SAP. Grain  $\Delta^{13}\text{C}$  significantly decreased by 12.4% and showed a significant negative correlation with grain WUE under SAP treatments ( $r = -0.670$ ,  $P < 0.05$ ). These results suggest that in the presence of SAP, maize leaf and grain  $\Delta^{13}\text{C}$  could be good indicators for evaluating maize WUE during periods of low rainfall.

**Keywords:** water-saving polymer; *Zea mays* L.; physiological traits; soil moisture; carbon isotope composition

Multi-functional chemicals, superabsorbent polymers (SAPs), as good water-saving additives and soil amendments, have been widely used in cropping production systems in semiarid and arid regions (Guilherme et al. 2015, Satriani et al. 2018). The materials can imbibe water hundred times of their mass. The water absorbed by SAP can release slowly to soil through osmotic potential gradient (Cao et al. 2017), reducing rainwater and irrigation water loss below the root-zone (Liao et al. 2017), thus promoting an efficient use of soil water under water-limited conditions (Suresh et al. 2018). The use of soil SAP improves plant growth performance (El-Rehim et al. 2004, Islam et al. 2011a) and enhances water use efficiency (WUE)

(Suresh et al. 2018). Few studies have reported that mixing soil with SAP promotes photosynthetic gas exchange and yield under moderate water deficit conditions (Islam et al. 2011b, Eneji et al. 2013, Yang et al. 2018).

In general, WUE can be defined in the following two ways: WUE at the leaf level and WUE at the crop yield level (Bacon 2004). The first expression is the ratio of net photosynthesis rate to transpiration rate (or stomatal conductance) within a short-time gas exchange period; it is mainly used to describe the behavior of water loss and instantaneous photosynthesis of leaf, plant or canopy. The second is the ratio of crop yield (or shoot biomass) to the crop total water consumption during the entire

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crop growth period. Gas exchange parameters and instantaneous WUE at leaf level are readily determined with photosynthesis analyser systems, but these variables are vulnerably affected by surrounding environmental factors (Bacon 2004). WUE at the yield level requires data on the actual crop water consumed during the season, which is difficult to measure. The weighing lysimeter and soil water balance equation are often used to estimate crop water consumption. However, such efforts are time-consuming, laborious and costly.

Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) is increasingly being used in agriculture to indirectly assess crop yield and WUE of plants (Ellsworth and Cousins 2016). For  $\text{C}_4$  plants, the  $\Delta^{13}\text{C}$  is calculated by the following expression (Henderson et al. 1992):

$$\Delta^{13}\text{C} = a + [b_1 + \phi (b_2 - s) - a]C_i/C_a$$

Where:  $a$  – fractionation under diffusion of  $\text{CO}_2$  in air;  $b_1$  – fractionation of the preceding isotopic equilibrium and phosphoenolpyruvate carboxylase under dissolution and hydration of  $\text{CO}_2$ ;  $b_2$  – fractionation regulated by critical carboxylating enzymes;  $\phi$  – approximately 0.34;  $C_i/C_a$  – ratio of intercellular  $\text{CO}_2$  concentration ( $C_i$ ) to ambient  $\text{CO}_2$  concentration ( $C_a$ );  $s$  – fractionation during leakage.

Obviously, variations in gas exchange may lead to the changes in plant  $\Delta^{13}\text{C}$ , yield and WUE. Many studies reported the positive or negative correlations between  $\Delta^{13}\text{C}$  and gas exchange parameters with WUE in plants under water deficit conditions (Najafinezhad et al. 2015, Raimanová et al. 2016).

