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Full Length Article

Effects of Soil Nitrification on Compensatory Growth upon Post-Drought Rewatering of Corns Based on Cytokinin

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Abstract

This study aimed to investigate the effect of soil nitrification on corn (*Zea mays* L.) compensatory growth of post-drought rewatering (CGPDR) based on root-induced leaf cytokinin. Potted corn seedlings were used as test materials. The nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) was added to soil to inhibit soil nitrification. The experiment consisted of four treatments: (1) wetness, (2) wetness with DMPP addition, (3) rewatering and (4) rewatering with DMPP addition. Results indicated that drought stress and DMPP addition to soil decreased the aboveground and total biomasses and the net photosynthetic rates of corn. Without DMPP added, the aboveground and total biomasses and net photosynthetic rates in rewatered corns were significantly higher than those in wet corns during the rewatering period. As a consequence, the CGPDR occurred. However, rewatering could not increase the aboveground and total biomasses and the net photosynthetic rates when DMPP was added, and the CGPDR did not occur. The DMPP addition to soil reduced the soil nitrification rate and the decreased zeatin riboside (ZR) concentrations in the leaf and xylem sap. Without DMPP added, the increased soil nitrification rate caused by rewatering improved the ZR concentrations in the leaf and xylem sap. No significant differences were found on soil nitrate contents amongst wet and rewatered corns without DMPP added during drought stress and rewatering periods. A high leaf cytokinin concentration caused an increase in the corn net photosynthetic rate and induced rapid growth. Therefore, soil nitrification was a key soil environmental factor regulating the CGPDR.

Key words: Soil nitrification; Corn; Compensatory growth; Cytokinin; Seedling stage; Rewatering upon drought stress

Introduction

Water is considered as one of the most critical resources for human beings. Over the past 60 years, global demand for water has increased because of many reasons, mainly including rapid population and economic growth (Kaur *et al.* 2010). As population and economic growth will continue, more food will be needed to be produced in the future. Therefore, water demand will have been increased by more than 40% by 2050 (Nazari *et al.* 2018). Water scarcity will be a remarkable issue in the near future (Doungmanee 2016). Agriculture is the sector responsible for the highest amount of water use, consuming 70% of the total water use worldwide (Yavuz *et al.* 2012; Kang *et al.* 2017). As such, improving agricultural water productivity is an important measure for ensuring global food security. In this regard, researchers must develop ways to improve the efficiency of water consumption in agriculture and to implement agricultural water saving technologies (Farooq *et al.* 2009; Chai *et al.* 2016; Xu *et al.* 2017).

Plant CGPDR theory states that reduced plant growth due to drought stress is offset or exceeded when water supply becomes available. Based on the theory, agricultural water-saving technologies, including deficit irrigation, regulated deficit irrigation and supplemental irrigation in dryland farming, have been widely used in crop production (Rowland *et al.* 2018; Farooq *et al.* 2019; Ramlow *et al.* 2019). Therefore, crop CGPDR is important for water-saving agriculture.

Zhang *et al.* (2018) found that applying fertilisers containing nitrogen, phosphorus pentoxide and potassium can increase the water use efficiencies of corn and cotton. Wang *et al.* (2011) reported that improving soil fertility can enhance the compensation growth of rewatered drought-stressed winter wheat. Wang *et al.* (2016) indicated that root-induced leaf cytokinin caused by root nitrate (NO_3^-) absorption increases corn CGPDR. Thus, soil fertiliser, especially NO_3^-, plays a vital role in crop CGPDR. In

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general, soil releases NO_3^- via nitrification. Soil nitrification likely participates in crop CGPDR. However, the connection between soil nitrification and the CGPDR has not been reported. Thus, the effect of soil nitrification on the CGPDR mechanism should be further investigated to provide insights into the underlying mechanism in plants and soils.

Corn is a high-water-consuming crop and the third-largest produced crop worldwide. It is also the most produced crop in China. In northern China, corn production is usually hampered by water scarcity, indicating that the efficient use of water in corn production should be increased. Corn seedlings are sensitive to drought, and their growth variation can be easily detected as they grow rapidly. Therefore, in the present study, corn seedlings were used to reveal the regulatory mechanism of soil nitrification in crop CGPDR. The nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP) was added to soils to retain soil nitrification. Plant hormones in leaves and xylem saps, photosynthesis indices and soil nitrification rate were estimated to ascertain the regulatory mechanism.

