Journal of Plant Physiology 193 (2016) 57–63

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

Graphene oxide modulates root growth of *Brassica napus* L. and regulates ABA and IAA concentration

Fan Cheng[a,1], Yu-Feng Liu[a,1], Guang-Yuan Lu[b], Xue-Kun Zhang[b], Ling-Li Xie[a], Cheng-Fei Yuan[a], Ben-Bo Xu[a,*]

[a] College of Life Science, Yangtze University, Jingzhou 434025, China
[b] Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences/Key Laboratory of Biology and Genetic Improvement of Oil Crops, Ministry of Agriculture, Wuhan 430062, China

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 28 January 2016
Accepted 5 February 2016
Available online 3 March 2016

Keywords:
ABA
Graphene oxide
IAA
Root growth
Transcript abundance

ABSTRACT

Researchers have proven that nanomaterials have a significant effect on plant growth and development. To better understand the effects of nanomaterials on plants, Zhongshuang 11 was treated with different concentrations of graphene oxide. The results indicated that 25–100 mg/l graphene oxide treatment resulted in shorter seminal root length compared with the control samples. The fresh root weight decreased when treated with 50–100 mg/l graphene oxide. The graphene oxide treatment had no significant effect on the Malondialdehyde (MDA) content. Treatment with 50 mg/l graphene oxide increased the transcript abundance of genes involved in ABA biosynthesis (*NCED*, *AAO*, and *ZEP*) and some genes involved in IAA biosynthesis (*ARF2*, *ARF8*, *IAA2*, and *IAA3*), but inhibited the transcript levels of *IAA4* and *IAA7*. The graphene oxide treatment also resulted in a higher ABA content, but a lower IAA content compared with the control samples. The results indicated that graphene oxide modulated the root growth of *Brassica napus* L. and affected ABA and IAA biosynthesis and concentration.

1. Introduction

Since the discovery of graphene in 2004 (Geim and Novoselov, 2007), increasing attention has been placed on exploring its physical properties (Service, 2009). Nanotechnology has been increasingly applied to new materials, medicines, energy, electronics, and environmental protection (Kim et al., 2013; Nair et al., 2012; Sharma, 2014; Zhang et al., 2012). The application of nanomaterial biosensors in photo-electronics has increased in the last few years because of their relatively high sensitivity (Germarie et al., 2009; Holzinger et al., 2009). Nanomaterials are currently successfully applied in medicine and medical devices, and novel nano-drug delivery systems have been demonstrated to be more effective and convenient than common materials (Li et al., 2009; Sharma et al., 2009).

However, limited research has been conducted on the application of graphene in agriculture. Considering that plants are primary producers in ecosystems, understanding the cross-talk between nanomaterials and plants is important to manipulate the effect of the nanomaterials on ecosystems. It is therefore essential to understand the ecological risk and health significance of the graphene material before using it in agriculture. Many researchers have demonstrated that nanomaterials can be released into the environment. However, some researchers have reported the potential risks associated with the use of nanomaterials. Some reports have been published on the effects of nanomaterials in biology (Shen et al., 2009; Xue et al., 2000), while others have proven that nanoparticles can potentially enter cells and accumulate in tissues, resulting in damaged cells and tissue lesions (Liu et al., 2009; Tang et al., 2009).

The application of nanomaterials in agriculture started in 2007 and since then, much attention has been paid to this field (Lin and Xing, 2007; Liu et al., 2015; Mushtaq, 2011). For example, researchers (Khodakovskaya et al., 2009) reported that nanotubes penetrated the husk of tomato seeds and greatly increased the rate of seed germination. Engineered nanomaterials are constantly being deployed in agricultural fields with unknown impacts on crop species (Bandyopadhyay et al., 2013; Stampoulis et al., 2009). Lin

Abbreviations: AAO, abscisic acid aldehyde oxidase; ABA, abscisic acid; ARF, auxin response factor; IAA, indole-3-acetic acid; GA, gibberellin; MDA, malondialdehyde; NCED, 9-*cis*-epoxycarotenoid dioxygenase; ZEP, zeaxanthin epoxidase.

* Corresponding author. Fax: +86 716 8066257.
E-mail address: benboxu@yangtzeu.edu.cn (B.-B. Xu).

[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.jplph.2016.02.011
0176-1617/

Table 1
Primers for real time PCR.

