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SHORT COMMUNICATION

Nod factor supply under water stress conditions modulates cytokinin biosynthesis and enhances nodule formation and N nutrition in soybean

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ABSTRACT

Nod factors (NF) are molecules produced by rhizobia which are involved in the N_2-fixing symbiosis with legume plants, enabling the formation of specific organs called nodules. Under drought conditions, nitrogen acquisition by N_2-fixation is depressed, resulting in low legume productivity. In this study, we evaluated the effects of NF supply on nitrogen acquisition and on cytokinin biosynthesis of soybean plants grown under drought. NF supply to water stressed soybeans increased the CK content of all organs. The profile of CK metabolites also shifted from t-Z to cis-Z and an accumulation of nucleotide and glucoside conjugates. The changes in CK coincided with enhanced nodule formation with sustained nodule specific activity, which ultimately increased the total nitrogen fixed by the plant.

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In legumes, lipo-chitooligosaccharides secreted by rhizobia, also called nodulation factors (NFs), are signals involved in the establishment of the symbiosis enabling the initiation of dinitrogen-fixing nodules.[1] These molecules induce a cascade of responses within cells of the host plant such as calcium spiking, root hair curling, formation of pre-infection threads and nodule morphogenesis.[2-4] Several categories of NFs can be synthesized by rhizobia, which can structurally differ in chitin chain, fatty acylation, acetylation, etc. enabling specific plant host recognition and biological activity.[5-7] Previous works demonstrated that an exogenous supply in NFs could enhance seed germination and plant growth of a large range of crop species including Brassica napus, Zea mays, Gossypium hirsutum, Beta vulgaris and Glycine max.[8,9] NF signaling seems to interact with programs controlled by phytohormones,[10] in particular with cytokinins (CK) as NF recognition in plant epidermal cells promotes CK signaling in the cortex, most likely by increasing CK levels.[11] Evidence that CK are a key differentiation signal for nodule organogenesis[12] are numerous, although the underlying mechanisms still need to be further investigated.[13]

Symbiotic N_2-fixing activity depends on 2 components, namely structural components such as total nodule biomass (specifically the number of nodules and the average biomass of one nodule) and functional components such as nodule-specific activity (g of fixed N_2 per g of nodule and per day). In previous works, it has been demonstrated that a water stress occurring during the vegetative development of soybean led to a strong decrease in plant N acquisition via N_2-fixation due to both a decrease of nodule biomass and a decrease of nodule specific activity.[13-15] This study investigated how NF application impacted nitrogen (N) acquisition of soybean plants for which N_2-fixation had been depressed by a water stress. In order to evaluate the effect of an exogenous supply of NF to soybean (*Glycine max* L. Merr. cv. OAC Champion) in symbiosis with N_2-fixing rhizobia (*Bradyrhizobium japonicum* strain 532C), we followed the protocol previously described in Prudent et al.[13] for plant growth and water stress imposition and treated soybean plants by adding 10–7 M nodulation factor Nod Bj (C18:1, MeFuc) isolated from Bradyrhizobium japonicum strain 532C[16] to the nutrient solution, as soon as the water stress began. Plant N acquisition was based on N_2 fixation only, via the use of an inert substrate and N-free nutrient solution. After 16 d of treatment, NF application under water stress increased the amount of N symbiotically fixed by plant by 25% (Fig. 1) over that of the water stressed plants. Because the lowered nodule specific activity observed for water stressed plant was not increased by NF application, their increase in total N resulted from an intensification of nodule initiation as NF application increased the number of nodules by 71% and the nodule biomass by 40%. Because roots are potential sites for nodule initiation, we observed some traits of the root system, however, NF did not impact root length or the number of root tips (Fig. 1).

Given that CK signaling plays a key role in nodule organogenesis,[12] and in lateral root formation,[17,18] we decided to identify which, if any, steps of CK biosynthesis were impacted by NF, by quantifying various forms of CK at 5, 10 and 16 d after the beginning of the treatments, following the protocol described by others.[19-21] Total CK content was decreased by drought in all tissues, although higher in nodules than in roots, which is in agreement with studies in soybean[22] or in other species.[23-25] When NF was

