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Silver nanoparticles with different concentrations and particle sizes affect the functional traits of wheat

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Abstract

The response of functional traits of plants to external environment can influence their competitive ability because these functional traits are required for the acquisition of resources. The overuse of silver nanoparticles (AgNPs) has gained attention due to their environmental toxicity. This study aimed to examine the effects of AgNPs with different concentrations and particle sizes on functional traits of wheat. It was observed that AgNPs significantly reduced the plant height and so decrease its competitive ability. Ag ions decreased leaf chlorophyll and nitrogen content and specific leaf area more than AgNPs, but the opposite was true for leaf length, single leaf fresh mass, and shoot fresh mass. Hence, the toxicity of AgNPs may be higher than that of Ag ions in some cases. In this study, leaf chlorophyll and nitrogen content decreased with increasing concentration of AgNPs (with size 30 nm). The AgNPs with smaller particle size exerted higher toxicity on leaf chlorophyll and N content than those with larger particle size at the same concentration. However, AgNPs with larger particle size reduced more aboveground fresh mass than those with smaller particle size at the same concentration.

Additional key words: Ag ions, chlorophyll, competitive ability, nitrogen, resource acquisition, specific leaf area, toxicity.

Introduction

Plant species needs their functional traits to respond to the variations in environmental conditions (Pietsch *et al.* 2014, Wang *et al.* 2017a,b, Jiang *et al.* 2019). In particular, the response of leaf functional traits to the external environment can help to expand their habitat niches because leaves play a vital role in the acquisition of resources, such as sunlight (Liu *et al.* 2010a, Meng *et al.* 2014, Jiang *et al.* 2019). Thus, the response of leaf functional traits is regarded as an important factor for good growth and development (Vile *et al.* 2005, Wang *et al.* 2017a, b, 2018a-d, Jiang *et al.* 2019). As one of the most important functional traits, specific leaf area (SLA, defined as the leaf area per unit leaf biomass), determines the resource acquisition strategies, such as trade-offs between storage resource and rapid growth (Kardel *et al.* 2010, Scheepens *et al.* 2010, Pietsch *et al.* 2014). In general, high values of SLAs are closely related to greater acquisition

and utilization efficiency for available resources and less investment in leaf structures. Further, low values of SLAs have been found to be strongly related to greater amount of investment in leaf structures and relatively low growth rates (Kardel *et al.* 2010, Scheepens *et al.* 2010, Pietsch *et al.* 2014). In addition, leaf size, leaf shape index, and leaf chlorophyll and nitrogen content are essential leaf functional traits, which are highly correlated to plant resource-utilization strategy (Wang and Zhang 2012, Wang *et al.* 2016, 2017a,b). Moreover, plant height also contributes towards the competitive ability for sunlight acquisition (Gross *et al.* 2007, Thomson *et al.* 2011).

Presently, one of the important environmental issues is the extensive use of nanomaterials. Their excellent physicochemical properties and the correspondingly environmental activities are attributed to the small particle size, which usually ranges from 1 to 100 nm (Nowack and Bucheli 2007, Pulit-Prociak *et al.* 2015). Among the various nanoparticles, silver nanoparticles (AgNPs)

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Abbreviations: AgNPs - silver nanoparticles; Chl - chlorophyll; SLA - specific leaf area.

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are extensively used due to their unique antimicrobial performance and other physiochemical properties (Pulit-Prociak *et al.* 2015, Batista *et al.* 2017). Global AgNPs production is estimated over 400 tons annually, and 30 % of its production is mainly used in medical applications (Pourzahedi and Eckelman 2014, Batista *et al.* 2017). The widespread and increasing production and use of AgNPs generate the concern of their release into environment (such as farmland, rivers, lakes, *etc.*) due to their toxicity to living organisms (Batista *et al.* 2017, Rahmatpour *et al.* 2017, Schlich *et al.* 2017). In higher plants, they can cause membrane disruption (Farkas *et al.* 2011, Wang *et al.* 2017c), oxidative stress (through Fenton reactions) (Huang *et al.* 2010, Li *et al.* 20017), chromosomal aberrations, DNA mutations, DNA repair inhibition, DNA methylation (Ng *et al.* 2010), cell death (Wan *et al.* 2012), reduction of the growth and development (Jasim *et al.* 2017), seed germination and seedling growth (Yin *et al.* 2012, Vannini *et al.* 2014, Zuverza-Mena *et al.* 2016, Wang *et al.* 2018a), plant transpiration (Gubbins *et al.* 2011, Kim *et al.* 2011), and enzyme activities (McGee *et al.* 2017, Rahmatpour *et al.* 2017).

