

RESEARCH ARTICLE

# Response of root growth and distribution of maize plants to foliar-sprayed antitranspirant and soil-amended hydrogel polymer

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## ABSTRACT

Root growth and development of maize (*Zea mays* L.) is an important process in determining grain yield. The effects of combined fulvic acid (FA, potassium humate as FA source) and super absorbent polymer (SAP) uses on maize root growth and yield were studied. A 2×3 factorial field experiment was applied by combining two SAP doses (0, 45 kg ha<sup>-1</sup>) and three FA rates (0, 1, and 2 g L<sup>-1</sup>) over 2 yr. Super absorbent polymer was applied to upper 20 cm soil layer at sowing, and FA solutions (1 or 2 g L<sup>-1</sup>, corresponding to FA<sub>1</sub> and FA<sub>2</sub>) were sprayed onto the canopy three times during growing season. Root parameters, including root biomass, root length density (RLD), root surface-area density (RSD), and root diameter, were determined in the top 40 cm soil layer. Relative to control plants, both chemicals significantly increased root biomass, but these effects were not significantly different from their combined use. Compared to individual FA or SAP treatment, combined applications increased RLD and RSD in 0-20 cm depth. The combined SAP and FA<sub>2</sub> improved RLD at 0-10 cm by 32% compared to FA<sub>2</sub> treatment alone and by 82% compared to non-chemicals control plots over 2 yr. Averaged root diameters also significantly increased in the 40 cm soil layer in FA and SAP treatments alone compared to control, but they were not significantly different with the combined treatment. Similar patterns were found for maize yield each year. The combined application of SAP (45 kg ha<sup>-1</sup>) and FA<sub>2</sub> (2 g L<sup>-1</sup>) greatly increased root length density and biomass than their individual application, and it could be suitable for high production of maize grown under low rainfall conditions.

**Key words:** Humic substances, logistic model, maize, root biomass, root length density, soil water deficit, super absorbent polymer, *Zea mays*.

## INTRODUCTION

Root system is closely linked with growth and development of the crop's above ground organs, as coarse roots provide abundant moisture and nutrients and maintain a stable posture for the crop (Li et al., 2017). Larger and more roots in the soil profile are conducive to high harvest yields and promote the utilization efficiency of water and nutrients (Zhou et al., 2012). Root has considerable adaptive plasticity in the soil under various environmental factors (Bian et al., 2016). Root length density (RLD), as a sensitive indicator, can be broadly used to assess the water uptake capacity of roots in soils. It is closely correlated with root morphological, physiological adaptiveness, and crop productivity (Guan et al., 2014). For field crops, fine roots with root diameter < 0.5 mm have higher root length density and surface area, but the weight of root biomass is relatively slight; coarse roots have larger biomass, but the RLD is relatively short (Domingos et al., 2009). These differences in RLD and root biomass between fine and coarse roots reflect the potential of water uptake and transport by roots (Zhou et al., 2012). Therefore, the formation of a better root system and greater root growth is favorable to obtain efficient utilization of water and nutrients resources and agricultural crop production.

Most districts in Inner Mongolia of China are in semiarid areas with low annual rainfall with an average of 250 ~ 400 mm and high annual soil surface evaporation of more than 1800 mm. Normal maize (*Zea mays* L.) growth is often limited by the rainfall, although around ~ 65% of annual total rainfall was in the crop growing season. In addition, most soils in the district are characterized by low water-holding capacity due to few organic matter and a high sandy fraction (Fan et al., 2005), so irrigation water and rainstorms occurred in summer growing season are easily lost as deep percolation below the root-zone, resulting in greater limitations of crop root growth, yield and water use efficiency (Yu et al., 2011; Yang et al., 2017). It is crucial to take agronomic practice to improve root growth and grain of crops in the cropping system under conditions with low rainfall and high soil surface evaporation.

Water-saving super absorbent polymers (SAPs) have been widely accepted as playing a favorable role in the cropping system in water deficient districts (Zhang et al., 2016; Satriani et al., 2018). When SAPs are applied to soil, they can absorb a larger amount of soil water as much as hundreds of times relative to its weights (Akhter et al., 2004). This positive role directly reduces the degree of water deficiency, promoting crop growth, and improving the effective utilization of water and nutrient resources (Yang et al., 2019a). Addition of hydrogel into the soil also changes soil physics properties and affects root growth and distribution (Bai et al., 2010; Liao et al., 2018). A soil-culture pot test study demonstrated that mixing soil with SAP reduces total root length, root surface area, and root volume of maize, while promoting absorbing and transmitting ability by larger root diameter (Zhou et al., 2012). Few studies found that soil SAP not only increases root biomass and adjusts root distribution, but improves the amount of water absorbed through increasing RLD at the upper soil layer under a dryland cropping system (Liao et al., 2014). Foliar antitranspirant, fulvic acid (FA), can be widely extracted from various sources, such as brown coals, peat, composts, and raw organic wastes (Nardi et al., 2002; Rose et al., 2014; Lotfi et al., 2015), which is the most active ingredient of humic substances because of the large number of total carboxyl groups and higher cation exchange and absorption capacities (Bocanegra et al., 2006; Domingos et al., 2009). It has physiological behaviors similar to auxin and foliar antitranspirant (Yazdani et al., 2014; Zhang et al., 2016). They are broadly applied to various types of species of plants such as vegetable crops, field crops, and tree species, with various methods of application (Calvo et al., 2014; Canellas et al., 2015; Iqbal et al., 2020). Foliar spray of FA onto maize plant canopy improves the tolerance to water deficiency (Anjum et al., 2011; Rose et al., 2014), reducing luxury crop transpiration and increasing photosynthesis (Zhang et al., 2016; Yang et al., 2017), promoting growth of root system (Maibodi et al., 2015), improving biological production and yield (Zhang et al., 2016; Yang et al., 2019a). Fulvic acid is more favorable on crop growth and water use efficiency (WUE) under water deficit than under well-irrigated condition (Xudan, 1986; Anjum et al., 2011). Fulvic acid increased the length and number of lateral roots and root surface area of plant seedlings conducted in greenhouse conditions (Canellas et al., 2009; El-Desuki et al., 2012). Later root emergence increased in *Arabidopsis thaliana* and tomato plants after applying FA (Canellas et al., 2002; Dobbss et al., 2010). However, some publications pointed out that humic substances had no effect on the growth of roots (Aguirre et al., 2009) and even induced the potential negative effects on plant's growth and yield (Santiago et al., 2010; Hartz and Bottoms, 2010).