Recently, few studies have tested the change in plant  $\Delta^{13}\text{C}$  and its correlation with plant physiological parameters under drought conditions for SAPs. Chirino et al. (2011) suggested that leaf  $\Delta^{13}\text{C}$  was used to assess transpiration and water potential of plant seedlings in the SAP-applied dryland ecosystems. Liao et al. (2017) suggested that the stable isotope discrimination was considered as a practical indicator to quantify root water uptake by maize at dry land amended with soil-saving polymers. However, the associations between plant  $\Delta^{13}\text{C}$  and photosynthetic physiological parameters with WUE are less clear under SAP-based maize production in the semiarid districts. The objective of this study was to determine the relationship of leaf  $\Delta^{13}\text{C}$  with gas exchange and the changes in grain WUE and its association with grain  $\Delta^{13}\text{C}$  after applying SAP to the soil.

## MATERIAL AND METHODS

**Lysimeter experiment.** A lysimeter study with maize plants was conducted in 2016 at the Central Station for Irrigation Experiment Research, Inner Mongolia, China (40°16'N, 111°46'E, and 1130 m a.s.l.). Annual precipitation, annual pan evaporation and annual average temperature at the location are 395 mm, 1850 mm, and 19.5°C, respectively (Yang et al. 2018). During the whole crop growth period, total precipitation was 389 mm and the mean air temperature was 19.2°C.

The cylindrical weighing lysimeter made of iron with 1.15 m inner diameter and 2.0 m depth was used. The lysimeter was fitted with an automatic weighing system with an electrical balance to record the weight on a continuous basis. Soil moisture sensors were installed and used to measure daily volumetric water content at depths of 0–20 cm and 20–40 cm from planting to harvest. The real-time records of lysimeter weighing and soil water content were transferred to computer through wireless internet. A drainage port was placed at the bottom of lysimeter and controlled by tap which was closed during the experiment period. The lysimeter was placed in the crop canopy with a spacing between two lysimeters of approximately 1.35 m.

The lysimeter was filled with soil collected from the experimental field. The soils in upper 0–20 cm and 20–40 cm soil layers were classified as sandy loam. The soil pH in the top 20 cm layer was 8.21 and the soil organic matter content was 2.18 g/kg. The available N, available P, and available K contents were 73.6, 42.2, and 20.3 mg/kg, respectively. Maize (cv. Qiangsheng No. 31) used in this study was provided by Shan'Xi Qiang-Sheng Seed Industry Co. LTD, China. SAP used in the study is an organic and cross-linked polymer with 0.4–1.5 mm of particle size and 200 g/g of deionized water absorption ratio. It consists of 30% attapulgite and 70% negatively charged acrylic-acrylamide polymers.

Two SAP treatments (a no SAP (CK) and 45 kg/ha SAP) were tested as a randomized completely block design with three replicates per treatment. In total, 14 seeds were sowed at a depth of 3–4 cm in the lysimeters on 22 May. When plants had developed three leaves, 7 seedlings per experimental lysimeter were retained and grown until maturity. For the SAP-applied lysimeters, the SAP (45 kg/ha corresponding to 4.5 g/lysimeter) was mixed completely into the top 10 cm depth at sowing. During

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the growing season, all experimental lysimeters were fertilized with N, P, and K of 150, 59.4, and 74.7 kg/ha, respectively, corresponding to 15, 13.5, and 9 g/lysimeter. All the P and K fertilizers and 60% of the N fertilizer were applied prior to sowing; the remaining 40% of N was applied with watering at the jointing stage. At the jointing and grain filling stages, the lysimeters were watered with underground water at a rate of 30 mm per watering (Figure 1).

**Gas exchange and photosynthetic pigments.** Leaf gas exchange was measured on three plants from each lysimeter on 98, 102, 107, 110 and 114 days after sowing (DAS), corresponding to 8/27 (month/day), 8/31, 9/5, 9/8 and 9/12, respectively (Figure 1). The measurements by a portable photosynthesis system (ADC Bioscientific Ltd., Hoddesdon, UK) included net photosynthesis rate ( $P_n$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $T_r$ ), ratio of intercellular  $\text{CO}_2$  concentration to ambient  $\text{CO}_2$  concentration ( $C_i/C_a$ ). Intrinsic WUE at the leaf level ( $\text{WUE}_i$ ) was calculated as a ratio between  $P_n$  and  $g_s$ . The air temperature of the leaf chamber was maintained at 25°C and the photosynthetic photon flux density was set at 1200  $\mu\text{mol}/\text{m}^2/\text{s}$ . Subsequently, chlorophyll content (*Chl*) was measured with a portable chlorophyll system CCM-300 (Opti-Sciences, Inc.