Materials and Methods

Experimental design

The study took place at the experimental farm in Henan University of Science and Technology (34°32′ N, 112°16′ E, altitude 138m) in Luoyang City, Henan Province. This area receives an annual rainfall of 601 mm with a temperature of 14.2°C. 'Zhengdan-958' was selected as the type of corn (*Zea mays* L.) cultivar because it has the advantages of drought resistance, wide adaptability and wide cultivation in China. The study was carried out under a rain shelter with potting.

The experiment was conducted for 41 days from 13 May 2018 to 23 June 2018. On 13 May 2018, 12 corn seeds were planted in 150 pots for the selection of pots with uniformly grown seedlings. The diameter of the pot mouth was 21.5 cm, and the height of each pot was 20.0 cm. Each pot contained 5.8 kg of soil with organic carbon and total nitrogen contents of 24.3 and 2.16 g/kg, respectively. Only five seedlings showing strong growth were selected 6 days after emergence, and they grew for 10 days. Other seedlings were pulled out. Then, 36 pots with uniformly grown seedlings were selected from 150 pots. The schematic in Fig. 1 clearly displays the trial time course, treatment setting and related indicators measured in the 36 pots.

Afterwards, 36 pots were split into four groups, with 9 pots in each group. They were then subjected to two growth periods for 10 days: drought stress and rewatering. During the drought stress period, the first two groups were given sufficient water, while the remaining groups were subjected to drought stress. Then, 13 mL of DMPP solution (1 g/L) was added to the second and fourth groups every day during the drought stress period to restrain soil nitrification during

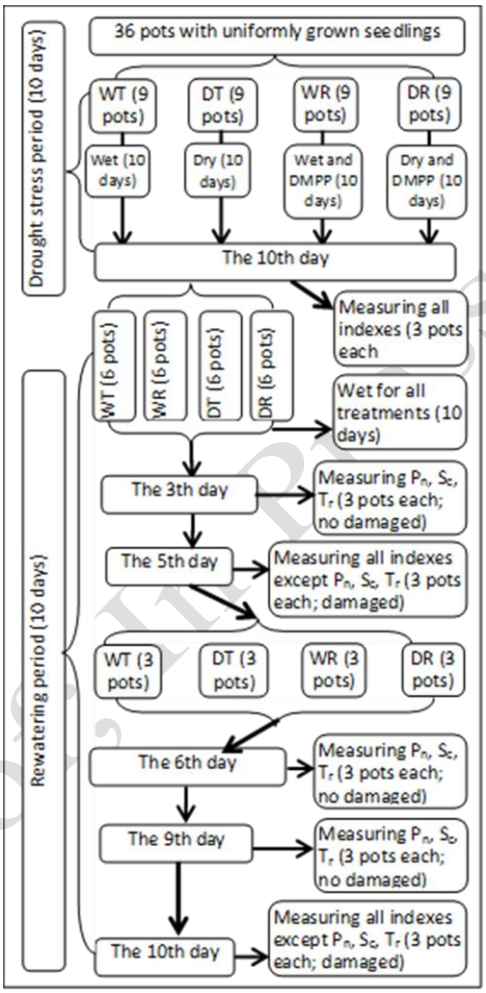

Fig. 1: Schematic diagram of the experimental design
The 3th, 5th, 6th, 9th and 10th days refer to the 3th, 5th, 6th, 9th and 10th days of the 10-day drought stress or rewatering periods. WT, WR, DT and DR indicate treatments of wetness, wetness with DMPP added, rewatering and rewatering with DMPP added, respectively. "Wet" and "Dry" refer to the exposure of corns to wetness and drought stress, respectively. "DMPP" indicates the DMPP added to soil. "3 pots each" corresponds to the three pots in each treatment. "10 days" denotes the 10-day duration. "Damaged" implies that the corns were taken to lab to be completely harmed for measurement. "No damage" means that corns were not harmed during measurement. All indices refer to indices that were measured in the present study. P_n, S_c, and T_r represent the net photosynthetic rate, stomatal conductance and transpiration rate, respectively

the rewatering period. In the rewatering period, all the groups received sufficient water. Thus, the four treatments comprising nine pots each were as follows: (1) wetness (WT), (2) wetness with DMPP added (WR), (3) rewatering (DT) and (4) rewatering with DMPP added (DR).

At the beginning of the drought stress, the corns in this trial were in the growth period of 4–5 leaves. At the end of the rewatering period, the corns were in the growth period of 7–8 leaves. Our preliminary experiment found that after adding DMPP to the soil, its inhibitory effect on soil nitrification appeared after several days. This phenomenon was the main reason for choosing this time to add DMPP to soil. Wetness and drought stress were induced by the addition of the weighed amount of water that allowed the

soil water content to be maintained at 75–80% and 50–55% of the field water capacity, respectively. During the time period 6:00 a.m. to 9:00 p.m., when the soil water content reached lower than 75% of the field water capacity, the water content was maintained at 75–80% of the field water capacity by adding water. Water was not added to the soil on the first 2–3 days to allow soil water dissipation. Afterwards, the pots were weighed, and the soil water content was maintained at 50–55% of the field water capacity by adding water.