Gene name	Primers	Sequence	Annealing temperature (°C)
ACT	ACTF	5′-CTGGAATTGCTGACCGTATGAG-3′	58
	ACTR	5′-ATCTGTTGGAAAGTGCTGAGGG-3′	
NCED	*NCEDF*	5′-GTCGCGTCAACCTCCAAGCT-3′	56
	NCEDR	5′-TCTGTTTCTCCCCGGAGAGG-3′	
AAO	*AAOF*	5′-CAACAGTGGACACCACAAGAAC-3′	56
	AAOR	5′-CAACAGTGGACACCACAAGAAC-3′	
ZEP	ZEPF	5′-CAACAGTGGACACCACAAGAAC-3′	56
	ZEPR	5′-CAACAGTGGACACCACAAGAAC-3	
IAA2	IAA2F	5′-CAACAGTGGACACCACAAGAAC-3′	56
	IAA2R	5′-CAACAGTGGACACCACAAGAAC-3′	
IAA3	IAA3F	5′-CAACAGTGGACACCACAAGAAC-3′	56
	IAA3R	5′-CAACAGTGGACACCACAAGAAC-3′	
IAA4	IAA4F	5′-CAACAGTGGACACCACAAGAAC-3′	56
	IAA4R	5′-CAACAGTGGACACCACAAGAAC-3′	
IAA7	IAA7F	5′-CAACAGTGGACACCACAAGAAC-3′	56
	IAA7R	5′-CAACAGTGGACACCACAAGAAC-3′	
ARF2	ARF2F	5′-CAACAGTGGACACCACAAGAAC-3′	56
	ARF2R	5′-CAACAGTGGACACCACAAGAAC-3′	
ARF8	ARF8F	5′-CAACAGTGGACACCACAAGAAC-3′	55
	ARF8R	5′-CAACAGTGGACACCACAAGAAC-3′	

and Xing (2007) found that nanoparticles significantly inhibited seed germination and root growth. Graphene oxide was also found to decrease biomass and root number and increase oxidative stress, all thought to be regulated by its metabolism (Hu et al., 2014).

To better understand the effects of graphene on plants, rape seedlings were used as study materials in this study. The rape seedlings were treated with different concentrations of graphene and as a result, we found that 25–100 mg/l of graphene had a significant effect on root growth. These findings motivated us to study the underlying mechanisms.

2. Materials and methods

2.1. Materials

All experiments were performed on cultivated "Zhongshuang 11" from the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences. Rapeseeds were germinated on wet sterilized filter paper in 80-cm petri dishes in a growth chamber at 25 ± 1 °C, with a photoperiod of 24 h in the dark.

2.2. Effects of graphene on the growth of rape roots

Seedlings (8 days old), with uniform growth, were chosen for the experiments. The seedlings were placed on sterilized sponges in a plastic bowl in a growth chamber at 25 ± 1 °C, with a photoperiod of 16 h of light and 8 h of dark. The sponges were soaked in distilled water or graphene oxide to expose the seedlings to different stress treatments (0, 5, 10, 25, 50 and 100 mg/l graphene oxide). The seedlings were harvested for measurements after treatment.

2.3. Determination of root length and fresh weight

At least three seedlings were randomly selected from those that received the graphene oxide treatment for 15 days. Maximum root length was measured using a ruler, and root length (cm) was defined as the length from the root tip to the base of root.

The seedling roots were cut at the base and weighed to determine the fresh root weight.

2.4. Determination of the MDA content

MDA was measured by reaction with 2-thiobarbituric acid according to a previously reported method (Cakmak and Marschner, 1992). Briefly, 0.3 g of fresh material from each seedling was homogenized in 3 ml of 50 mM PBS (pH 7.8) containing 0.2 M EDTA and the homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C. Then, 0.5 ml of supernatant was added to 2 ml of 10% (w/v) TCA and 2 ml of 0.5% (w/v) TBA (2-thiobarbituric acid). The mixture was heated in a water bath shaker at 95 °C for 30 min and then rapidly cooled in an ice-bath. The absorbance was measured at 532 nm after centrifugation at 5000 × *g* for 10 min, and the value for non-specific absorption at 600 nm was measured using a spectrophotometer. The concentration of MDA was calculated from the absorbance at 532 nm (correction was performed for unspecific turbidity by subtracting the absorbance at 600 nm).

2.5. Determination of transcript abundance

Total RNA was extracted from seedling or root samples using Trizol (Invitrogen, US) and treated with RNase-free DNase I (Sangon Biotech, China) to remove contaminating genomic DNA. First-strand complementary DNA (cDNA) was synthesized according to the Superscript Reverse Transcriptase manual (Invitrogen, US).

Quantitative real-time PCR was performed with a 7300 Real-Time PCR System (Applied Biosystems) using SYBR Green SuperReal qPCR PreMix (Tiangen, China). The β-*Actin* gene was used as the internal control to normalize the sample variance. Relative quantification of the transcript abundance of each gene was performed using the Livak method (Livak and Schmittgen, 2001). The primers used for determining transcript abundance are listed in Table 1.

2.6. IAA and ABA quantification

A 0.1-g sample was ground using a chilled mortar and liquid N_2, and transferred to a tip containing 1.2 ml cold 80% methanol and 10 mg/l butylated hydroxytoluene. The pellet was incubated in ice for 30 min, centrifuged for 5 min at 1000 × *g* and the supernatant was collected. The pellet was extracted twice with 0.5 ml cold 80% methanol and 10 mg/l butylated hydroxytoluene. All of the supernatant was transferred to a 4 seppak C_{18} column (Millipore Waters, USA) and eluted with 80% methanol. All elute was collected and evaporated under vacuum at 45 °C. The dry sample was analyzed according to the manuals of the Phytodeted IAA Test Kit and the Phytodeted ABA Test Kit (Agdia, France).