Hudson, USA) on fully expanded leaves (6<sup>th</sup> from top) from three plants in each lysimeter.

**Biomass yield and water use efficiency.** At plant physiological maturity, all plants from each lysimeter were harvested to determine aboveground biomass and yield (Yang et al. 2018). The harvested biomass and grains were dried in an oven at 105°C until constant weight. Grain WUE ( $\text{WUE}_g$ ) was defined as a ratio between grain yield and actual evapotranspiration. The evapotranspiration for each lysimeter was calculated based on the difference of lysimeters weight, as measured every 7 or 10 days.

**Carbon isotope discrimination.** On 98, 102, 107, 110 and 114 DAS, fresh leaves were collected from each treatment and dried in an oven at 105°C for 12 h. The dried plant samples were ground to pass 80-mesh sieve (screen size = 0.18 mm), and then 0.1 g of samples was used to determine the carbon isotope composition using the Isotope Ratio Mass Spectrometer 2000 (Thermo Fisher, Waltham, USA). Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) for grains and leaves was calculated using the equation provided by Farquhar et al. (1989).

**Statistical analysis.** The data were subjected to one-way analysis of variance (ANOVA) using SigmaPlot (version 12.5, Systat Software Inc., San Jose, USA). Pearson's correlation coefficients among  $\Delta^{13}\text{C}$ , photosynthetic gas exchange and WUE were also performed with SigmaPlot.

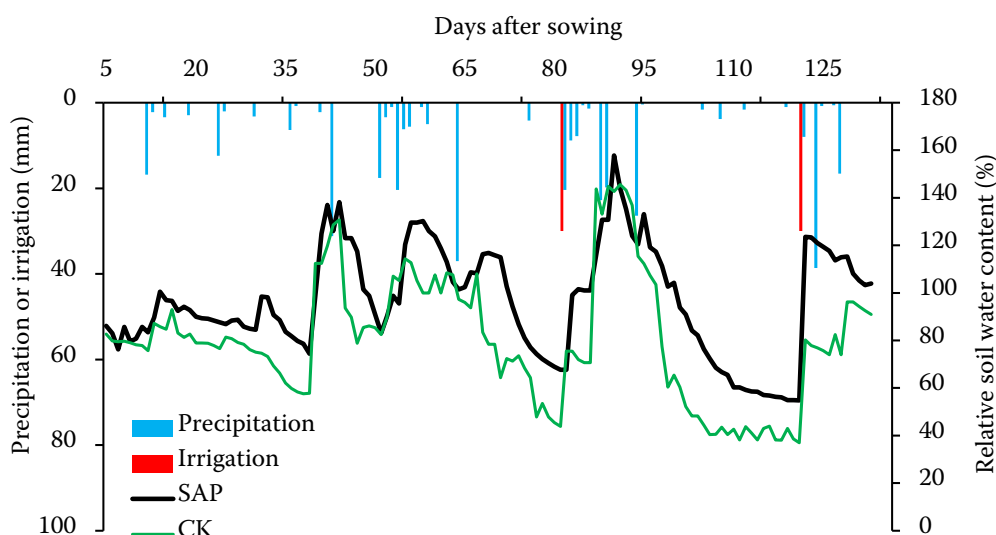


Figure 1. Precipitation, irrigation and relative soil water content at the depth of 40 cm during the whole maize growth period. Sampling period was on August 25 (96 days after sowing) through September 14 (116 days after sowing) with very dry climate (precipitation accumulated was 7.0 mm). Relative soil water content at the depth of 40 cm is defined as a ratio between the averaged soil water content and averaged field capacity at depths of 0–20 cm and 20–40 cm. SAP – superabsorbent polymer; CK – control plants not treated with SAP. Field capacities at depths of 0–20 cm and 20–40 cm are 0.22  $\text{cm}^3/\text{cm}^3$  and 0.21  $\text{cm}^3/\text{cm}^3$