The objective of this study was to gain insights into the response of several traits (plant height, leaf size, leaf shape index, SLA, leaf chlorophyll content, and leaf N content) of wheat to AgNPs with different concentrations and particle sizes.

Materials and methods

Experimental design: Wheat (*Triticum aestivum* L.) was cultivated in pots (4 seedlings per pot) with humus (pH ~6.5, organic matter ≥ 40 %, electrical conductivity ≤ 3 mS cm^{-1} ; Hongyang Agricultural Science & Technology Co., Huai'an, China). The potted seedlings were then exposed to AgNPs obtained from Shanghai ST-Nano Science & Technology Co., Shanghai, China (purity ≥ 99.8 %) with concentrations: L - 100 mg kg (soil) $^{-1}$, M - 200 mg kg (soil) $^{-1}$, and H - 400 mg kg (soil) $^{-1}$ and particle sizes 30, 50, and 70 nm. Distilled water was used as a control (CK). AgNPs suspensions were continuously stirred for complete distribution to avoid particle polymerization using an ultrasonic generator (KQ-250DE, Kunshan Ultrasonic Instruments, Suzhou, China). The operating parameters were: 40 kHz with a maximum power output of 100 W at room temperature (Cvjetko *et al.* 2017, Rahmatpour *et al.* 2017, Wang *et al.* 2018a). In addition, the potted seedlings were also treated with AgNO₃ solution (purity ≥ 99.8 %, Sinopharm Chemical Reagent Co., Shanghai, China) with concentrations 100, 200, and 400 mg kg (soil) $^{-1}$. Each experiment was performed in triplicates (*i.e.* three pots). The experiments were conducted in a glasshouse at Jiangsu University in Zhenjiang, China (32.206 °N, 119.512 °E) under natural sunlight from February to April, 2017. The site has a north subtropical monsoon humid climate, with an annual mean temperature of ~16.1 °C, annual mean precipitation of ~1 150.6 mm, and mean duration of sunshine ~1 986.9 h. The mean monthly values of the above parameters from February to April were ~10.0 °C,

~73.7 mm, and ~154.0 h, respectively. The summaries of the site climate were obtained from local records (Hang and Wu 2017).

All wheat samples were collected at the end of the cultivation period. Five fully developed, mature, and undamaged leaves from each individual were randomly selected to evaluate their functional traits. All samples were stored in sealed bags and immediately transported back to the laboratory to determine the corresponding indices.

Determination of functional traits: Plant height was estimated by measuring the distance between the base of the stem and apical shoot or tallest leaf with a ruler (Wang *et al.* 2017a,d,e, 2018a-d, 2019, Jiang *et al.* 2019). Leaf shape index was determined using the ratio of the leaf length to the corresponding leaf width (Jeong *et al.* 2011, Wang and Zhang 2012, Li *et al.* 2016). Leaf length (the maximum value along midrib) and leaf width (the maximum width perpendicular to midrib) were evaluated using a ruler.

Relative N content in the leaves were measured using a hand-held plant nutrient meter (TYS-3N, TOP Instrument Co., Hangzhou, China). The chlorophyll (Chl) content was determined in "SPAD units" based on absorbances at 650 nm and 940 nm (Jiang *et al.* 2019).

The shoot and single-leaf fresh and dry masses were estimated using an electronic balance with 0.001 g accuracy; dry mass after drying the samples in oven at 60 °C for 24 h when a constant mass was achieved (Wang *et al.* 2016). SLA was calculated as a ratio of the leaf area to corresponding leaf dry mass (Kardel *et al.* 2010).