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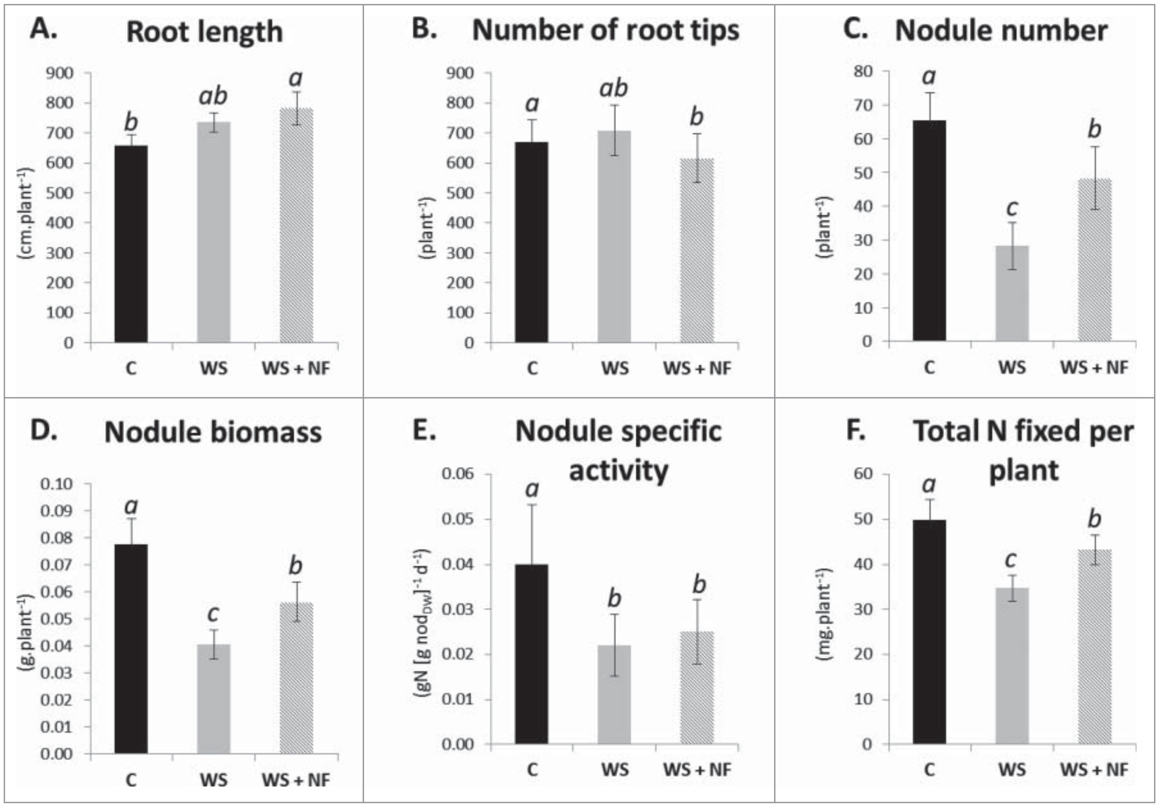

Figure 1. Nodulated root phenotypic traits of soybean plants grown for 15 d either under well-watered conditions (C), or subjected to a water deficit with (WS+NF) or without (WS) NF supply in the nutrient solution. Phenotypic traits comprised: root length (A), number of root tips (B), nodule number (C), nodule biomass (D), nodule specific activity (E), and the total amount of N symbiotically fixed per plant (F). Error bars represent standard deviation (n = 10), and differences among treatments significant at the 0.05 probability level, are denoted by different letters.

applied under water stress conditions, CK content increased as soon as 5 d after the treatment in all plant organs (leaves, roots and nodules) till the end of the experiment (Fig. 2). In the case of leaves, the increase in total CK was significantly above the WS plants but not recovered to the level of unstressed controls. In roots the recovery to control CK levels was complete and in nodules NF treatment actually enabled the CK levels to surpass those of unstressed control plants. These results suggest that following NF application to the root system under drought conditions, a systemic signal within plant allowed a stimulation of CK biosynthesis thus increasing CK content in all plant organs, but particularly effective in roots and nodules.

Then, the abundance of each CK derivative was measured (Table 1) and it appeared that in addition to increasing the total CK content, the NF treatment also significantly altered the individual CK metabolite profiles (Fig. 2). The most clear effect of NF treatment to WS plants was the accumulation of glucoside (i.e. (trans)(OG)[9R]Z and (OG)DHZ) and nucleotide ((trans)[9RMP]Z) conjugates and the production of greater levels of (cis)Z. Interestingly the latter coincided with a decrease in (trans) CK forms (i.e (trans)Z and (trans)[9R]Z (Fig. 2), suggesting that the profile of CK metabolites shifted from t-Z to cis-Z. The increase in cis-CK following NF application in roots and nodules is particularly interesting as the role of cis-CK has been hypothesized to maintain a minimal CK activity under growth-limiting conditions in order to enable the plant to consume its energy for more vital processes.[26]