**RESULTS AND DISCUSSIONS**

**Yield and WUE and their association with grain  $\Delta^{13}\text{C}$ .** Under low rainfall conditions, SAP, relative to the control plots not treated with SAP (CK), significantly increased aboveground biomass, yield, and grain WUE ( $\text{WUE}_g$ ) (Figure 2a,b). Some studies have reported such favourable effects in SAP-treated maize production under moderate water-deficit conditions (Islam et al. 2011b, Satriani et al. 2018). Possible reason for this response was that SAP treatment improved soil moisture conditions at the root-zone during the plant growth period compared to CK (Figure 1). Under good watering conditions, N and K contents in plants were significantly enhanced (Islam et al. 2011b, Eneji et al. 2013), which led to improved photosynthetic capacity and *Chl* levels (Figure 3a,b). When SAP was applied to soil under drought conditions, the high  $P_n$  and *Chl* levels for maize plants were important contributors for improved yield and  $\text{WUE}_g$  (Yang et al. 2017). As shown in Figure 2b,

compared with CK, under the SAP-applied plots, grain  $\Delta^{13}\text{C}$  significantly decreased by 12.4%, and it showed a negative correlation with  $\text{WUE}_g$  ( $r = -0.67, P < 0.01$ ; Figure 2d). There was a significant negative correlation between grain  $\Delta^{13}\text{C}$  and  $\text{WUE}_g$  under good water supply conditions as reported by Rytter (2005) and Zhang et al. (2015).

**Photosynthetic gas exchange and leaf  $\Delta^{13}\text{C}$ .** Compared with CK, application of SAP increased  $P_n, g_s, T_r,$  and  $\text{WUE}_i$  and decreased  $C_i$  and  $C_i/C_a$  from 102 DAS through 120 DAS (Figure 3a–g). The maximum percentage of differences in  $P_n, g_s, T_r, \text{WUE}_i, C_i$  and  $C_i/C_a$  between two treatments were 57.0, 41.5, 32.6, 14.2, 23.1 and 51.4%, respectively. Averaged over all samplings ( $n = 5$ ), soil SAP, relative to CK, significantly increased  $P_n, g_s,$  and  $\text{WUE}_i$  by 28.0, 28.5 and 17.2%, respectively, but reduced  $C_i$  by 18.5% and  $C_i/C_a$  by 27.4% (Table 1), indicating that adding SAP to soil promotes plant photosynthetic activity and WUE. The main reason for this response was that the application of SAP directly enhances water-holding capacity and increases soil