Determination of Ag content: Ag content in soil and plant was estimated with acid digestion methods (HNO₃-H₂O₂-HF) using iCAP 6300 inductively-coupled plasma atomic emission spectrometer (ICPAES, Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis: Differences in the values of functional traits of wheat among various treatments were evaluated by analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparisons, when required. Two-way ANOVA was used to assess the effects of different concentration and particle size of AgNPs on the functional traits of wheat. Meanwhile, the partial eta-squared (η^2) values were determined to evaluate the effect of size of each factor for use in the two-way ANOVA. Correlation analysis was performed using Pearson product-moment correlation coefficient to evaluate the relationships between functional traits of wheat and Ag content in plant and soil and $P \leq 0.05$ was considered as statistically significant. All statistical analyses were performed using IBM SPSS statistics (version 22.0, IBM Corp., Armonk, NY, USA).

Results

ANOVA results showed that the concentration of AgNPs significantly affected leaf width, leaf Chl and N content,

and shoot fresh mass of wheat ($P < 0.05$; Table 1 Suppl.). The particle size of AgNPs and also AgNO₃ significantly affected plant height, leaf width, aboveground dry mass, and plant Ag content. The interaction between the concentration and particle size significantly affected plant height, and leaf chlorophyll and N content as well as soil Ag content. Plant height of wheat was significantly reduced under all treatments, when compared to control. Similarly, leaf length was significantly decreased under moderate concentration of AgNPs with size 70 nm compared to control. Further, the leaf width was significantly decreased under low concentration of AgNPs with size 70 nm and

moderate concentration of AgNPs with size 50 or 70 nm compared to control. Leaf Chl content was significantly reduced under low concentration of AgNPs with size 70 nm, moderate concentration of AgNPs with size 50 nm, and high concentration of AgNPs of all particle sizes compared to control ($P < 0.05$; Table 1). Leaf N content was significantly reduced under all treatments (except under low and moderate concentrations of AgNPs with size of 30 nm). Similarly, aboveground fresh mass was found to be significantly lower under low and moderate concentrations of AgNPs with size of 70 nm compared to control ($P < 0.05$; Table 1).

Table 1. Differences in the functional traits of wheat under AgNPs and AgNO₃. Means $\pm$ SEs, $n = 3$, different lowercase letters indicate significant differences ($P < 0.05$) determined with an analysis of variance among groups followed by the Student-Newman-Keuls test for multiple comparisons (ns - no significant difference). Abbreviations: CK - control; AgNP30L - low concentration of AgNPs with size of 30 nm; AgNP30M - moderate concentration of AgNPs with size of 30 nm; AgNP30H - high concentration of AgNPs with size of 30 nm; AgNP50L - low concentration of AgNPs with size of 50 nm; AgNP50M - moderate concentration of AgNPs with size of 50 nm; AgNP50H, high concentration of AgNPs with size of 50 nm; AgNP70L - low concentration of AgNPs with size 70 nm; AgNP70M - moderate concentration of AgNPs with size of 70 nm; AgNP70H - high concentration of AgNPs with size of 70 nm; AgNO₃L - low concentration of AgNO₃; AgNO₃M - moderate concentration of AgNO₃; AgNO₃H - high concentration of AgNO₃; PH - plant height; LL - leaf length; LW - leaf width; LSI - leaf shape index; LCC - leaf chlorophyll content; LNC - leaf N content; SLFM - single leaf fresh weight; SLDM - single leaf dry mass; SLA - specific leaf area; AFM - aboveground fresh mass; ADM - aboveground dry mass.