Taken together, these results highlight that NF application under drought conditions increased CK production in soybeans, and enabled the plant to initiate more nodules, thus enhancing plant N acquisition. Interestingly, Muños et al.[27] demonstrated that under stress conditions, the N_2-fixing B. japonicum USDA 138 produces NF with altered biological activities, suggesting that the addition of exogenous NF could compensate the natural decrease of efficient NF under drought. This NF supply to the plant was a way to manipulate plant hormonal status, in particular CK, whose content was increased. In the literature, increases in CK content (obtained by over expression of IPT genes encoding an enzyme involved in CK biosynthesis pathway) led to a higher tolerance to water stress[28] as plants had higher photorespiration,[23,29] higher stomatal conductance, higher transpiration,[30] better osmotic adjustment by accumulation of amino acids and carbohydrates[28,31] and enhanced root growth and root viability.[32]

In this study, soybean N acquisition was enhanced by NF supply under drought conditions, but further studies are still needed in order to estimate whether the use of NF as plant growth regulator under fluctuating environment could enhance the overall plant productivity in the field.

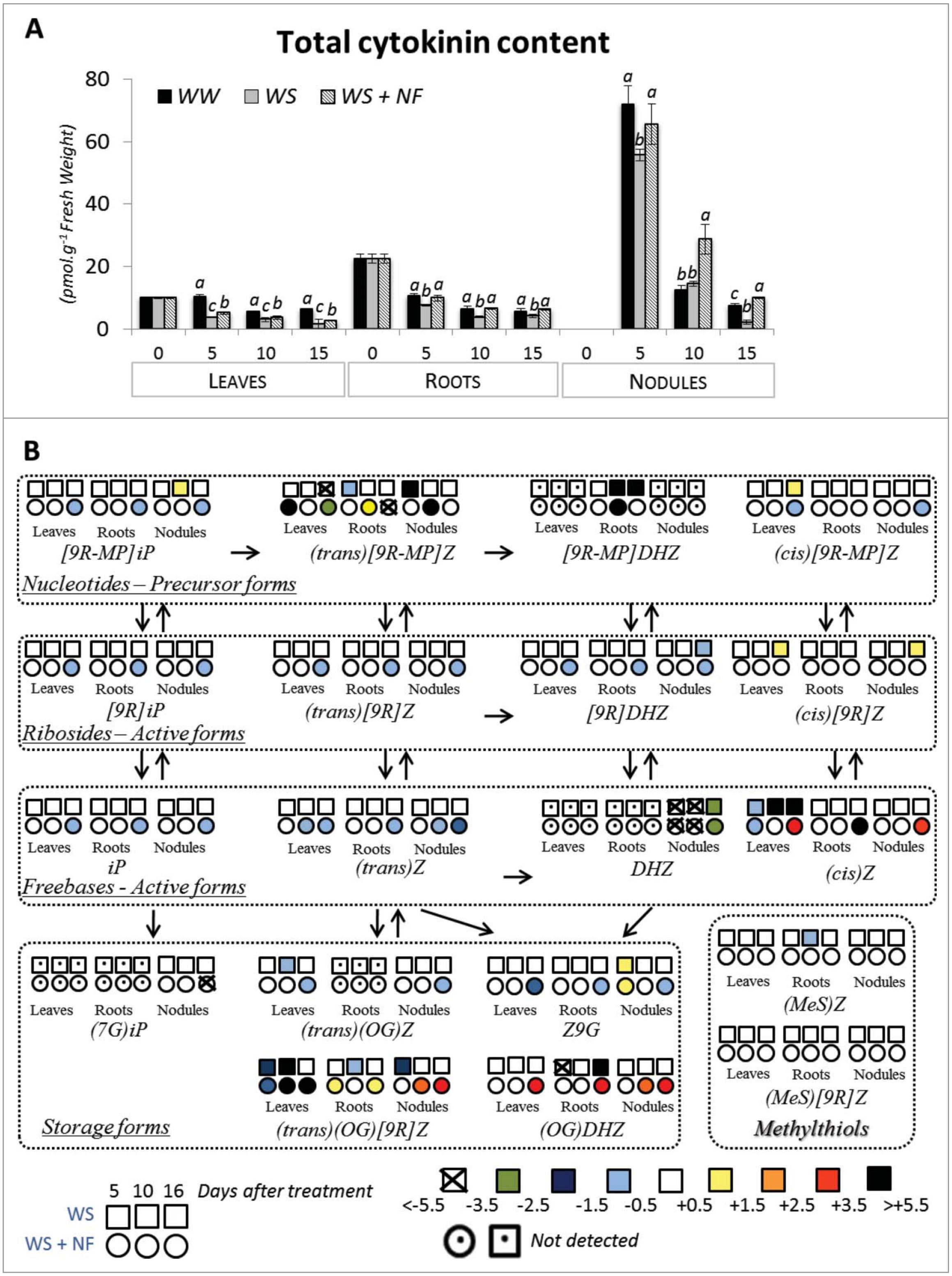

Figure 2. Effect of NF application after 5, 10, 15 d of treatment on total cytokinin content (A) of leaves, roots and nodules, and the relative abundance of each CK form (B) of plants grown for 5, 10, 15 d either under well-watered conditions (C), or subjected to a water deficit with (WS+NF) or without (WS) NF supply in the nutrient solution. Error bars represent standard deviation ($n = 3$ pools of 3 plants), and differences among treatments significant at the 0.05 probability level, are denoted by different letters. The colors indicate the extent of abundance modification when compared to the control plants, expressed as log (WS/C) for WS treatment and expressed as log(WS+NF/C) for WS+NF treatment. As such, a negative value means that CK form is more abundant in the control plants.

Table 1. Abundance of cytokinin derivatives measured in leaves, roots and nodules of well-watered control plants grown for 5, 10 and 16 d. Data are means and standard deviations (s.d) calculated on 3 pools of 3 plants and are expressed in pmol per g of fresh weight.

Days after treatment		0		5		10		16	