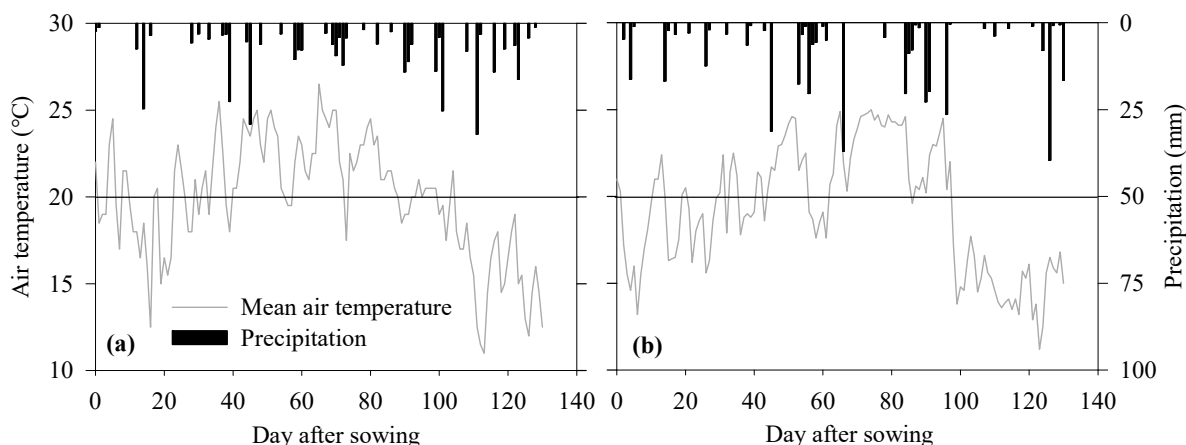
Recently several studies addressed that the combined use of soil SAP and foliar antitranspirant FA have shown the synthetic benefits under water-limited resources conditions, such as to improve physiological performance (Liao et al., 2017; Yang et al., 2017; 2019b), enhance yield (Liao et al., 2017; Zhang et al., 2018; Yang et al., 2019a), and facilitate efficient use of N and water (Eneji et al., 2013; Yang et al., 2017; Satriani et al., 2018) of maize grown under low rainfall conditions. However, little is known about the effects of combined SAP and FA use on root growth and distribution and its correlation with crop grain yield. The objective of this study was to determine the changes of root parameters and their distribution in the soil after applying two chemicals to the maize production system under low rainfall conditions.

## MATERIALS AND METHODS

The study was conducted during the 2015 and 2016 crop growth seasons at Helin Experiment Center for Agricultural Water-Saving and Irrigation, Hohhot, Inner Mongolia (40°16' N, 111°46' E), China. The annual average, highest, and lowest temperatures at the site are 5.6, 37.5 and -34.5 °C. Annual total rainfall is 395 mm and the annual evaporation is 1850 mm. During the 2015 and 2016 maize (*Zea mays* L.) growth period, total rainfalls were 365 and 389 mm, and the mean air temperatures were 19.2 and 19.8 °C, respectively, as recorded by the weather station (T-warner 300, STEP Systems GmbH, Nuremberg, Germany) close to the field (Figure 1). The soil from a depth of 20 cm was classified

as sandy loam. The principal physical and chemical properties of the soil used were measured at the beginning of the experiment in 2015 (Table 1).

Maize ‘Qiangsheng No.31’ was purchased from Shanxi Qiang Sheng Seed, Taiyuan, China. Super absorbent polymer (SAP) used in the study is a crossed-linked and artificial-made material (Chang’an Group Co. Ltd., Dongying, China). The particle size of SAP ranged 0.4-1.5 mm and the deionized water absorption ratio was 200 g g<sup>-1</sup>. It contains 70% negatively charged acrylic-acrylamide polymers and 30% atpulgite. Potassium-humate consists of 50% fulvic acid (FA), 12% potassium oxide, and 3% phosphorus pentoxide (Xinjiang Double Dragons Humic Acid Co. Ltd., Urumchi, China) and it was applied as a source of FA.



**Figure 1.** Daily air temperature and precipitation during the maize growth periods in 2015 (a) and 2016 (b).

**Table 1.** Major physical and chemical properties of soil collected from the top 20 cm depth at the beginning of the experiment. BD: Bulk density; FC: field capacity; OM: organic matter; TN: total nitrogen; AN: alkali-hydrolyzable N; AP: available P; AK: available K.

Texture	Sand	Silt	Clay	BD	pH	FC	OM	TN	AN	AP	AK
	%			g cm <sup>-3</sup>		cm <sup>3</sup> cm <sup>-3</sup>	mg kg <sup>-1</sup>				
Sandy loam	68.6	12.3	19.1	1.45	8.53	0.21	4470	780	80.3	21.7	14.4

### Experimental design

A 2×3 factorial field experiment was applied by combining two doses of SAP (0, 45 kg ha<sup>-1</sup>) and three rates of FA (0, 1, and 2 g L<sup>-1</sup>). Experiment was performed as a randomized complete block design with three replicates for each treatment. The SAP treatments assigned in the main plot and the FA to the subplots. The plants not treated with any chemicals were considered as control. For each plot (10 m by 4 m), SAP was broadcasted onto the soil surface, and then mixed into 20-cm soil depth while sowing. FA solutions was sprayed onto maize plant canopy three times at bell, tassel, and grain fill stages, respectively, using an electric-driven sprayer between 16:00 and 20:00 h. Total amount of spray was 21 L per plot, with 7 L per plot for each growth period. Similarly, an equal amount of groundwater was sprayed to control plant canopy in the corresponding stages.

Maize was sown at 5500 seeds ha<sup>-1</sup> under film mulching on 19 May 2015 and 22 May 2016. Seeds were planted in a wide-narrow row pattern, with a distance of 0.4 and 0.8 m between narrow and wide rows, respectively. There were two rows of maize under each strip of plastic film. The drip-irrigation line was placed at the center of two planting rows for each individual strip. The diameter and head spacing of the drip-irrigation pipe were 16 and 300 mm, respectively, and the head flow was 1.68 L h<sup>-1</sup>. During the jointing and tassel stages, plants were drip-irrigated with groundwater at a rate of 40 mm per irrigation (total amount of 80 mm). All experimental plots received 150 kg N ha<sup>-1</sup>, 135 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>,

and 90 kg K<sub>2</sub>O ha<sup>-1</sup> during the growing season. The P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O fertilizers and 60% N fertilizer were applied while sowing; the remaining N was applied at the jointing stage.

### Root sampling and analysis

Root samples were collected during grain filling stage on 30 August 2015 and 10 September 2016. In the individual plot, three consecutive plants were randomly selected from the middle 3<sup>rd</sup> or 4<sup>th</sup> row to avoid side effects and root sampling. The root samples were taken with the soil-cores method using a hand-held power sampler. The size of the sampler was 8.5 cm in inner diameter and 10 cm in length, equivalent to 567 cm<sup>3</sup> volume. Soil cores were vertically sampled from three directions at a distance of 5 cm to the plant, on the basis of four soil depths: 0-10, 10-20, 20-30, and 30-40 cm (Zhou et al., 2016). A total of 532 root samples were thus collected.

Root samples were put in plastic bags, then rinsed with groundwater which is also used for irrigation. After rinsing, roots were stored in a refrigerator until measurements. Root samples were completely dispersed on a transparent tray with distilled water and were scanned to acquire images using Epson Perfection 4990 Photo Scanner (Seiko Epson Corporation, Nagano, Japan). Root length, root surface area, and root diameter were determined from these images using the software WinRHIZO Professional (Regent Instruments Inc., Sainte-Foy, Canada). Root length density (RLD) was calculated as the ratio between root length and soil-core volume. Root surface-area density (RSD) was calculated as the ratio between root surface area and soil-core volume. After scanning, fresh roots were placed in an oven at 105 °C for 12 h until obtain constant weight. Root biomass density was calculated as the ratio between dry root biomass and soil-core volume.