The soil water content at each treatment was calculated using Formula (1) in accordance with previously described methods (Xiong *et al.* 2007):

$$SWC = \frac{W_t - W_d - W_e - W_p}{W_d \times FWC} \times 100\% \quad (1)$$

where SWC is the soil water content, W_t is the temporary whole pot weight, W_d is the net weight of dried soil in the pot, W_e is the weight of the empty pot, W_p is the estimated fresh weight of all plants in the pot, and FWC is the field water capacity. The estimated fresh weight of all plants in one pot was determined in advance by measuring the fresh weight of the plants in extra pots in each test period.

Measurements and data analysis

At the end of the drought stress period and 3, 6 and 9 days post rewatering, net photosynthetic rate (P_n), transpiration rate (T_r) and stomatal conductance (G_s) were measured using LI-6400 photosynthesis equipment at 11:00 a.m. in each treatment.

At the end of the drought stress period and 5 and 10 days post rewatering, three pots from each group were taken to lab. After the stem bases were clipped, the stem wounds were immediately covered with 1.0 g of absorbent cotton. After 12 h, the cotton's weight was determined. The quantity of xylem was equal to the increased weight in the absorbent cotton. The volume of sap was determined by dividing the increased weight by 1 g/cm^3. Then, the cotton was compactly placed at the end of a 10 mL syringe by using a piston. The sap was pooled from each sample to 5 mL centrifuge tubes to measure the concentrations of zeatin riboside (ZR) and abscisic acid (ABA) in the xylem sap. Immediately after the stem bases were clipped, the weights of the clipped stems and leaves were determined, and leaf samples were collected for the measurement of indole-3-acetic acid (IAA), gibberellic acid (GA_3), ZR and ABA concentrations in leaves.

After the xylem sap was collected, soil samples were obtained to measure soil ammonium (NH_4^+) and NO_3^- contents and soil nitrification rate. The roots of the corn seedlings were separated from the soil by washing, and the roots and stem bases were weighed. Root samples were also collected to measure the root activity and solute carbohydrate content in roots. The fresh samples of roots, stems and leaves were dried at 65°C for 72 h to determine their water and dry matter contents. The product of the fresh weight and dry matter content of the sample was adopted to determine the biomasses. Aboveground biomass was calculated as the sum of the stem and leaf biomasses. The sum of the stem, leaf, and root biomasses was then used to calculate the total biomass.

IAA, GA_3, ZR and ABA concentrations in the leaves or xylem saps were estimated via an enzyme-linked immunosorbent assay in accordance with previously described methods (Qin and Wang 2019). The test kits for IAA, GA_3, ABA and ZR were produced at the Phytohormone Research Institute of China Agricultural University. Soil NH_4^+ and NO_3^- contents were measured through indophenol blue and phenol disulfonic acid colorimetry, respectively (Lu 1999).

The soil net nitrification rate was determined via the culture method (Lu 1999), which was slightly modified. All the soil samples except the soil samples of DT and DR at the end of the drought stress period were cultured at 25°C for 7 days under the SWC of 75–80% of the field water capacity to simulate the wetness condition. The soil samples of DT and DR at the end of the drought stress period were cultured at 25°C for 7 days under the SWC of 50–55% of the field water capacity to simulate the drought stress condition. The NO_3^- contents of the soil before and after being cultured were measured, and their differences were divided by the culturing time of 7 days to obtain the soil net nitrification rates. Anthrone method was used to measure the soluble carbohydrate contents in roots (Li *et al.* 2000). Root activity was determined using the triphenyltetrazolium chloride method (Li *et al.* 2000). The abbreviations used in the text are listed in Table 1.

Statistical analysis

Statistical analyses were conducted with Microsoft Excel 2007. The values in the tables or graphs corresponded to average values. The effects of water, including drought stress and rewatering, and DMPP addition to soil on corn growth were analysed using two-way variance analysis and Duncan's multiple range tests (P= 0.05).