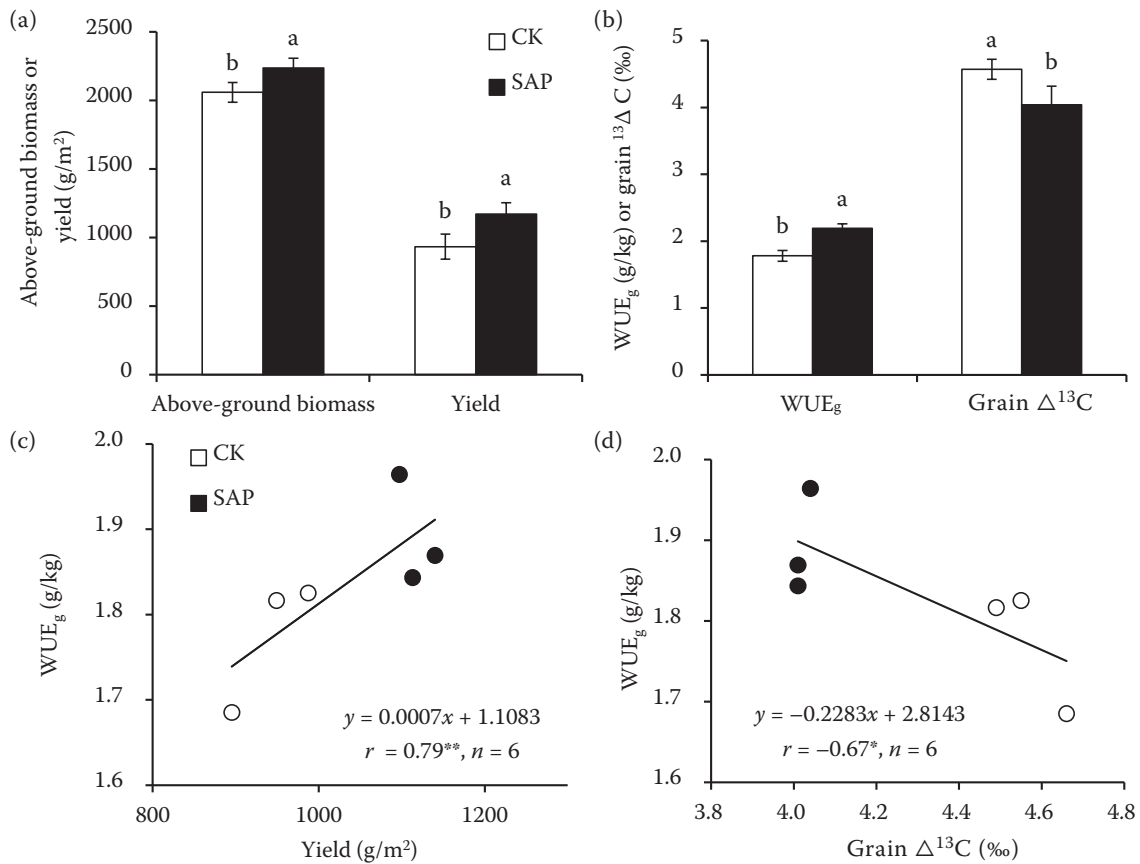


Figure 2. Above-ground biomass, yield, grain water use efficiency ( $\text{WUE}_g$ ), and grain carbon isotope discrimination (grain  $\Delta^{13}\text{C}$ ) and their correlations for maize plants subjected to treatments with superabsorbent polymer (SAP) or without SAP under low rainfall conditions. Different letters above columns indicate significant difference at  $P < 0.05$ .  $*P < 0.05$ ;  $**P < 0.01$ ; CK – control plants not treated with SAP