### Biomass accumulation and crop harvest

Five plants from every plot of each treatment were collected on 20, 40, 60, 80, 100, and 130 d after sowing, calculating eigenvalues of above ground biomass. Above ground organs of the plants were dried in an oven at 80 °C for 72 h to obtain constant dry mass and weighted. To quantify the above ground biomass over time for each treatment, the logistic growth model was applied to evaluate the eigenvalues. Above ground biomass overtime during the whole growth period was estimated by Equation 1:

$$DM = \frac{DM_{max}}{1 + a \times \exp(-b \times T)} \quad (1)$$

where DM is the above ground biomass, T is the days after sowing, DM<sub>max</sub> is the theoretical maximum of above ground biomass, a and b are regression parameters. The starting time (T<sub>1</sub>), terminating time (T<sub>2</sub>), and duration (ΔT = T<sub>2</sub> - T<sub>1</sub>) of the above ground biomass rapid-accumulation period, and the maximum accumulation rate (V<sub>max</sub>) were calculated from the following Equations 2, 3, and 4:

$$T_1 = \frac{1}{b} \times \ln\left(\frac{a}{2 + \sqrt{3}}\right) \quad (2)$$

$$T_2 = \frac{1}{b} \times \ln\left(\frac{a}{2 - \sqrt{3}}\right) \quad (3)$$

$$V_{max} = \frac{b \times DM_{max}}{4} \quad (4)$$

At plant physiological maturity, 10 plants were harvested on 1 October 2016. Stem, leaf, cob, and grain were separated and dried in an oven at 80 °C for 72 h to determine dry weight. Grain yield was measured from the middle two rows of each plot on 27 September 2015 and 3 October 2016. All yield results were adjusted to 15.5% (2015) and 12.0% (2016) moisture content by determining 100 grains moisture using an oven at 105 °C for 12 h.

### Statistical analysis

The mean values for observed parameters were subjected to a two-factor ANOVA using SPSS, version 19.0 (IBM, Armonk, New York, USA). The main and interactive effect of SAP and FA on root parameters, above ground biomass, and yield components in each year were tested. Total root growth parameters in top 40 cm soil were obtained by summarizing the results of the depths of 0-10, 10-20, 20-30, and 30-40 cm, significant difference was tested using Duncan's method at a significance level of p < 0.05.

## RESULTS

### Maize yield and above ground biomass

In both years, FA showed the significant main effects on grain number, grain weight, yield, and above ground biomass, as displayed in the ANOVA test results in Table 2. The SAP showed significant main effects on grain weight, yield, and biomass, but did not show the main effect on grain number. The combined use of the two chemicals did not show the interactive effects on the values of these variables except for grain number. Relative to plants in the control treatment, SAP, FA<sub>1</sub>, and FA<sub>2</sub> alone increased grain number by 22.3%, 11.6%, and 12.8% in 2015, and 8.2%, 6.3%, and 10.4% in 2016. Over 2 yr, FA<sub>1</sub>+SAP and FA<sub>2</sub>+SAP treatments also increased grain number by 12.5% and 11.0%, respectively, compared with control plots. In comparison to the control plants in 2015 and 2016, the FA<sub>1</sub>+SAP treatment improved grain weight by 8.9% and 7.7%, yield by 21.6% and 18.2%, and biomass by 28.3% and 25.3%. The grain weight and yield in the SAP+FA<sub>1</sub> treatment were also greater, respectively, 11.0% and 9.6% than in the SAP treatment alone. However, there were not significantly differences in yield and biomass under SAP-based conditions with FA<sub>1</sub> or FA<sub>2</sub> spraying. The increases in plant biomass and yield were thus greater in the combined FA and SAP treatment than in their individual applications.

**Table 2.** Maize yield and yield components subjected to treatments with foliar fulvic acid (FA) and soil superabsorbent polymer (SAP). FA<sub>x</sub>: 1 or 2 g L<sup>-1</sup> FA spraying during the crop growth periods. Within each column, means followed by the different letters are significantly different at 5% each year. \*, \*\* p < 0.05 and p < 0.01, respectively; ns: nonsignificant.

Treatment		Grain number	100-grain weight	Shoot biomass	Grain yield	Harvest index
		nr cob <sup>-1</sup>	g	t ha <sup>-1</sup>		
2015	Control	608c	27.9b	18.2c	8.3c	0.45a
	FA <sub>1</sub>	679b	27.2b	21.3ab	8.9b	0.44a
	FA <sub>2</sub>	686b	28.9ab	21.9ab	9.7b	0.45a
	SAP	744a	27.4b	20.7b	9.4b	0.45a
	FA <sub>1</sub> +SAP	688b	30.4a	22.8a	10.3a	0.45a
	FA <sub>2</sub> +SAP	693b	30.7a	22.0a	10.1a	0.46a
2016	Control	627c	32.3b	19.4c	9.3c	0.47a
	FA <sub>1</sub>	667ab	31.8b	21.1b	10.2b	0.46a
	FA <sub>2</sub>	692a	34.9a	20.5bc	10.1b	0.47a
	SAP	667b	34.3a	22.5b	10.4b	0.46a
	FA <sub>1</sub> +SAP	702a	34.8a	24.9a	11.4a	0.46a
	FA <sub>2</sub> +SAP	678ab	35.1a	24.2a	11.0a	0.47a
ANOVA analysis						
2015	FA	*	ns	*	*	ns
	SAP	ns	**	**	**	ns
	FA×SAP	**	ns	ns	ns	ns
2016	FA	*	*	**	**	ns
	SAP	ns	**	**	**	ns
	FA×SAP	**	ns	ns	ns	ns

As shown in Table 3, the logistic growth model described the accumulation of above ground biomass well with SAP or FA alone and their combined use within two consecutive experimental periods. There was nonsignificant difference between FA<sub>1</sub> and FA<sub>2</sub> in terms of these eigenvalues from the model in each year. The FA spraying generally extended the period of above ground biomass rapid-accumulation (T<sub>2</sub>), increased the average growth rate during the rapid-accumulation period (V<sub>T</sub>), but it did not significantly change the starting time of above ground biomass rapid-accumulation period (T<sub>1</sub>). Averaged over 2 yr, as compared with control plants, the T<sub>2</sub> value was prolonged by 1.8 d for FA<sub>1</sub> treatment, and 2.4 d for FA<sub>2</sub> treatment. In contrast, compared to control plots, SAP applications reduced T<sub>1</sub> values and increased T<sub>2</sub> values each year. However, the application of combining SAP and FA, relative to control, significantly reduced T<sub>1</sub> values and increased T<sub>2</sub> and V<sub>T</sub> values

each year. The  $T_1$ ,  $T_2$ , and  $V_T$  values were not significantly different among SAP, SAP+FA<sub>1</sub>, and SAP+FA<sub>2</sub> treatments. Generally, co-application of FA<sub>2</sub> and SAP treatment had the greatest average growth durations of fastest growing point ( $T_0$ ) within 2 yr (53.6 d in 2015 and 56.7 d in 2016), and followed by combining FA<sub>1</sub> and SAP use, and the control plants had the lowest  $T_0$  (47.8 d in 2015 and 50.6 d in 2016). The  $T_0$  for the separated use of chemicals ranged 48.2 d (FA<sub>1</sub> treatment in 2015) and 54.4 d (SAP treatment in 2016). The maximum accumulation rates during the fastest growing point ( $V_{max}$ ) in the combined treatment were superior to the separated use of the chemicals. Across 2 yr, the  $V_{max}$  values under the combined use of SAP+FA<sub>2</sub> were higher 8.4%, 10.0%, and 9.0% on average than under the control plots, FA<sub>2</sub>, and SAP treatments.