Results

Biomass and photosynthetic characteristics

Table 2 shows that the aboveground and total biomasses in WT significantly increased compared with that in WR, DT and DR during drought stress and 5 days after rewatering. These biomasses still significantly increased in WT compared with those in WR and DR, but no significant differences in these biomasses were found between WT and DT 10 days after rewatering. Furthermore these biomasses in WT, DT and WR also significantly increased compared with those than in DR during drought stress and rewatering period. P_n, T_r and S_c in WT significantly increased compared with

Table 1: Symbol definition

Symbol	Definition	Symbol	Definition
CGPDR	compensatory growth of post-drought rewatering	WT	wetness
DMPP	Nitrification inhibitor 3,4-dimethylpyrazole phosphate	WR	wetness with DMPP added
ZR	zeatin riboside	DT	rewatering
NO_3^-	Soil nitrate	DR	rewatering with DMPP added
NH_4^+	soil ammonium	SWC	soil water content
GA_3	gibberellic acid	FWC	field water capacity
ABA	abscisic acid	W_t	temporary whole pot weight
IAA	indole-3-acetic acid	W_d	net weight of dried soil in the pot
P_n	net photosynthetic rate	W_e	weight of empty pot
T_r	transpiration rate	W_p	fresh weightof all plants in the pot
S_c	stomatal conductance		

Table 2: Aboveground and total biomasses, P_n, S_c, and T_r in different treatments

	Days after rewatering (d)	Treatments				F-Value		
		WT	WR	DT	DR	DMPP effect	Water effect	Water×
Abovegro-und biomass (g/pot)	0	3.44^{a}±0.13	3.02^{b}±0.34	2.44^{c}±0.22	1.79^{d}±0.28	13.07*	57.46*	0.63
	5	3.68^{a}±0.12	3.11^{b}±0.33	3.24^{b}±0.25	1.93^{c}±0.27	41.80*	30.84*	6.54*
	10	4.00^{a}±0.18	3.22^{b}±0.32	3.81^{a}±0.23	2.31^{c}±0.28	59.14*	13.65*	5.76*
Total biomass (g/pot)	0	5.66^{a}±0.24	5.00^{b}±0.48	4.20^{c}±0.08	3.11^{d}±0.27	25.53*	92.91*	1.51
	5	6.07^{a}±0.21	5.12^{b}±0.49	5.42^{b}±0.25	3.21^{c}±0.29	68.94*	45.45*	11.17*
	10	6.63^{a}±0.18	6.33^{b}±0.39	5.31^{a}±0.47	3.83^{c}±0.26	93.47*	20.41*	9.14*
P_n (μmol/m^2•s)	0	14.67^{a}±0.84	5.88^{c}±0.49	9.11^{b}±0.83	3.83^{d}±0.24	351.94*	102.77*	21.82*
	3	11.57^{b}±0.81	3.55^{c}±0.30	15.50^{a}±1.31	4.35^{c}±0.29	435.12*	26.54*	11.63*
	6	10.60^{b}±0.95	2.27^{c}±1.69	16.87^{a}±0.19	1.77^{c}±0.14	431.26*	26.09*	35.93*
	9	10.19^{b}±1.10	3.95^{c}±1.42	13.27^{a}±0.25	3.32^{c}±0.14	237.19*	5.42*	12.44*
S_c (μmol/m^2•s)	0	0.12^{a}±0.005	0.06^{b}±0.005	0.06^{b}±0.006	0.03^{c}±0.004	222.07*	191.68*	27.65*
	3	0.08^{bc}±0.003	0.08^{c}±0.007	0.09^{a}±0.006	0.04^{d}±0.004	89.82*	18.51*	72.54*
	6	0.07^{b}±0.007	0.02^{c}±0.010	0.10^{a}±0.001	0.02^{c}±0.002	289.62*	12.85*	28.86*
	9	0.06^{b}±0.006	0.03^{c}±0.009	0.08^{a}±0.001	0.02^{d}±0.002	216.78*	3.84	28.85*
T_r (mmol/m^2•s)	0	1.66^{a}±0.10	1.01^{b}±0.12	0.99^{b}±0.05	0.64^{c}±0.07	92.44*	98.35*	8.17*
	3	2.70^{b}±0.11	2.35^{b}±0.26	3.36^{a}±0.37	1.55^{c}±0.13	60.61*	0.26	27.86*
	6	0.81^{b}±0.07	0.28^{c}±0.10	1.22^{a}±0.03	0.20^{c}±0.02	411.23*	18.17*	41.83*
	9	1.27^{b}±0.11	0.65^{c}±0.06	1.68^{a}±0.06	0.40^{d}±0.06	460.211*	3.20	56.22*

The values are the mean ± 1 standard deviation (n = 3). Different letters in each row indicate significant differences (P <0.05). "*" means "P<0.05". P_n, S_c and T_r represent the net photosynthetic rate, stomatal conductance, and transpiration rate, respectively. WT, WR, DT and DR indicate treatments of wetness, wetness with DMPP added, rewatering and re-watering with DMPP added, respectively

those in WR, DT and DR prior to rewatering. These parameters also significantly increased in DT compared with those in WT, WR and DR and in WT compared with those in WR and DR 6 and 9 days after rewatering. No significant differences in P_n were found between WR and DR during the drought rewatering period. The interaction between water and DMPP significantly affected corn growth and its photosynthetic characteristics during drought stress and rewatering periods. Therefore, drought stress and DMPP addition to soil inhibited corn growth and photosynthesis, but rewatering without adding DMPP to soil increased them obviously.