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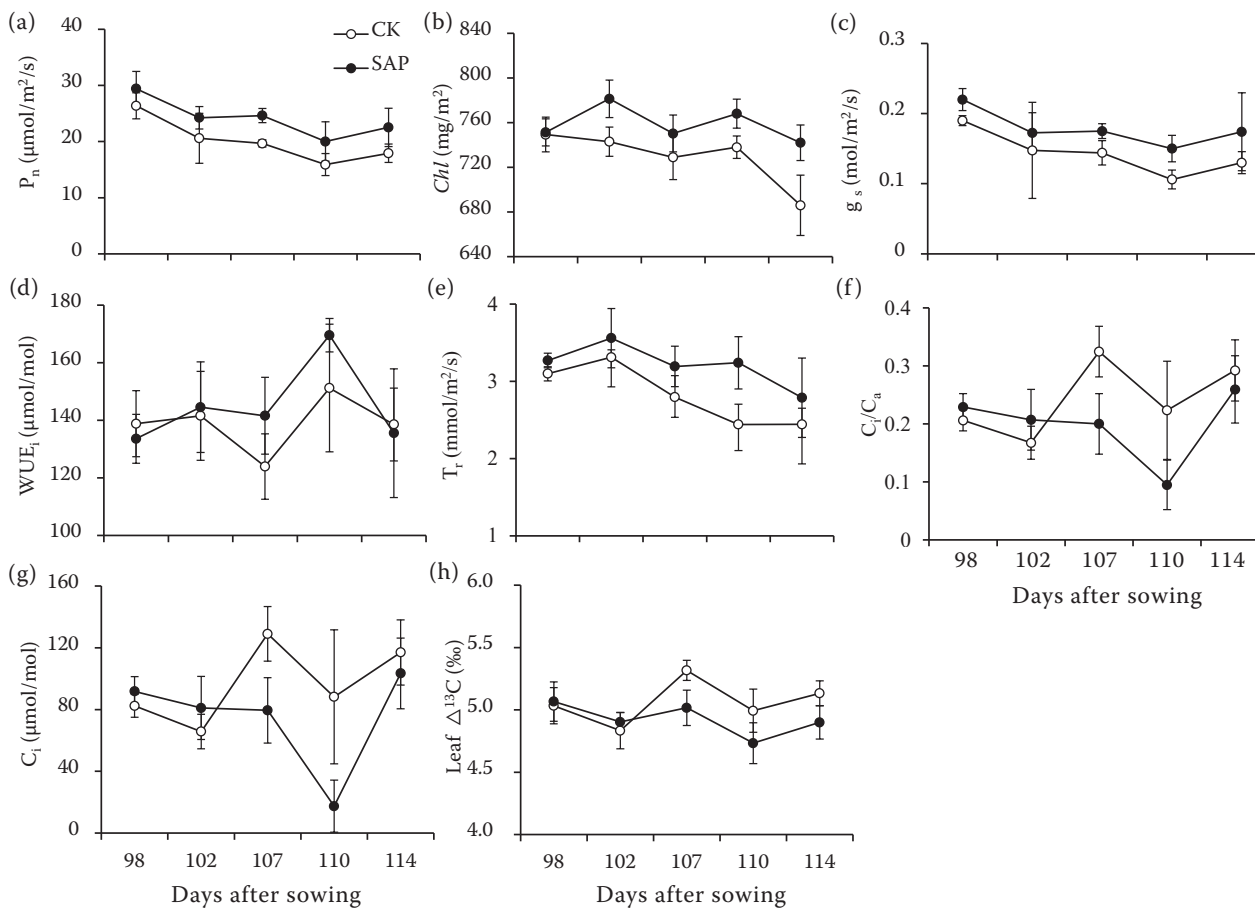


Figure 3. Photosynthetic gas exchange parameters, intrinsic water use efficiency at leaf level ( $WUE_i$ ), and leaf carbon isotope discrimination (leaf  $\Delta^{13}\text{C}$ ) of maize plants subjected to treatments with superabsorbent polymer (SAP) or without SAP under low rainfall conditions.  $P_n$  – net photosynthesis rate;  $Chl$  – chlorophyll content;  $g_s$  – stomatal conductance;  $T_r$  – transpiration rate;  $C_i/C_a$  – ratio of intercellular  $\text{CO}_2$  concentration ( $C_i$ ) to ambient  $\text{CO}_2$  concentration ( $C_a$ ); CK – control plants not treated with SAP

moisture to 40 cm of soil depth (Figure 1). When SAP is applied to sandy loam soil under water deficiency conditions, the greater the  $g_s$ , the more  $\text{CO}_2$  that enters the cell, and the less  $\text{CO}_2$  between cells, the more  $\text{CO}_2$  was converted to sugars through photosynthesis processes (Eneji et al. 2013).