Leaves		mean	s.d	mean	s.d	mean	s.d	mean	s.d
	iP	0.161	3.0E-02	0.057	4.5E-03	0.037	2.9E-03	0.030	3.8E-03
	[9R]iP	0.847	6.4E-02	0.739	1.1E-01	0.716	1.3E-01	0.335	5.7E-02
	[9R-MP]iP	1.050	8.7E-02	0.280	4.1E-02	0.134	2.9E-02	0.141	2.6E-02
	(trans)Z	0.369	2.0E-02	0.379	4.8E-02	0.249	1.5E-02	0.496	8.1E-02
	(trans)[9R]Z	0.111	7.0E-03	0.072	8.4E-03	0.032	6.4E-03	0.034	2.7E-03
	(trans)[9R-MP]Z	nd		nd		0.017	1.3E-03	0.033	2.3E-03
	(cis)Z	0.234	4.7E-02	1.984	2.2E-01	nd		nd	
	(cis)[9R]Z	0.241	3.4E-02	0.097	7.6E-03	0.148	3.8E-02	0.079	4.7E-03
	(cis)[9R-MP]Z	0.483	8.4E-02	0.284	4.3E-02	0.402	4.7E-02	0.285	2.5E-03
	DHZ	nd		nd		nd		nd	
	[9R]DHZ	0.037	4.6E-03	0.024	3.6E-03	0.014	4.6E-03	0.010	2.5E-03
	[9R-MP]DHZ	nd		nd		nd		nd	
	(trans)(OG)Z	0.134	2.5E-02	0.143	2.5E-02	0.065	5.6E-03	0.069	2.5E-03
	(OG)[9R]Z	3.684	5.9E-01	4.366	3.1E-01	nd		nd	
	(cis)(OG)Z	nd		nd		nd		nd	
	(OG)DHZ	0.242	3.5E-02	0.246	3.1E-02	0.135	3.3E-02	0.152	2.5E-02
	(OG)[9R]DHZ	nd		nd		nd		nd	
	[Z9G]	0.116	2.6E-02	0.052	3.4E-03	0.053	9.2E-04	0.063	4.9E-03
	(7G)iP	nd		nd		nd		nd	
	(MeS)Z	1.622	2.8E-01	1.353	2.6E-01	0.823	7.4E-02	0.699	6.5E-02
	(MeS)[9R]Z	0.663	4.2E-02	0.210	3.0E-02	0.170	2.9E-02	0.136	9.5E-03
	(MeS)iP	nd		nd		nd		nd	
	(MeS)[9R]iP	nd		nd		nd		nd	
	BA	nd		nd		nd		nd	
	[9R]BA	nd		nd		nd		nd	
Roots									
	iP	0.787	4.9E-02	0.160	1.5E-02	0.160	1.9E-02	0.132	2.0E-02
	[9R]iP	0.979	1.9E-01	0.661	4.8E-02	1.504	4.1E-01	0.585	6.2E-02
	[9R-MP]iP	2.837	5.2E-01	0.782	5.5E-02	0.574	5.1E-02	0.636	3.1E-02
	(trans)Z	0.572	2.4E-02	0.533	4.4E-02	0.311	3.8E-02	0.198	1.8E-02
	(trans)[9R]Z	0.232	2.3E-02	0.284	4.7E-02	0.111	3.0E-02	0.144	1.9E-02
	(trans)[9R-MP]Z	1.392	1.7E-01	1.736	1.0E-01	0.153	4.0E-02	0.107	1.5E-02
	(cis)Z	nd		nd		nd		nd	
	(cis)[9R]Z	0.690	5.1E-02	0.470	4.1E-02	0.619	8.9E-02	0.489	2.4E-02
	(cis)[9R-MP]Z	4.669	2.3E-01	1.623	8.6E-02	1.145	2.6E-01	0.415	5.8E-02
	DHZ	nd		nd		nd		nd	
	[9R]DHZ	0.080	3.2E-03	0.096	1.1E-02	0.039	7.7E-03	0.025	1.0E-03
	[9R-MP]DHZ	nd		nd		nd		nd	
	(trans)(OG)Z	nd		nd		nd		nd	
	(OG)[9R]Z	0.989	2.2E-02	0.157	2.5E-02	0.163	1.0E-02	0.122	2.8E-02
	(cis)(OG)Z	nd		nd		nd		nd	
	(OG)DHZ	0.147	3.2E-02	0.076	1.1E-02	nd		nd	
	(OG)[9R]DHZ	nd		nd		nd		nd	
	[Z9G]	0.038	2.9E-03	0.075	2.1E-03	0.067	5.2E-03	0.086	5.4E-03
	(7G)iP	nd		nd		nd		nd	
	(MeS)Z	8.547	7.6E-01	3.162	7.2E-01	1.283	9.1E-01	2.698	6.7E-01
	(MeS)[9R]Z	0.435	5.9E-02	0.193	2.6E-02	0.181	1.9E-02	0.157	3.0E-02
	(MeS)iP	nd		nd		nd		nd	
	(MeS)[9R]iP	nd		nd		nd		nd	
	BA	nd		nd		nd		nd	
	[9R]BA	nd		nd		nd		nd	