**Table 3.** Dynamic-accumulation of shoot biomass of maize plants subjected to treatments with foliar fulvic acid (FA) and soil superabsorbent polymer (SAP) under low rainfall conditions. FA<sub>x</sub>: 1 or 2 g L<sup>-1</sup> FA spraying during the crop growth periods;  $T_1$  and  $T_2$ : starting and terminating time of the rapid accumulation period, respectively;  $\Delta T$ : duration of above ground biomass rapid-accumulation period;  $V_T$ : average growth rate during rapid-accumulation period;  $T_0$ : average growth duration of fastest growing point;  $V_{max}$ : maximum accumulation rate during fastest growing point. Values are mean  $\pm$  SD (n = 3). Different letters in columns indicate significant difference at  $p < 0.05$ . \*\*  $p < 0.01$ .

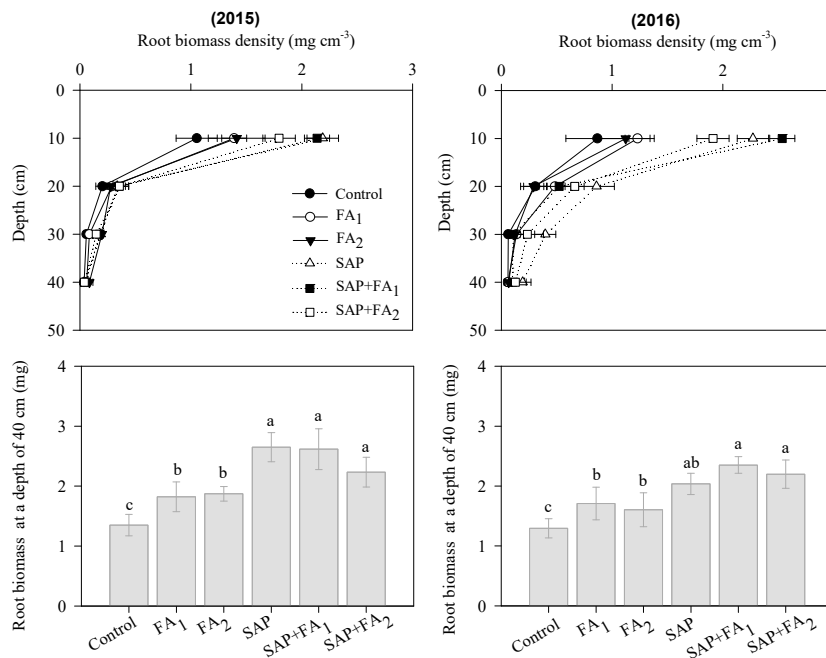
Year	Treatment	$r^2$	Rapid growth period			$V_T$ t ha <sup>-1</sup> d <sup>-1</sup>	The fastest growing point	
			$T_1$	$T_2$	$\Delta T$		$T_0$ d	$V_{max}$ t ha <sup>-1</sup> d <sup>-1</sup>
2015	Control	0.991**	58.1a	89.2b	31.1c	0.30c	47.8b	0.33c
	FA <sub>1</sub>	0.990**	57.5a	90.6b	34.0b	0.33b	48.2b	0.36b
	FA <sub>2</sub>	0.991**	57.3a	91.4b	33.1b	0.32bc	49.1b	0.35b
	SAP	0.989**	55.3b	91.3ab	36.0a	0.34ab	52.3a	0.37b
	FA <sub>1</sub> +SAP	0.995**	55.8b	94.7a	37.9a	0.37a	53.1a	0.40a
	FA <sub>2</sub> +SAP	0.993**	55.9b	93.4a	37.5a	0.36a	53.6a	0.41a
2016	Control	0.995**	59.0a	91.7b	31.7c	0.34b	50.6b	0.38c
	FA <sub>1</sub>	0.989**	60.6a	93.9ab	33.3b	0.37ab	52.6ab	0.42b
	FA <sub>2</sub>	0.994**	59.6a	92.1ab	33.5b	0.34b	51.3b	0.41bc
	SAP	0.993**	56.4b	94.6a	35.2a	0.36ab	54.4ab	0.40bc
	FA <sub>1</sub> +SAP	0.990**	56.7b	94.5a	38.8a	0.39a	56.1a	0.45a
	FA <sub>2</sub> +SAP	0.996**	55.3b	94.1a	37.8a	0.38a	56.7a	0.44a

### Root biomass

Treatments FA and SAP alone and their combined use significantly increased root biomass in the upper 40 cm depth (Figure 2). As averaged across 2 yr, compared with control plants, total root biomass density was increased by 34% (35% in 2015 and 32% in 2016) for FA<sub>1</sub> treatment, 32% for FA<sub>2</sub> treatment (39% in 2015 and 24% in 2016), and 77% (96% in 2015 and 57% in 2016) for SAP treatment. In contrast, relative to control plants, FA<sub>1</sub>+SAP and FA<sub>2</sub>+SAP treatments increased root biomass density by 88% and 68%, respectively. Moreover, these increases using both FA and SAP were higher than these using only either FA<sub>1</sub> or FA<sub>2</sub>. Application of FA and SAP led to the change in root biomass distribution in the 40 cm soil profile (Figure 2a). The obvious change in root biomass density between applied chemicals and a control plant was in the top 20 cm soil, though these changes were not significantly different, especially in 2016. In both years, more than 90% of total root biomass was in the top 20 cm soil for FA and SAP treatments, approximately 10% of which was in 20-40 cm layer. As for the mean values over the 2 yr, the percentage of root biomass ranged 63% ~ 70% in the upper 10 cm soil layer, 20% ~ 24% in the 10-20 cm layer, 6% ~ 10% in 20-30 cm, and 2% ~ 5% in the 30-40 cm layer. The largest percentage of root biomass in the top 10 cm soil layer was 72% in SAP+FA<sub>1</sub> treatment, and the lowest was 63% for control plots. When averaged over 2 yr, relative to control plots, spraying FA<sub>1</sub> and FA<sub>2</sub> reduced the percentage of root biomass in 10-20 cm but increased the percentage of that in 0-10 cm depth, whereas it was not different in 0-20 cm layer. The SAP treatment alone did not change the percentage of root biomass at any soil layers above 40 cm each year. In contrast, the percentage of root biomass in a depth of 10 cm was 14% greater in the SAP+FA<sub>1</sub> treatment than in the control plots, and it was greater than in the in the FA or SAP treatment alone.