Leaf  $\Delta^{13}\text{C}$  was higher under water-deficit control conditions than under SAP-treated soil which had good moisture conditions (Figure 3h). Water stress induced the increase of leaf  $\Delta^{13}\text{C}$  and largely contributed to an increase of  $C_i/C_a$  under the reduction of photosynthetic capacity (Farquhar et al. 1989). Monneveux et al. (2007) reported that water-deficit stress increases leaf  $\Delta^{13}\text{C}$  under different inbred lines and cultivars of maize. SAP treatment resulted in a slight increase of chlorophyll levels. Najafinezhad et al. (2015) tested the use of soil SAP under low rainfall conditions, which improves total  $Chl$  content and decreases carotenoid contents in  $C_4$  plants.

**Correlation between leaf  $\Delta^{13}\text{C}$  and photosynthetic gas exchange.** Leaf  $\Delta^{13}\text{C}$  showed significant and positive correlations with  $C_i$  and  $C_i/C_a$ , and it showed significant and negative correlations with  $P_n$ ,  $g_s$ , and  $T_r$  under these two treatments (Table 2). The coefficients for these correlations in SAP treatment were generally greater compared with the coefficients in the CK. Thus the changes in gas exchange parameters were more closely related with leaf  $\Delta^{13}\text{C}$  to plots with SAP than plots with non-SAP. Under the absence of SAP applications, the correlation coefficient of leaf  $\Delta^{13}\text{C}$  with  $g_s$  was  $-0.835$  ( $P < 0.01$ ) and that with  $P_n$  and  $C_i$  was  $-0.668$  and  $-0.615$ , respectively. Under water deficit conditions, stomatal closure reduces excessive water loss via transpiration but reduces the diffusion of  $\text{CO}_2$  into leaves, so the ratio of  $P_n$  to  $g_s$  was generally increased (Farquhar et al. 1989). These favourable adaptations to moderate

Table 1. Comparison of averaged leaf  $\Delta^{13}\text{C}$ , photosynthetic gas exchange parameters, and intrinsic water use efficiency at leaf level ( $\text{WUE}_i$ ) in maize plants subjected to treatments with superabsorbent polymer (SAP) or without SAP under low rainfall conditions

Trait	CK ( $n = 5$ )	SAP ( $n = 5$ )	Difference
Leaf $\Delta^{13}\text{C}$ (‰)	5.06 ± 0.13	4.88 ± 0.16	-0.18*
$P_n$ ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	19.7 ± 2.2	25.2 ± 2.7	5.5*
$g_s$ ( $\text{mol}/\text{m}^2/\text{s}$ )	0.13 ± 0.02	0.18 ± 0.03	0.05*
$T_r$ ( $\text{mmol}/\text{m}^2/\text{s}$ )	2.82 ± 0.18	3.01 ± 0.20	0.39
$C_i$ ( $\text{mol}/\text{m}^2/\text{s}$ )	96.5 ± 18.2	78.6 ± 18.2	-17.9**
$\text{WUE}_i$ ( $\mu\text{mol}/\text{mol}$ )	128.8 ± 14.6	150.0 ± 13.1	21.2**
$C_i/C_a$	0.25 ± 0.06	0.18 ± 0.03	-0.07*
$\text{Chl}$ ( $\text{mg}/\text{m}^2$ )	729.1 ± 17.1	768.6 ± 14.9	29.5

Difference = SAP – CK. \* $P < 0.05$ ; \*\* $P < 0.01$ . The value for each trait was shown as mean ± standard deviation. Average value was calculated from five field measurements as shown in Figure 3.  $P_n$  – net photosynthesis rate;  $g_s$  – stomatal conductance;  $T_r$  – transpiration rate;  $C_i/C_a$  – ratio of intercellular  $\text{CO}_2$  concentration ( $C_i$ ) to ambient  $\text{CO}_2$  concentration ( $C_a$ );  $\text{Chl}$  – chlorophyll content

water deficiency are conducive to improve plant survival and promote WUE during late growth stages when evapotranspiration is larger. Under SAP-induced good soil moisture conditions, leaf