Plant hormones and xylem sap quantity

Table 3 shows that the leaf ZR contents and its concentration in the xylem saps significantly increased in WT compared with that in DT prior to rewatering and in DT compared with that in WT 5 days post rewatering. The leaf ZR content significantly increased in WT compared with that in WR and in DT compared with that in DR during the rewatering period. No significant differences in leaf ZR and its concentration in the xylem saps were detected between WR and DR during the rewatering period and between DT and WT 10 days post rewatering. The interaction between drought stress and DMPP significantly affected the leaf ZR contents and its concentrations in the xylem saps. The primary type of cytokinin was ZR. These findings indicated that drought stress and DMPP addition to soils decreased the leaf cytokinin content. Rewatering without DMPP addition increased the cytokinin contents in the leaves and the xylem saps during the rewatering period, but this effect gradually diminished during rewatering.

The leaf IAA contents in WR were significantly higher than those in WT. Similarly, the leaf IAA contents in DR were significantly higher than those in DT before rewatering and 5 and 10 days post rewatering. The leaf ABA content and its concentration in the xylem saps were significantly higher in DT and DR than in WR and WT prior to rewatering, respectively. The xylem sap quantity per unite increased in WT compared with that in DT, DR and WR. This parameter also significantly increased in DT compared with that in DR prior to rewatering. Conversely, the xylem sap quantity per unit time significantly decreased in WR compared with that in WT and in DR compared with that in DT 5 and 10 days post rewatering. The interaction between water and DMPP slightly affected the leaf IAA content, but

Table 3: ZR, ABA, IAA, and GA_3 concentrations in the leaves; ZR and ABA concentrations in the xylem saps; and xylem sap quantity in different treatments

	Days after rewatering (d)	Treatments				F-Value		
		WT	WR	DT	DR	DMPP effect	Water effect	Water × DMPP
ZR content in leaves (μg/g)	0	119.41^{a} ± 6.41	95.24^{b} ± 9.72	68.47^{c} ± 9.22	78.67^{c} ± 8.90	1.95	45.62*	11.83*
	5	111.53^{b} ± 12.50	93.08^{c} ± 8.14	137.51^{a} ±10.16	98.32^{c} ± 8.28	25.27*	7.41*	3.27
	10	122.10^{a} ± 9.39	100.12^{b} ± 4.57	128.80^{a} ± 11.02	104.58^{b} ± 10.84	18.40*	1.08	0.04
ZR content in xylem sap (μg/ml)	0	78.19^{c} ± 7.62	57.94^{c} ± 9.42	308.40^{a} ±32.47	218.18^{b} ± 21.45	22.04*	275.31*	8.84*
	5	63.72^{a} ± 5.30	68.45^{ab} ±9.26	78.79 ^{a} ± 4.09	77.79 ^{a} ± 9.96	0.18	7.78*	0.43
	10	57.85^{b} ± 9.69	65.12 ^{a} ± 12.68	63.47 ^{a} ± 6.34	71.96 ^{a} ± 2.31	2.48	1.55	0.02
IAA content in leaves (μg/g)	0	294.36^{bc}±30.78	360.10^{a} ±33.30	266.82^{c} ±35.75	342.60^{ab}±33.00	13.58*	1.38	0.07
	5	295.93^{b} ±31.99	413.59^{a} ±37.10	237.09^{b} ±32.02	395.49^{a} ± 17.24	60.33*	4.69	1.31
	10	241.62^{b} ±32.02	314.03^{a} ±32.85	244.03^{b} ±23.51	323.08^{a} ± 21.51	22.06*	0.13	0.04
ABA content in xylem sap (μg/mL)	0	192.33d±26.54	356.53^{c} ±54.96	798.18^{b} ±32.98	917.60^{a} ± 45.17	35.21*	596.12*	0.88
	5	146.68^{b} ±22.09	163.63^{b} ± 6.89	64.50^{c} ± 13.00	200.24^{a} ± 34.08	37.48*	3.34	22.69*
	10	75.45^{c} ± 1.66	148.49^{a} ±14.50	121.33^{b} ±14.14	88.82^{c} ± 6.01	10.98*	1.27	74.46*
ABA content in leaves (μg/g)	0	192.33d±26.54	356.53^{c} ±54.96	798.18^{b} ±32.98	917.60^{a} ± 45.17	35.21*	596.12*	0.88
	5	146.68^{b} ±22.09	163.63^{b} ±6.89	64.50^{c} ± 13.00	200.24^{a} ± 34.08	37.48*	3.34	22.69*
	10	75.45^{c} ± 1.66	148.49^{a} ±14.50	121.33^{b} ±14.14	88.82^{c} ± 6.01	10.98*	1.27	74.46*
GA_3 content in leaves (μg/g)	0	8.88^{b} ± 0.94	7.77^{b} ± 0.33	11.14^{a} ± 0.94	10.39^{a} ± 1.49	2.52	17.42*	0.10
	5	14.83^{a} ± 2.14	13.22^{a} ± 0.77	10.59^{b} ± 0.91	5.75^{c} ± 0.57	19.72*	64.77*	4.93
	10	9.18^{ab} ± 1.03	6.56^{c} ± 0.77	8.17^{b} ± 0.68	9.99^{a} ± 1.22	0.54	4.89	16.44*
Root xylem sap quantity (mg/plant•h)	0	42.50 ^{a} ± 4.38	27.19^{b} ± 2.61	8.71^{c} ± 0.73	7.68^{c} ± 0.66	29.70*	316.00*	22.69*
	5	48.57 ^{a} ± 4.61	39.54^{b} ± 2.72	49.20^{a} ± 4.52	32.89^{c} ± 2.40	35.19*	1.98	2.90
	10	52.38^{a} ± 5.22	37.34^{b} ± 2.34	56.98^{a} ± 5.38	42.59^{b} ± 4.54	31.40*	0.02	3.51