### Root morphological parameters and distribution

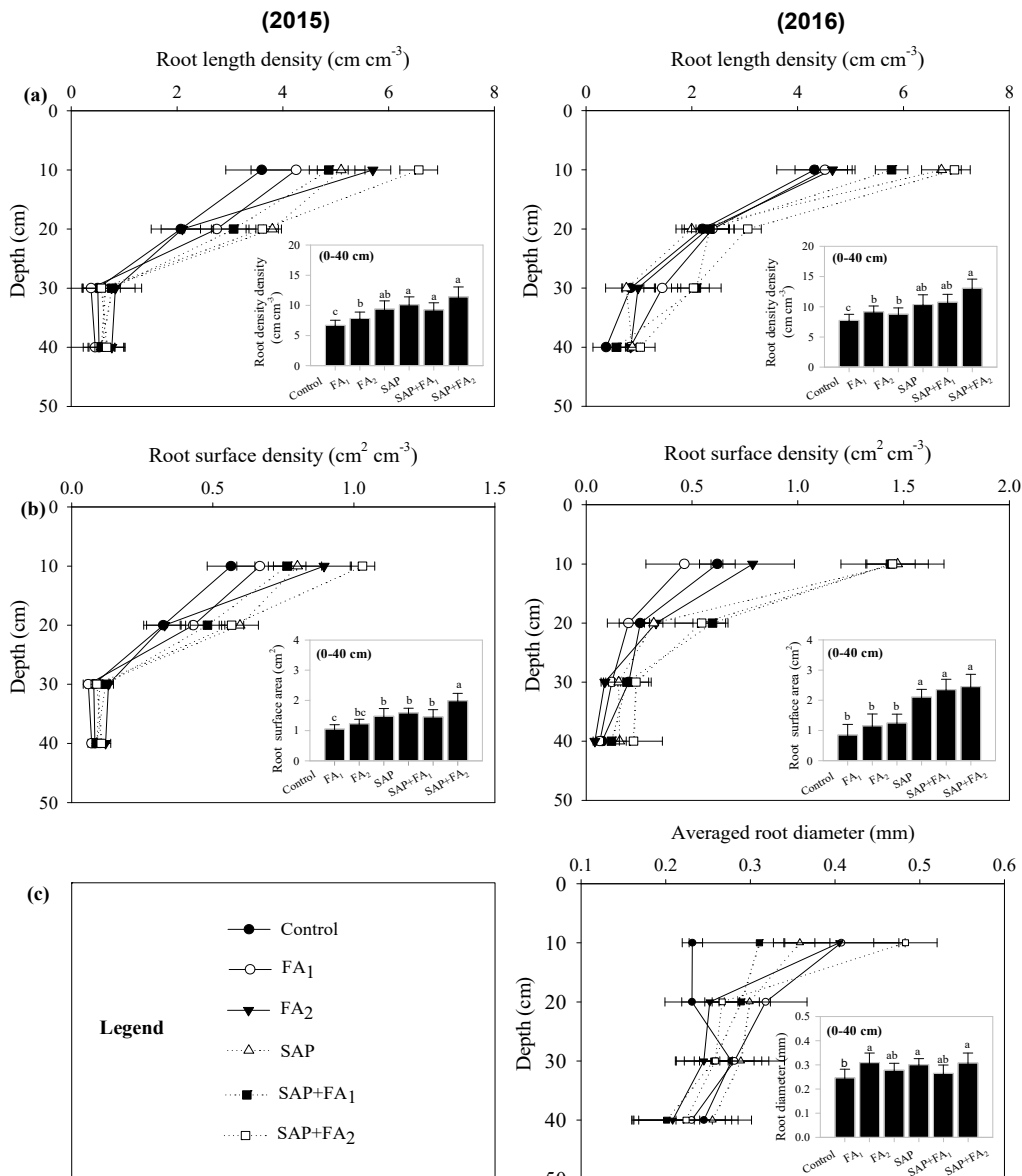
Root length density (RLD) and root surface-area density (RSD) generally decreased over soil depth above 40 cm in both years (Figure 3). The obvious changes in RLD and RSD among FA and SAP treatments occurred in the 0-10 cm layer each year. There were nonsignificant differences between the two parameters below the depth of 10 cm (10-20, 20-30, and 30-40 cm) between chemicals-applied and control plots. For the upper 10 cm soil layer, RLD and RSD were generally shown a slight improvement in FA<sub>1</sub> or FA<sub>2</sub> treatment alone in both years, but they were significantly increased in SAP treatment. The two parameters in 0-10 cm were also greater in combined FA and SAP treatment than under the individual addition of the chemicals. Based on mean values across 2 yr, SAP+FA<sub>2</sub> treatment significantly improved RLD and RSD values in 0-10 cm soil layer by 32% and 37% compared to FA<sub>2</sub> treatment alone and 82% and 85% compared to control plots. In the entire 0-40 cm soil profile, as compared with control, the RLD and RSD were significantly increased by FA<sub>1</sub> or FA<sub>2</sub> treatment, but they were significantly lower than these under the combined SAP and FA<sub>2</sub> application. There was nonsignificant difference in RLD and RSD values in 0-10 cm and 0-40 cm soil layers among SAP, SAP+FA<sub>1</sub>, and SAP+FA<sub>2</sub> treatments. Root diameter showed a decreasing trend over soil depth. Relative to control plots, root diameter in 10 cm soil layer increased by 76% as averaged two FA treatments and by 54% for SAP treatment alone. Combing two chemicals use, relative to control, increased root diameter at 10 cm depth by 34% in SAP+FA<sub>1</sub> treatment and by 108% in SAP+FA<sub>2</sub> treatment. Averaged soil layers, compared to control plots, root diameter increased by 13% and 26% for FA<sub>1</sub> or FA<sub>2</sub> treatment, 22% for SAP treatment, 7% for SAP+FA<sub>1</sub>, and 25% for SAP+FA<sub>2</sub> treatment.



**Figure 2.** Maize root biomass density at different soil depths (0-10, 10-20, 20-30, and 30-40 cm) subjected to treatments with foliar fulvic acid (FA) and soil superabsorbent polymer (SAP) under low rainfall conditions in 2015 and 2016. FA<sub>x</sub>: 1 or 2 g L<sup>-1</sup> FA spraying during the crop growth periods. Values are means  $\pm$  SD (n = 3). Different letters above columns indicate significant difference at  $p < 0.05$ .

Approximately 75%-80% of RLD with root diameter  $\leq 0.5$  mm was within a 30 cm soil profile; remaining 20%-25% of which with root diameter  $> 0.5$  mm was within 30 cm soil profile in 2015 and 2016 (Figure 4). Within 2 yr, foliar spray with FA<sub>1</sub> or FA<sub>2</sub> did not change the percentage of RLD between the two diameters of roots in depths of 0-10 cm and 10-20 cm. As compared to control plants, soil SAP reduced the percentage of RLD for root diameter  $>$

0.5 mm, but increased the percentage with root diameter  $\leq 0.5$  mm. Regardless of root diameter with  $> 0.5$  mm or  $\leq 0.5$  mm, the combined use of these two chemicals did not change RLD compared to control plants at 0-10 cm, whereas it was higher than that in SAP treatment and lower than in FA treatment. For the 10-20 cm layer, relative to control plants, SAP application increased the percentage of RLD for roots with diameter  $> 0.5$  mm and reduced the percentage of which with root diameter  $\leq 0.5$  mm; FA combined with SAP did not change the percentage of RLD regardless of root diameter. Compared with control plants, SAP, FA<sub>1</sub>, or FA<sub>2</sub> treatment reduced the percentage of RLD at a diameter  $> 0.5$  mm and increased the percentage of which with diameter  $\leq 0.5$  at depths of 20-30 and 30-40 cm; the decreasing extent in the SAP treatment was higher than in the FA treatment. In contrast, applying both FA and SAP, relative to control, increased the percentage of which with diameter  $\leq 0.5$  at depths of 20-30 and 30-40 cm; the increasing level was higher than that in the individual use of the chemicals.