Treatment	PH	LL	LW	LSI	LCC	LNC	SLFM	SLDM	SLA	AFM	ADM
CK	26.83 $\pm$ 0.62a	22.89 $\pm$ 0.53ab	1.26 $\pm$ 0.01a	18.16 $\pm$ 0.56ns	41.89 $\pm$ 1.15a	3.51 $\pm$ 0.06a	0.37 $\pm$ 0.04ab	0.09 $\pm$ 0.00ns	172.92 $\pm$ 4.83ab	14.60 $\pm$ 0.10a	2.63 $\pm$ 0.02ab
AgNP30L	21.58 $\pm$ 1.21b	21.87 $\pm$ 0.85abc	1.13 $\pm$ 0.01ab	19.40 $\pm$ 0.85ns	40.97 $\pm$ 0.74ab	3.43 $\pm$ 0.06ab	0.33 $\pm$ 0.01ab	0.08 $\pm$ 0.00ns	173.58 $\pm$ 3.70ab	11.23 $\pm$ 0.03abc	2.33 $\pm$ 0.04ab
AgNP30M	21.82 $\pm$ 1.19b	21.15 $\pm$ 0.79abc	1.12 $\pm$ 0.03ab	18.90 $\pm$ 1.13ns	39.57 $\pm$ 1.31abc	3.33 $\pm$ 0.09abc	0.33 $\pm$ 0.04ab	0.08 $\pm$ 0.01ns	207.31 $\pm$ 16.11a	12.60 $\pm$ 1.42ab	2.59 $\pm$ 0.23ab
AgNP30H	20.46 $\pm$ 0.72bc	20.51 $\pm$ 0.56abc	1.14 $\pm$ 0.01ab	18.02 $\pm$ 0.64ns	35.03 $\pm$ 0.77d	3.00 $\pm$ 0.05d	0.36 $\pm$ 0.06ab	0.07 $\pm$ 0.01ns	177.20 $\pm$ 9.18ab	13.30 $\pm$ 0.95ab	2.65 $\pm$ 0.02ab
AgNP50L	18.9 $\pm$ 0.82bc	20.12 $\pm$ 0.18bc	1.11 $\pm$ 0.01ab	18.12 $\pm$ 0.41ns	39.08 $\pm$ 0.78abc	3.29 $\pm$ 0.05bc	0.31 $\pm$ 0.01ab	0.08 $\pm$ 0.00ns	184.03 $\pm$ 11.95ab	12.37 $\pm$ 0.63ab	2.84 $\pm$ 0.22a
AgNP50M	19.4 $\pm$ 0.76bc	21.44 $\pm$ 0.61abc	1.08 $\pm$ 0.01b	19.92 $\pm$ 0.78ns	37.16 $\pm$ 0.10cd	3.17 $\pm$ 0.01cd	0.41 $\pm$ 0.03ab	0.08 $\pm$ 0.01ns	194.02 $\pm$ 7.98ab	11.23 $\pm$ 0.75abc	2.55 $\pm$ 0.14ab
AgNP50H	18.46 $\pm$ 0.15bc	21.29 $\pm$ 0.55abc	1.13 $\pm$ 0.05ab	19.05 $\pm$ 1.34ns	38.58 $\pm$ 0.22bc	3.27 $\pm$ 0.01bc	0.38 $\pm$ 0.04ab	0.08 $\pm$ 0.00ns	185.33 $\pm$ 8.61ab	12.70 $\pm$ 1.76ab	2.58 $\pm$ 0.29ab
AgNP70L	18.96 $\pm$ 0.85bc	20.48 $\pm$ 1.28abc	1.08 $\pm$ 0.04b	19.02 $\pm$ 0.60ns	37.74 $\pm$ 0.51bc	3.21 $\pm$ 0.04bc	0.35 $\pm$ 0.01ab	0.07 $\pm$ 0.01ns	202.44 $\pm$ 10.83a	9.90 $\pm$ 0.21bc	1.99 $\pm$ 0.11ab
AgNP70M	17.40 $\pm$ 0.55bc	19.10 $\pm$ 0.78c	0.98 $\pm$ 0.02b	19.43 $\pm$ 0.81ns	38.87 $\pm$ 0.74abc	3.24 $\pm$ 0.07bc	0.27 $\pm$ 0.01b	0.07 $\pm$ 0.01ns	192.48 $\pm$ 9.98ab	8.40 $\pm$ 0.49c	2.03 $\pm$ 0.06ab
AgNP70H	21.05 $\pm$ 0.25bc	21.78 $\pm$ 0.29abc	1.15 $\pm$ 0.03ab	18.97 $\pm$ 0.57ns	38.69 $\pm$ 0.12bc	3.26 $\pm$ 0.01bc	0.39 $\pm$ 0.02ab	0.07 $\pm$ 0.01ns	176.86 $\pm$ 8.65ab	14.33 $\pm$ 1.46a	2.41 $\pm$ 0.27ab
AgNO ₃ L	19.64 $\pm$ 1.72bc	23.09 $\pm$ 0.34ab	1.12 $\pm$ 0.04ab	20.75 $\pm$ 0.97ns	38.90 $\pm$ 0.32abc	3.29 $\pm$ 0.02bc	0.40 $\pm$ 0.01ab	0.08 $\pm$ 0.00ns	166.65 $\pm$ 9.06ab	13.20 $\pm$ 0.79ab	2.13 $\pm$ 0.19ab
AgNO ₃ M	19.85 $\pm$ 0.50bc	23.64 $\pm$ 0.12a	1.14 $\pm$ 0.07ab	20.92 $\pm$ 1.32ns	38.18 $\pm$ 0.38bc	3.24 $\pm$ 0.02bc	0.42 $\pm$ 0.02a	0.08 $\pm$ 0.00ns	152.29 $\pm$ 10.28b	13.40 $\pm$ 0.42ab	2.19 $\pm$ 0.05ab
AgNO ₃ H	16.83 $\pm$ 1.31c	22.27 $\pm$ 0.73abc	1.11 $\pm$ 0.04ab	20.23 $\pm$ 1.29ns	34.83 $\pm$ 0.63d	3.03 $\pm$ 0.07d	0.34 $\pm$ 0.01ab	0.09 $\pm$ 0.00ns	171.27 $\pm$ 3.23ab	10.43 $\pm$ 0.32abc	1.87 $\pm$ 0.02b