The values are the mean ± 1 standard deviation (n = 3). Different letters in each row indicate significant differences ($P < 0.05$). "*" means "$P < 0.05$". WT, WR, DT and DR indicate treatments of wetness, wetness with DMPP added, rewatering and rewatering with DMPP added, respectively

Table 4: Soil nitrification rate, soil ammonium and nitrate nitrogen, solute carbohydrate content in roots, and root activity in different treatments

	Days after rewatering (d)	Treatments				F-Value		
		WT	WR	DT	DR	DMPP effect	Water effect	Water×DMPP
Soil nitrification rate (mg/kg•d)	0	0.31 ^{a} ± 0.03	0.08^{c} ± 0.01	0.18^{b} ± 0.03	0.09^{c} ± 0.03	88.65*4	14.427*	16.625*
	5	0.33^{a} ± 0.04	0.08^{b} ± 0.01	0.27^{a} ± 0.03	0.09^{b} ± 0.05	101.1*39	1.600	2.209
	10	0. 23^{a} ± 0.01	0.11^{b} ± 0.01	0.28^{a} ± 0.05	0.14^{b} ± 0.01	50.58*6	4.804	0.505
NH_4^+-N content in soil (mg/kg)	0	14.85^{c} ± 1.49	18.21^{b} ± 1.15	13.51^{c} ± 1.23	20.94^{a} ± 1.00	57.73*	0.95	8.26*
	5	11.11^{b} ± 0.88	16.43^{a} ± 1.10	12.17^{b} ± 0.67	17.99^{a} ± 1.53	78.25*	4.31	0.17
	10	8.08^{c} ± 0.60	12.41^{b} ± 0.88	10.12^{c} ± 0.89	17.38^{a} ± 1.00	121.95*	40.64*	13.77*
NO_3^--N content in soil (mg/kg)	0	9.97^{a} ± 0.93	2.03^{c} ± 0.14	7.70^{b} ± 0.84	8.51^{b} ± 0.41	87.76*	30.46*	131.54*
	5	7.08^{a} ± 0.62	3.87^{b} ± 0.30	7.94^{a} ± 0.38	7.34^{a} ± 0.71	38.74*	50.00*	18.28*
	10	6.06^{a} ± 0.29	4.42^{c} ± 0.35	5.48^{ab} ± 0.54	5.09^{b} ± 0.34	21.97*	0.55	9.75*
Solute carbohydrate content in roots (mg/kg)	0	121.54^{b} ±12.61	135.63^{b} ±14.84	178.89^{a}±16.94	169.39^{a} ±11.62	0.079	31.082*	2.084
	5	128.95^{b} ±9.84	119.75^{b} ±8.59	157.67^{a}±20.16	149.85^{ab} ±12.62	1.181	14.102*	0.008
	10	137.57^{a} ±14.37	129.86^{a} ±8.46	143.58^{a}±11.20	139.55^{a} ±10.74	0.798	1.424	0.078
Root activity (mg/pot•h)	0	219.17^{b} ±20.65	200.91^{b} ±21.09	274.96^{a}±24.58	267.54^{a} ±20.91	1.03	23.50*	0.18
	5	257.49^{b} ±17.71	233.52^{b} ±14.43	302.98^{a}±30.71	285.26^{a} ±21.37	2.71	14.76*	0.06
	10	235.8^{ab} ± 22.82	208.44^{b} ±12.08	268.58^{a}±18.33	242.49^{ab} ±20.92	5.95*	9.30*	0.01