**Figure 3.** Maize root surface density (a), root length density (b), and root diameter (c) at different soil depths (0-10, 10-20, 20-30, and 30-40 cm) subjected to treatments with foliar fulvic acid (FA) and soil super absorbent polymer (SAP) under low rainfall conditions. FA<sub>x</sub>: 1 or 2 g L<sup>-1</sup> FA spraying during the crop growth periods. Values are means  $\pm$  SD (n = 3).



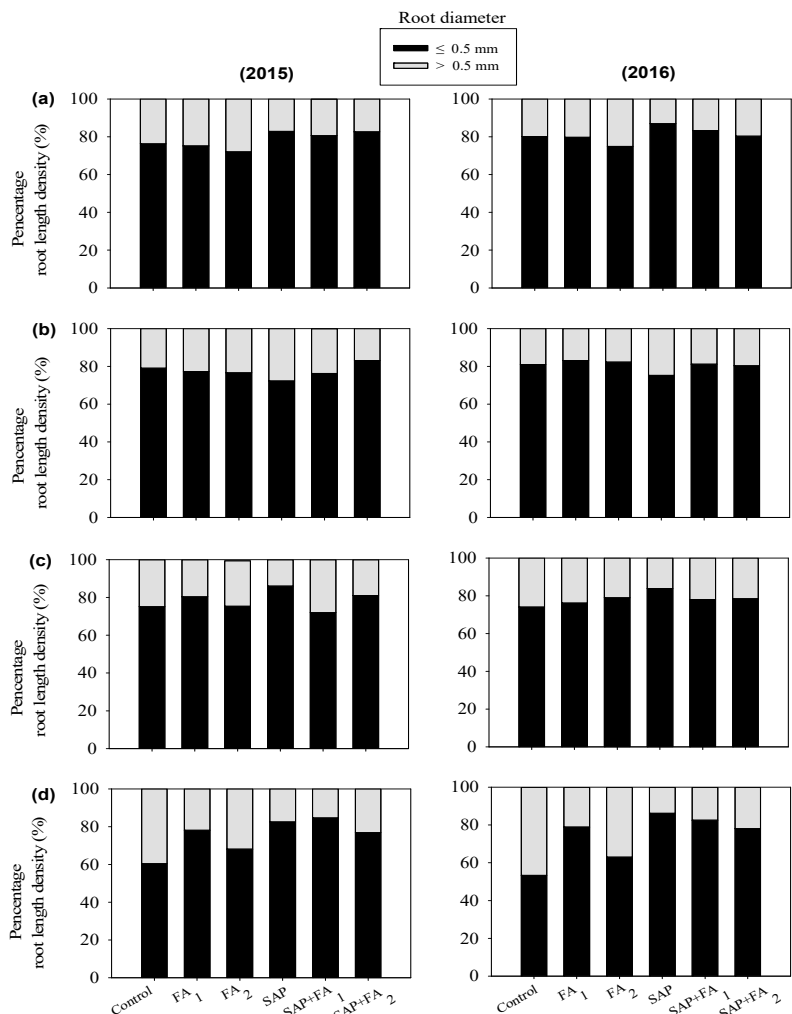
## DISCUSSION

In the study, the effect of the combined use of FA and SAP was attributed to maize yield and aboveground biomass, this effect was superior compared with the effects of their individual applications (Tables 2, 3). The yield increase was principally attributed to the increment in grain weight. Possible explanations are, on the one hand, SAP is a good soil-conditioning additive, its application in the study improves the field capacity of sandy loam soils used in the study (Yang et al., 2019a), further bettering the soil environment for crop growth. Foliar FA application reduces leaf stomatal opening while maintaining higher photosynthesis (Anjum et al., 2011; Yang et al., 2017), and it significantly improves kernel number by promoting pollen viability under limited irrigation (Weerasinghe et al., 2016; Yang et al., 2019a). On the other hand, these two chemicals application significantly promoted crop root growth, especially root length density and root biomass in the upper layer. Larger root length density and root biomass promote water and nutrients uptake by roots and increases crop yield under water deficiency (Zhou et al., 2012; Liao et al., 2018). Thus, the combined use of FA and SAP was conducive to grain yield for the low rainfall and high soil surface evaporation conditions.

The application rate of foliar FA should be a critical factor in determining plant root growth under water deficit conditions (Calvo et al., 2014; Canellas et al., 2015). Previous study results regarding FA spraying effects on root growth were sporadic and inconsistent in various species of crops. However, increased rooting and enhanced root elongation with the application of FA were found on maize plants (Canellas et al., 2009). The studies showed that application of FA with concentration (30-250 mg L<sup>-1</sup>) had no impact on root growth of cucumber plants (Aguirre et al., 2009) and creeping bent grass (Hunter and Anders, 2004). The optimal concentrations of humic substances to improve root biomass were 50 to 300 mg L<sup>-1</sup>, varied for various species of plants (Calvo et al., 2014; Maibodi et al., 2015). The concentrations of FA (0.6-2.0 g L<sup>-1</sup>) were recommended as foliar canopy application in late growth periods of field crops (Wang et al., 2013; Zhang et al., 2016; Yang et al., 2017). In the present study, maize root biomass was improved in a low or high concentration of FA with 1 or 2 g L<sup>-1</sup> (Figure 2). It was probably attributed to increased plant water content under FA-sprayed conditions (Anjum et al., 2011). Fulvic acid worked better under sufficient water supply than water deficit for wheat plants grown in soil-culture pots (Zhang et al., 2016), who found that root biomass was increased by 38% under moderate drought and by 56% under well-watered conditions. The activity of FA used may be easier to interact with leaves containing higher water content, and it makes a considerable contribution to root system development during the growing season.

The SAP increased root biomass compared to non-chemicals treatments; in contrast, the percentage of root biomass in the upper 10-20 cm was higher than that in the 30-40 cm (Figure 3). It could be explained that the soil-amended SAP improves soil water-holding capacity and increases soil moisture when applied to a sandy loam (Yu et al., 2011). Root biomass was further increased compared to FA<sub>1</sub> and FA<sub>2</sub> treatments and control plots, but it was not different from SAP treatment, indicating that soil SAP is the main contributor to root growth but not foliar FA. The plant had a better root system under soil mixing with SAP, and it has grown well under low rainfall conditions compared to FA treatments and control (Liao et al., 2017). Under those conditions, the potential role of FA was possibly shown in the improvements of biological production and yield components while maintaining better root system (Tables 2, 3). Yang et al. (2017) demonstrated that the combined use of FA and SAP significantly increased grain number, which is largely attributed to crop yield and grain WUE in a pot experiment. These effects were superior to those of individual chemicals.