Fig. 1. Differences in Ag content in soil and plants per gramme of dry mass under silver nanoparticles (AgNPs) with different particle sizes. Means $\pm$ SEs, $n=3$, different lowercase letters indicate significant differences ($P < 0.05$) determined by analysis of variance followed by the Student-Newman-Keuls test for multiple comparisons. Abbreviations have the same meanings as described in Table 1.

It was further observed that leaf Chl and N content decreased more under high concentration of AgNO₃ than under high concentration AgNPs with sizes of 50 and 70 nm (Table 1). SLA was more reduced under moderate concentration of AgNO₃ compared to that under moderate concentration of AgNPs with size 30 nm. Similarly, leaf length, single leaf fresh mass, and whole shoot fresh mass were less reduced under moderate concentration of AgNPs with size of 70 nm than under moderate concentration of AgNO₃. No significant differences were observed for all measured traits of wheat between AgNPs and AgNO₃ under low concentration (Table 1).

Leaf Chl and N content decreased with the increasing concentration of AgNPs with size of 30 nm and with increasing concentrations of AgNO₃ (Table 1). Leaf Chl and N content decreased more under high concentration of AgNPs with size 30 nm compared to those under high concentration of AgNPs with size of 50 nm and 70 nm. Shoot fresh mass was more decreased under low and moderate concentrations of AgNPs with size of 70 nm when compared to that under high concentration of AgNPs with the same size (Table 1). No significant difference was

observed for all measured traits of wheat among different concentrations of AgNPs with size of 50 nm ($P > 0.05$; Table 1).

Similarly, aboveground fresh mass was more reduced under moderate concentration of AgNPs with size of 70 nm compared to that under moderate concentration of AgNPs and size of 30 nm. No significant difference was observed among the different particle sizes of AgNPs under low concentration ($P > 0.05$; Table 1).

Further, it was observed that soil Ag content under AgNPs and AgNO₃ treatments was significantly higher when compared to control treatment (Fig. 1A). Similarly, plant Ag content under high concentration of AgNPs with size of 70 nm was significantly higher than that under high concentration of AgNPs with size of 30 nm (Fig. 1B).

No significant correlations were observed between plant Ag content and functional traits of wheat (Table 2). However, soil Ag content was negatively correlated with plant height, leaf width, and leaf Chl and N content (Table 2).

Table 2. A relationship between the functional traits of wheat and Ag concentrations in soil (AgS) and plant (AgP). *P*-values equal to or less than 0.05 are shown in **bold**. Abbreviations have the same meanings as described in Table 1.