Days after treatment			5		10		16	
Nodules		0	mean	s.d	mean	s.d	mean	s.d
	iP		1.325	2.6E-01	0.142	2.2E-02	0.097	3.2E-03
	[9R]iP		5.794	6.6E-01	1.383	1.2E-01	0.623	2.4E-02
	[9R-MP]iP		10.373	2.6E+00	0.485	2.6E-02	0.165	1.8E-02
	(trans)Z		1.849	4.1E-01	4.083	3.8E-01	1.122	1.4E-01
	(trans)[9R]Z		0.713	4.7E-02	0.065	5.1E-03	0.110	1.5E-02
	(trans)[9R-MP]Z		nd		nd		nd	
	(cis)Z		nd		2.756	1.7E+00	1.853	4.0E-01
	(cis)[9R]Z		1.752	2.8E-01	0.412	1.0E-02	0.136	2.5E-02
	(cis)[9R-MP]Z		7.606	7.1E-01	0.607	1.9E-01	1.093	7.0E-02
	DHZ		1.564	1.6E-01	0.094	7.9E-03	0.030	2.4E-03
	[9R]DHZ		0.220	5.7E-02	0.052	7.7E-03	0.058	4.3E-03

(*Continued on next page*)

Table 1. (Continued).

Days after treatment Nodules		0	5 mean	5 s.d	10 mean	10 s.d	16 mean	16 s.d
	[9R-MP]DHZ		0.553	1.1E-01	nd		nd	
	(trans)(OG)Z		nd		0.380	1.5E-02	0.234	3.3E-02
	(OG)[9R]Z		22.800	2.7E+00	0.078	1.9E-03	0.158	1.8E-02
	(cis)(OG)Z		nd		nd		nd	
	(OG)DHZ		nd		0.063	5.9E-03	0.089	4.4E-03
	(OG)[9R]DHZ		nd		nd		nd	
	[Z9G]		0.068	7.6E-02	0.156	1.3E-02	0.478	3.8E-02
	(7G)iP		nd	nd	0.183	4.6E-02	0.058	3.7E-03
	(MeS)Z		17.872	4.9E+00	1.413	1.8E-01	1.407	8.5E-02
	(MeS)[9R]Z		nd		0.124	7.8E-03	0.120	1.2E-02
	(MeS)iP		nd		nd		nd	
	(MeS)[9R]iP		nd		nd		nd	
	BA		nd		nd		nd	
	[9R]BA		nd		nd		nd	

Disclosure of potential conflicts of interest

No potential conflict of interest was disclosed.

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