The RLD and RSA are essential parameters to indicate the capability of soil moisture and nutrients uptake by roots (Bian et al., 2016). Roots show the trends in the availability of water and fertilizer, so water and nutrients in shallow soil profile significantly affect root morphological parameters (Zhou et al., 2012). The RLD and RSA in the top 20 cm depth were not changed after FA<sub>2</sub> spraying, but RD in the top 10 cm depth increased (Figure 3). Increased RD at a depth of 0-10 cm may be attributed to the proton pump activity and endogenous hormones formation when spraying-FA flowed from leaf to soil (Calvo et al., 2014). The H<sup>+</sup>-ATPase activity supports a critical driving force to facilitate uptake and efflux of ions within plasma membranes by creating an electrochemical gradient. Mora et al. (2010) found that using FA promotes H<sup>+</sup>-ATPase activity, cytokinins, and polyamines levels in roots. The FA application to maize improved root growth by producing auxin that induce H<sup>+</sup>-ATPase activity in the cell membranes, leading to the apoplast acidification due to the loosening of cell walls (Canellas et al., 2002; Jindo et al., 2012). A gene-encoding H<sup>+</sup>-ATPase with and two assumed N transporters on maize were found and identified by humic substances (Quaggiotti et al., 2004).



**Figure 4.** Percentage of maize root length density at different soil depths, 0-10 (a), 10-20 (b), 20-30 (c), and 30-40 cm (d), for roots with diameters of  $\leq 0.5$  mm and  $> 0.5$  mm subjected to treatments with foliar fulvic acid (FA) and soil super absorbent polymer (SAP). FA<sub>x</sub>: 1 or 2 g L<sup>-1</sup> FA spraying during the crop growth periods.

In general, nutrients available in the upper soil profile at 10-20 cm depth were relatively adequate, so plant developed more and larger coarse root to transport nutrient elements to shoot organs by appropriate root area with root in diameter  $< 0.5$  mm for absorbing water and nutrients (Figure 4; Zhou et al., 2012). This activity contributed to reducing nutrients loss in the soil profile. Nitrate-N concentration in shoots dramatically increased, and that in roots rapidly reduced for cucumber plants by applying FA (Mora et al., 2010). The uptake of N, P, and K in shoots increased as spraying FA to plants (El-Desuki et al., 2012; Calvo et al., 2014). The local soil is mainly characterized by poor water holding capacity. Water and nutrients in maize fields are easy to lose as drainage below the root zone, while it was encountered with high-intensity rainfall or rainstorms (Fan et al., 2005).

## CONCLUSIONS

For maize plants grown under low rainfall conditions, co-application of two chemicals, fulvic acid (FA) and super absorbent polymer (SAP), is conducive to the improvements of root surface area and root length density at 0-20 cm depth especially at 0-10 cm, which are beneficial to maize above ground biomass accumulation and grain yield increase. These roots adaptations could alleviate the adverse effect of periods of soil water deficit on

crop growth, improving crop yield and water productivity for a sandy loam soil characterized with few organic matter and a high sandy fraction. If similar management is practiced, the combined use of FA and SAP could be effective for promoting root growth and yield formation under semiarid and arid conditions.

#### Author contributions

Methodology: R.S. Formal analysis: Y.Y. Investigation: R.S. Data curation: Y.Y. Writing-original draft: W.Y., G.F., Y.Y. Writing-review & editing: W.Y., Z.Q., G.F. Supervision: W.Y., Z.Q. Funding acquisition: W.Y., Z.Q. All co-authors reviewed the final version and approved the manuscript before submission.

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#### References

- Aguirre, E., Leménager, D., Bacaicoa, E., Fuentes, M., Baigorri, R., Zamarreño, A.M., et al. 2009. The root application of a purified leonardite humic acid modifies the transcriptional regulation of the main physiological root responses to Fe deficiency in Fe-sufficient cucumber plants. *Plant Physiology and Biochemistry* 47(3):215-223.
- Akhter, J., Mahmood, K., Malik, K.A., Mardan, A., Ahmad, M., Iqbal, M.M. 2004. Effects of hydrogel amendment on water storage of dandy loam and loam soils and seedlings growth of barely, wheat and chickpea. *Plant, Soil and Environment* 50(10):463-469.
- Anjum, S.A., Wang, L., Farooq, M., Xue L., Ali, S. 2011. Fulvic acid application improves the maize performance under well-watered and drought conditions. *Journal of Agronomy and Crop Science* 197(6):409-417.
- Bai, W., Zhang, H., Liu, B., Wu, Y., Song, J. 2010. Effects of super-absorbent polymers on the physical and chemical properties of soil following different wetting and drying cycles. *Soil Use and Management* 26(3):253-260.
- Bian, D., Jia, G., Cai, L., Ma, Z., Eneji, A.E., Cui, Y. 2016. Effects of tillage practices on root characteristics and root lodging resistance of maize. *Field Crops Research* 185:89-96.
- Bocanegra, M.P., Lobartini, J.C., Orioli, G.A. 2006. Plant uptake of iron chelated by humic acids of different molecular weights. *Communications in Soil Science and Plant Analysis* 37(1-2):239-248.
- Calvo, P., Nelson, L., Kloepper, J.W. 2014. Agricultural uses of plant biostimulants. *Plant and Soil* 383:33-41.
- Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D.L., Nebbioso, A., Mazzei, P., et al. 2015. Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae* 196:15-27.
- Canellas, L.P., Olivares, F.L., Okorokova-Façanha, A.L., and Façanha, A.R. 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H<sup>+</sup>-ATPase activity in maize roots. *Plant Physiology* 130(4):1951-1957.
- Canellas, L.P., Spaccini, R., Piccolo, A., Dobbss, L.B., Okorokova-Façanha, A.L., de Araújo Santos G., et al. 2009. Relationships between chemical characteristics and root growth promotion of humic acids isolated from Brazilian Oxisols. *Soil Science* 174(11):611-620.
- Dobbss, L.B., Canellas, L.P., Olivares, F.L., Aguiar, N.O., Peres, L.E.P., Azevedo, M., et al. 2010. Bioactivity of chemically transformed humic matter from vermicompost on plant root growth. *Journal of Agriculture and Food Chemistry* 58(6):3681-3688.
- Domingos, R.F., Tufenkji, N., Wilkinson, K.J. 2009. Aggregation of titanium dioxide nanoparticles: Role of a fulvic acid. *Environment Science and Technology* 43(5):1282-1286.
- El-Desuki, M., El-Bassiony, A.M., Fawzy, Z.F. 2012. Response of growth and yield of cucumber plants (*Cucumis sativus* L.) to different foliar applications of humic acid and bio-stimulators. *Australian Journal of Basic and Applied Sciences* 6(3):630-637
- Eneji, A.E., Islam, R., An, P., Amalu, U.C. 2013. Nitrate retention and physiological adjustment of maize to soil amendment with superabsorbent polymers. *Journal of Cleaner Production* 52(1): 474-480.
- Fan, T., Stewart, B.A., Payne, W.A., Yong, W., Luo, J.J., Gao, Y.F. 2005. Long-term fertilizer and water availability effects on cereal yield and soil chemical properties in Northwest China. *Soil Science Society of America Journal* 69:842-855.
- Guan, D., Al-Kaisi, M.M., Zhang, Y., Duan, L., Tan, W., Zhang, M., et al. 2014. Tillage practices affect biomass and grain yield through regulating root growth, root-bleeding sap and nutrients uptake in summer maize. *Field Crops Research* 157:89-97.