		PH	LL	LW	LSI	LCC	LNC	SLFM	SLDM	SLA	AFM	ADM
AgS	r	-0.55	-0.20	-0.38	0.11	-0.32	-0.34	-0.20	-0.28	-0.02	-0.29	-0.22
	<i>P</i>	<0.001	0.2272	0.0171	0.4907	0.0442	0.0351	0.2314	0.0883	0.8893	0.0699	0.1705
AgP	r	0.13	-0.18	0.15	-0.29	-0.26	-0.27	-0.13	-0.24	0.22	0.10	0.11
	<i>P</i>	0.4443	0.2694	0.3482	0.0732	0.1069	0.0989	0.4230	0.1435	0.1720	0.5463	0.4964

Discussion

Previous results showed that AgNPs can reduce the growth and development of many plant species (Lee *et al.* 2012, Yin *et al.* 2012, Zuverza-Mena *et al.* 2016, Wang *et al.* 2018a). In this study, it was observed that AgNPs, with all concentrations and particle sizes, significantly reduced the plant height of wheat. In addition, some treatments of AgNPs significantly reduced leaf length, leaf width, and leaf Chl and N content. Plant height and leaf functional traits play a critical role in the competitive ability and tolerance to environmental conditions (Gross *et al.* 2007, Thomson *et al.* 2011). Thus, the decrease in height and some leaf functional traits of wheat affected by AgNPs might suppress the competitive ability for resource acquisition, particularly sunlight. Thus AgNPs may be the ecological factor impacting the growth and development of plant species (Liu *et al.* 2010a, Meng *et al.* 2014).

Although the mechanisms of toxic effects of AgNPs have not been clearly elucidated, it is generally believed that the primary mechanisms of AgNPs toxicity is related to Ag ions released from AgNPs (Gubbins *et al.* 2011, Zhao and Wang 2012a, Guo *et al.* 2017, Wang *et al.* 2017c). Previous studies have reported that Ag ions highly accumulate in plant roots (Vannini *et al.* 2014, Zuverza-Mena *et al.* 2016). In addition, it has been observed that AgNPs and AgNO₃ significantly increase soil Ag content. The correlation analysis indicated that plant height, leaf width, and leaf Chl and N content of wheat were negatively affected by soil Ag content. Meanwhile, high AgNO₃ concentration decreased leaf Chl and N content of wheat leaves more than high concentration of AgNPs with sizes of 50 and 70 nm. Moreover, moderate AgNO₃ concentration decreased SLA of wheat more than moderate concentration of AgNPs with size of 30 nm. Thus, it was confirmed that Ag ions might be highly toxic to wheat plant. These results were in agreement with previous studies, which confirmed stronger toxic effects of Ag ions compared to AgNPs (Barbasz *et al.* 2016, Wang *et al.* 2017c). Reversed differences in toxic effects between AgNO₃ and AgNPs treatments were also observed in this study. In particular, moderate concentration of AgNPs with size of 70 nm reduced leaf length, single leaf fresh mass, and whole shoot fresh mass of wheat more than AgNPs at the same concentration. The result was consistent with previous studies which confirm stronger toxic effects of AgNPs compared to Ag ions (Yin *et al.* 2011, 2012, Lee *et al.* 2012, Krajcarová *et al.* 2017). Hence, the toxicity of AgNPs cannot be attributed to Ag ions alone, it is highly

likely that AgNPs itself has toxic properties (Lubick 2008, Yin *et al.* 2011, 2012, Jiang *et al.* 2012, Geisler-Lee *et al.* 2013). Further, lower accumulation of Ag ions was observed in treatments with AgNPs compared to AgNO₃ (Cvjetko *et al.* 2017, Krajcarová *et al.* 2017). This phenomenon may be attributed to the mechanisms changing their ability to be absorb by plant roots. Ag ions can form complexes by binding to ligands (such as chloride, phosphate, and/or sulfide) (Xiu *et al.* 2011, Reinsch *et al.* 2012, Yang *et al.* 2014). In addition, Ag ions can be absorbed by colloids and/or humic acid in the soil (Wie *et al.* 2012, Yang *et al.* 2014). Further, AgNPs can be converted to Ag ions and then rapidly transformed to AgNPs in the soil subsystem or under sunlight conditions (Glover *et al.* 2011).