$\Delta^{13}\text{C}$  showed significant correlations with  $g_s$  and  $C_i/C_a$ ,  $P_n$  ( $r = -0.860, 0.850, \text{ and } -0.742$ , respectively). Monneveux et al. (2007) reported similar correlation under good water supply regimes. For  $C_4$  plants, differential response in leaf  $\Delta^{13}\text{C}$  and  $C_i/C_a$  and their association largely depends on soil moisture and plant species (Chirino et al. 2011). Leaf  $\Delta^{13}\text{C}$  was not significantly correlated with  $\text{Chl}$  with or without SAP application. This may be caused by the lack of relationship between  $\text{Chl}$  with gas exchange parameters (Table 2).

There were significant positive relationships of  $g_s$  and  $P_n$  with  $\text{WUE}_i$  for both treatments. Under moderate water deficit conditions, Yang et al. (2017) reported strong positive correlations between  $g_s$  and  $P_n$  for maize plants without SAP ( $r = 0.707, P < 0.01$ ) or with SAP ( $r = 0.721, P < 0.01$ ). In plots that received SAP, the correlation between  $g_s$  and  $\text{WUE}_i$  was greater compared with correlation between  $P_n$  and  $\text{WUE}_i$ . This helps to improve plant WUE while maintaining high photosynthesis under the limitation of leaf stomatal transpiration under water-deficit conditions. Leaf  $\Delta^{13}\text{C}$  showed significant negative correlations with  $\text{WUE}_i$  with SAP ( $r = -0.728$ ) or without SAP ( $r = -0.626$ ) application. Yasir et al. (2013) suggested negative correlations between  $\Delta^{13}\text{C}$  and  $\text{WUE}_i$  under well-watered and water-deficit conditions. Therefore, under SAP-based

Table 2. Pearson’s correlation coefficient ( $r$ ) among leaf  $\Delta^{13}\text{C}$ , photosynthetic gas exchange, and intrinsic water use efficiency at leaf level ( $\text{WUE}_i$ ) in maize plants subjected to treatments with superabsorbent polymer (SAP) or without SAP under low rainfall conditions

	$\text{Chl}$	$P_n$	$g_s$	$T_r$	$\text{WUE}_i$	$C_i$	$C_i/C_a$	
CK ( $n = 15$ )	leaf $\Delta^{13}\text{C}$	0.172	-0.668**	-0.835**	0.413	-0.626*	0.615*	0.613*
	chlorophyll ( $\text{Chl}$ )		0.495	0.372	0.411	0.141	0.023	0.026
	net photosynthesis rate ( $P_n$ )			0.893**	0.605*	0.643**	-0.343	0.490*
	stomatal conductance ( $g_s$ )				0.620*	0.577*	0.681**	0.381
	transpiration rate ( $T_r$ )					-0.218	-0.026	-0.019
	$\text{WUE}_i$						-0.813**	-0.822**
SAP ( $n = 15$ )	intercellular $\text{CO}_2$ concentration ( $C_i$ )							0.981**
	leaf $\Delta^{13}\text{C}$	0.222	-0.742**	-0.864**	0.222	-0.728**	0.860**	0.850**
	$\text{Chl}$		0.671**	0.496	0.736**	0.206	0.171	0.136
	$P_n$			0.901**	0.524*	0.662*	-0.476	0.553*
	$g_s$				0.514*	0.619*	0.735**	0.752**
	$T_r$					-0.229	0.136	0.156
	$\text{WUE}_i$						-0.700**	-0.752**
$C_i$							0.992**	

\* $P < 0.05$ ; \*\* $P < 0.01$ ; CK – control plants not treated with SAP

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maize production,  $\Delta^{13}\text{C}$  can be a promising indirect indicator for assessing plant WUE at later growth stages under low rainfall conditions.

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