- Hartz, T.K., Bottoms, T.G. 2010. Humic substances generally ineffective in improving vegetable crop nutrient uptake or productivity. *HortScience* 45(6):06-910.
- Hunter, A., Anders, A. 2004. The influence of humic acid on turfgrass growth and development of creeping bentgrass. *Acta Horticulturae* 661(661):257-264.
- Jindo, K., Martim, S.A., Navarro, E.C., Pérez-Alfocea, F., Hernandez, T., Garcia, C., et al. 2012. Root growth promotion by humic acids from composted and non-composted urban organic wastes. *Plant and Soil* 353(1):209-220.
- Iqbal, H., Yaning, C., Rehman, H., Waqas, M., Ahmed, Z., Raza, S.T., et al. 2020. Improving heat stress tolerance in late planted spring maize by using different exogenous elicitors. *Chilean Journal of Agricultural Research* 80:30-40.
- Li, H., Mollier, A., Ziadi, N., Shi, Y., Parent, L.É., Morel, C. 2017. The long-term effects of tillage practice and phosphorus fertilization on the distribution and morphology of corn root. *Plant and Soil* 412:97-114.
- Liao, R., Ren, S., Yang, P. 2014. Quantitative fractal evaluation of herbicide effects on the water-absorbing capacity of superabsorbent polymers. *Journal of Nanomaterials* 10:1-9.
- Liao, R., Yang, P., Wang, Z., Wu, W., Ren, S. 2018. Development of a soil water movement model for the superabsorbent polymer application. *Soil Science Society of America Journal* 82(2):436-446.
- Liao, R., Zhang, L., Yang, P., Wu, W., Zhang, Z. 2017. Physiological regulation mechanism of multi-chemicals on water transport and use efficiency in soil-maize system. *Journal of Cleaner Production* 172(2):1289-1297.
- Lotfi, R., Pessarakli, M., Gharavi-Kouchebagh, P., Khoshvaghti, H. 2015. Physiological responses of *Brassica napus* to fulvic acid under water stress: Chlorophyll a fluorescence and antioxidant enzyme activity. *The Crop Journal* 3:434-439.
- Maibodi, N.D.H., Kafi, M., Nikbakht, A., Rejali, F. 2015. Effect of foliar applications of humic acid on growth, visual quality, nutrients content and root parameters of perennial ryegrass (*Lolium perenne* L.) *Journal of Plant Nutrition* 38(2):224-236.
- Mora, V., Bacaicoa, E., Zamarreno, A.M., Aguirre, E., Garnica, M., Fuentes, M., et al. 2010. Action of humic acid on promotion of cucumber shoot growth involves nitrate-related changes associated with the root-to-shoot distribution of cytokinins, polyamines and mineral nutrients. *Journal of Plant Physiology* 167(8):633-642.
- Nardi, S., Pizzeghello, D., Muscolo, A., Vianello, A. 2002. Physiological effects of humic substances on higher plants. *Soil Biology and Biochemistry* 34:1527-1536.
- Quaggiotti, S., Ruperti, B., Pizzeghello, D., Francioso, O., Tugnoli, V., Nardi, S. 2004. Effect of low molecular size humic substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.) *Applied Microbiology* 18(398):816-823.
- Rose, M.T., Patti, A.F., Little, K.R., Brown, A.L. 2014. A meta-analysis and review of plant-growth response to humic substances: practical implications for agriculture. *Advances in Agronomy* 124:37-89.
- Santiago, A.D., Expósito, A., Quintero, J.M. 2010. Adverse effects of humic substances from different origin on lupin as related to iron sources. *Journal of Plant Nutrition* 33(2):143-156.
- Satriani, A., Catalano, M., Scalcione, E. 2018. The role of superabsorbent hydrogel in bean crop cultivation under deficit irrigation conditions: A case-study in Southern Italy. *Agricultural Water Management* 195:114-119.
- Wang, X., Su, Y., Xu, X., Li, G. 2013. Effect of fulvic acid on growth and yield components of direct seeding rice. *Agricultural Science Technology* 14(7):966.
- Weerasinghe, M.M., Kettlewell, P.S., Grove, I.G., Hare, M.C. 2016. Evidence for improved pollen viability as the mechanism for film antitranspirant mitigation of drought damage to wheat yield. *Crop and Pasture Science* 67:137-146.
- Xudan, X. 1986. The effect of foliar application of fulvic acid on water use, nutrient uptake and yield in wheat. *Australian Journal of Agricultural Research* 37(4):343-350.
- Yang, W., Guo, S., Li, P., Song, R., Yu, J. 2019a. Foliar antitranspirant and soil superabsorbent hydrogel affect photosynthetic gas exchange and water use efficiency of maize grown under low rainfall conditions. *Journal of the Science of Food and Agriculture* 99(1):350-359.
- Yang, W., Li, P., Guo, S., Fan, B., Song, R., Zhang, J., et al. 2017. Compensating effect of fulvic acid and super-absorbent polymer on leaf gas exchange and water use efficiency of maize under moderate water deficit conditions. *Plant Growth Regulation* 83(3):351-360.
- Yang, W., Li, P., Guo, S., Song, R., Yu, J. 2019b. Co-application of soil superabsorbent polymer and foliar fulvic acid alleviates water deficit tolerance maize: photosynthesis, water parameters, and proline. *Chilean Journal of Agricultural Research* 79:435-446.
- Yazdani, B., Nikbakht, A., Etemadi, N. 2014. Physiological effects of different combinations of humic and fulvic acid on *Gerbera*. *Communications in Soil Science and Plant Analysis* 45(10):1357-1368.
- Yu, J., Shainberg, I., Yan, Y.L., Shi, J.G., Levy, G.J., Mamedov, A.I. 2011. Superabsorbents and semiarid soil properties affecting water absorption. *Soil Science Society of America Journal* 75(6):2305-2313.

- Zhang, X., Zhang, X., Liu, X., Shao, L., Sun, H., Chen, S. 2016. Improving winter wheat performance by foliar spray of ABA and FA under water deficit conditions. *Journal of Plant Growth Regulation* 35(1):83-96.
- Zhang, J.F., Zhao, T.N., Sun, B.P., Song, S.S., Guo, H.B., Shen, H.J., et al. 2018. Effects of biofertilizers and super absorbent polymers on plant growth and soil fertility in the arid mining area of Inner Mongolia, China. *Journal of Mountain Science* 15(9):1920-1935.
- Zhou, B., Liao, R., Li, Y., Gu, T., Yang, P., Feng, J., et al. 2012. Water-absorption characteristics of organic-inorganic composite superabsorbent polymers and its effect on summer maize root growth. *Journal of Applied Polymer Science* 126(2):423-435.
- Zhou, Y., Coventry, David, R., Denton, Matthew, D. 2016. A quantitative analysis of root distortion from contrasting wheat cropping systems. *Plant and Soil* 404:173-192.