The result of this study showed that leaf Chl and N content of wheat were decreased with increasing concentration of AgNPs with size of 30 nm. Previous studies also demonstrated that the toxic effects of AgNPs are dose-dependent, and higher concentrations usually lead to higher toxicity (Lee *et al.* 2012, Zuverza-Mena *et al.* 2016, Sheng *et al.* 2017, Wang *et al.* 2017c, 2018a). This can be attributed to the fact that AgNPs at higher concentration can release more Ag ions (Gubbins *et al.* 2011, Zhao and Wang, 2012a, Guo *et al.* 2017) and/or initiate higher ROS production (Lee *et al.* 2012, Zuverza-Mena *et al.* 2016, Cvjetko *et al.* 2017). However, some studies have shown positive response of some plant species to AgNPs (Sharma *et al.* 2012, Jasim *et al.* 2017). The plant growth promoting effects of AgNPs may be attributed to its effects on aminocyclopropane-1-carboxylic acid (ACC) content and production of phytohormones (Syu *et al.* 2014). It was also observed that low and moderate concentrations of AgNPs with size of 70 nm exhibit higher positive effects on wheat shoot fresh mass compared to high concentration of AgNPs. This finding may be ascribed to the fact that low and moderate concentrations of AgNPs can address hormesis effects on plant species, and thus promoting their growth (Duke *et al.* 2006, Jasim *et al.* 2017).

Further, the toxic effects of AgNPs were generally increased with decreasing particle size (Matzke *et al.* 2014, McGillicuddy *et al.* 2017). In this study, it was observed that AgNPs with smaller particle size (high concentration of AgNPs with size of 30 nm) exerted higher toxicity on leaf Chl and N content than AgNPs with sizes of 50 and 70 nm at the same concentration. The possible reason was that smaller particle size of AgNPs would upsurge the effective surface areas and dispersity which are related to toxicity (Yuan *et al.* 2013, McGillicuddy *et al.* 2017). Another explanation may be that smaller

particles of AgNPs can release more Ag ions than larger particles (Wang *et al.* 2013, Yuan *et al.* 2013). The result of this study also showed that plant Ag concentration under AgNPs with size of 70 nm was significantly higher than that under AgNPs with size of 30 nm. This may be further attributed to the fact that AgNPs of smaller particle size of can induce more ROS, resulting in higher corresponding toxicity (Choi and Hu 2008, Ma *et al.* 2012). However, previous studies on size-dependent effects of AgNPs obtained varied results. In particular, some studies reported contrasting results that AgNPs with larger particle size were more toxic than those with smaller particle size (Contreras *et al.* 2014, Zhai *et al.* 2016, Batista *et al.* 2017, Wang *et al.* 2018a). This was in agreement with the result of this study showing that AgNPs with larger particle size (moderate concentration of AgNPs with size of 70 nm) exhibited stronger toxicity on wheat shoot fresh mass than AgNPs with smaller particle size at the same concentration (a moderate concentration of AgNPs with size of 30 nm). Nanoparticles with a larger particle size can increase cell wall pore size, which in turn allows the plant roots to absorb a higher amount of substances (Wild and Jones, 2009, Liu *et al.* 2010b). Further, AgNPs with larger particle size can be accumulated for longer duration inside the plant body than those with smaller particle size (Contreras *et al.* 2014) due their less aggregation and higher stability (Zhao and Wang 2012b, Gomes *et al.* 2013, Batista *et al.* 2017). Thus, AgNPs with smaller particle size may not always exhibit a stronger toxicity on the functional traits than that with larger particle size.

In conclusion, the results of this study showed that AgNPs exhibited significant negative effects on the functional traits of wheat, but these effects varied with the functional traits, particle size, and AgNPs concentration. It can be ascertained that extended application of AgNPs to soil samples may result into a change of speciation of AgNPs and to an altered bioavailability (Schlich *et al.* 2017). Thus, more experimental factors will be incorporated in our future research to achieve more precise information about the toxic effects of AgNPs on the growth and development of wheat.

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