The values are the mean ± 1 standard deviation (n = 3). Different letters in each row indicate significant differences ($P < 0.05$). "*" means "$P < 0.05$".WT, WR, DT and DR indicate treatments of wetness, wetness with DMPP added, rewatering and rewatering with DMPP added, respectively

significantly influenced the leaf ABA content and its concentration in the xylem sap during rewatering. These results indicated that adding DMPP to soil increased the leaf IAA contents, and drought stress increased the ABA content in the leaves and xylem saps. Drought stress and DMPP addition to soil decreased the xylem sap quantity per unit time. Adding DMPP to soil and rewatering slightly affected leaf GA_3.

Soil nitrification rate, soil ammonium and nitrate nitrogen, root soluble carbohydrate and root activity

In Table 4, the nitrification rate significantly increased in WT compared with that in WR, DT and DR prior to rewatering. This parameter also significantly increased in WT and DT compared with those in WR and DR 5 and 10 days after rewatering, respectively. No significant differences were observed in soil nitrification between WT and DT and between WR and DR 5 and 10 days post rewatering. The soil NH_4^+ content significantly increased in WR compared with that in WT and in DR compared with that in DT before rewatering and 5 and 10 days after rewatering. The soil NO_3^- content significantly increased in WT compared with that in WR, DT and DR. It also significantly increased in DR compared with that in DT before rewatering. Similarly, the soil NO_3^- content significantly increased in WT compared with that in WR 5 and 10 days post rewatering. No significant differences in

the soil NO_3^- content were detected between WT and DT 5 and 10 days after rewatering. The interaction of water and DMPP significantly affected soil NH_4^+ and NO_3^- contents. Their interaction also significantly influenced the soil nitrification rate before rewatering. Thus, DMPP addition to soil and drought stress without the added DMPP restrained soil nitrification and decreased the soil NO_3^- content. Without DMPP, rewatering resumed soil nitrification, and the soil NO_3^- content was similar to wetness.

The root soluble carbohydrate contents and root activity were significantly higher in DT than in WT, WR and DR before rewatering. These parameters were also significantly higher in DT than in WT and in DR than in WR 5 days after rewatering. The interaction of water and DMPP slightly influenced the root soluble carbohydrate contents and root activity. These results showed that drought stress caused an increase in the root soluble carbohydrate content and root activity, and this effect decreased with rewatering days.

Discussion

When the reduction of the biomass of rewatered corns induced by drought stress was offset in the rewatering period, the CGPDR occurred. Drought stress decreased corn growth in DT compared with that in WT. After 10 days of rewatering, the similar aboveground and total biomass between WT and DT indicated that corns experienced the CGPDR without DMPP addition to soil. By contrast, 10 days of rewatering growth did not cause the CGPDR in DR when DMPP was added. Therefore, the absence of DMPP addition to soil played a key role in the CGPDR.

Without DMPP, the leaf cytokinin concentration in DT was higher than that in WT during the rewatering period. A previous study showed that cytokinin concentration in leaves has a vital role in corn's CGPDR (Wang *et al.* 2016, 2018). Increased cytokinin levels in leaves help enhance their net photosynthetic rate (Tamaki and Mercier 2007; Kobayashi *et al.* 2010, 2012). By nature, the CGPDR is a fast production and accumulation process of organic matter via photosynthesis. P_n in DT was higher than that in WT during the rewatering period, thereby causing the occurrence of CGPDR without DMPP added. However, no high leaf cytokinin content was observed in DR compared with that in WR during the rewatering period, resulting in similar P_n in them. As a result, no CGPDR occurred when DMPP was added. Thus, the absence of DMPP in soil was closely related to the CGPDR on the basis of leaf cytokinin.

In general, as the main sites of cytokinin synthesis, roots synthesise cytokinin and deliver them to leaves via the xylem sap (Zaicovski *et al.* 2008; Lu *et al.* 2009). The product of xylem sap quantity and its cytokinin concentration showed the quantity of cytokinin delivered from roots to leaves. Transpiration is an indispensable factor because it promotes the migration of sap from the roots to the leaves. During the rewatering period, the cytokinin concentration in the xylem sap and T_r increased in DT compared with that in WT, although the same xylem sap quantity was found in WT and DT treatments. This result showed that the roots of DT could deliver more cytokinins to the leaves, leading to an increased leaf cytokinin concentration. However, in the presence of DMPP, no high cytokinin concentration in the xylem sap, xylem sap quantity and T_r were found in DR compared with that in WR during rewatering period. As a result, no high leaf cytokinin content was observed in DR compared with that in WR. Therefore, the absence of DMPP addition to soil increased the leaf cytokinin content mainly by enhancing its delivery rate from roots to leaves.

Without DMPP, NO_3^- is released from soil via nitrification. Soil NO_3^- directly induces roots to synthesise cytokinins in various plants (Criado *et al.* 2009; Lu *et al.* 2009). In our study, similar soil nitrification rates and soil NO_3^- contents between WT and DT during the rewatering period showed the close amount of NO_3^- released from soil and the close amount of NO_3^- being in soil. Therefore, their roots had similar chances to approach NO_3^- in this case. However, the leaf cytokinin content in DT was higher than that in WT during the rewatering period. A previous study found that high root absorption in rewatering corns causes them to have more chances to approach NO_3^- when NO_3^- is added to the roots of corn planted in sand; as a result, large amounts of cytokinin are synthesised in the roots and delivered to the leaves to promote the CGPDR (Wang *et al.* 2016, 2018). In the present study, during the rewatering period, the high root absorption in DT promoted its roots to deliver more cytokinins to the leaves than it did in WT, so the CGPDR was promoted.

Although the soil NO_3^- content in DR was high, the similar cytokinin concentration of xylem sap, T_r and xylem sap quantity in DR and WR showed that the same amounts of cytokinins were synthesised and delivered to the leaves by the roots during the rewatering period. This phenomenon might occur because adding DMPP to soil decreased the soil nitrification rate, resulting in less NO_3^- released from the soil. This caused less stimulations of NO_3^- on roots; as a result, the roots synthesising cytokinins were inhibited, and the delivery of these substances to the leaves was retained in WR and DR. Under this condition, the high root absorption in DR did not increase its root-induced leaf cytokinin compared with that in WR. CGPDR was also retained. Thus, in comparison with soil NO_3^-, soil nitrification was a relatively reliable soil environmental factor that influenced root-induced leaf cytokinin.

The soil NO_3^- concentration was similar between DT and DR during the rewatering period. Although the cytokinin concentration of the xylem sap in DR and DT was similar, high T_r and xylem sap quantity in DT showed that more cytokinins were delivered to the leaves compared with those in DR. This finding could be attributed to the high soil nitrification rate in DT. This further indicated that in the

present study soil NO_3^- weakly affected root-induced leaf cytokinin, and soil nitrification was the key soil environmental factor regulating it. Under drought stress, the soil nitrification rate in DT was lower than that in WT, but rewatering changed this result. The soil nitrification rates were similar in the two treatments during the rewatering period. The increased soil nitrification rate was beneficial to root-induced leaf cytokinin. Thus, rewatering was a key factor influencing soil nitrification.

The soil NO_3^- concentration was lower in WR than in WT during the rewatering period because DMPP retained soil nitrification. However, the soil NO_3^- concentration was similar between DT and DR during the rewatering period. This finding could be mainly due to rapid growth after rewatering using a large amount of soil NO_3^- in DT, and in the same time slow growth caused by drought stress and DMPP added reduced the use of NO_3^- in soil in DR. The high root carbohydrate concentration during the rewatering period played a role in enhancing the root absorption of mineral nutrition and NO_3^- in DT. The root absorption of mineral nutrients required energy. With sufficient organic substances and adequate water, roots could increase their rate of mineral nutrient and NO_3^- absorption.

Conclusion

Corns without DMPP added to soil encountered CGPDR. In the absence of DMPP in soil, rewatering increased the leaf cytokinin content and its delivery rate from roots to leaves, and leaf cytokinin improved the growth of corn after being rewatered. Without DMPP in soil, soil nitrification increased the ability of rewatered corn to synthesise cytokinin and deliver it from roots to leaves. Therefore, soil nitrification is a key soil environmental factor that influences the CGPDR of corn.

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