Original Research Article

J. Funct. Mater. Biomol. 1(2)-2017 pp 70-75

SACRED HEART RESEARCH PUBLICATIONS

Journal of Functional Materials and Biomolecules

Journal homepage: www.shcpub.edu.in

ISSN: 2456-9429

Effect of ZnO nanostructures in improving seed vigour and viability in blackgram

*D. Jesuvathy Sornalatha, P. Murugakoothan**

Received on 03 May 2017, Accepted on 23 Nov 2017

Abstract

The effect of zinc oxide (ZnO) nanostructures in improving the seed vigour and viability in blackgram (Vigna mungo (L) Hepper) cv. VBN 4 were investigated. Black gram seeds were treated with different ZnO nanostructures each at 750, 1000 mg kg-1 and assessed for physiological characters, namely germination, seedling length and vigour index both immediately and after accelerated ageing. The nano ZnO treated seeds were compared with untreated seeds (control) in order to know the variation. Zinc oxide nanochips at a concentration of 1000 mg have shown significant increase in germination, shoot length, root length and vigor index over other treatments. The reason for these effects is likely to be due to the smaller particle size and higher concentration of zinc in the seed when treated with ZnO nanoparticles. Employing nanosized ZnO in suitable concentration could promote the germination of blackgram seeds and increase the vigour.

Keywords: Nanomaterial; Agriculture; Nanosized ZnO; Seed germination; Vigour index.

1 Introduction

Vigna mungo is also known as blackgram, originated in India, where it has been in cultivation from ancient times and is one of the most highly prized pulses of India. It is cultivated throughout India. Coastal Andhra Pradesh is famous for blackgram next to paddy. Blackgram has also been introduced to other tropical areas mainly by Indian immigrants. It is an erect, suberect or trailing, densely hairy annual herb. The tap root produces a branched root system with smooth, rounded nodules. The pods are narrow, cylindrical and long up to 6 cm. The seeds are rich in protein and starch.

Nanotechnology is a fascinating field of science dealing with atom by atom manipulation that yields, processes and products which are likely to transfer traditional farming into precision agriculture [1]. Nanomaterials have gained increasing attention because of their novel properties, including a large specific surface area and high reaction activity [2, 3]. Due to rapid development of nanotechnology, nanomaterials with various shapes and diameters have been prepared and used in some industrial products and commodities [4–6]. Nanomaterials have also been used for various fundamental and practical applications, such as drug delivery, cell imaging and cancer therapy [7–11]. Nanotechnology permits broad advances in agricultural research, such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful byproducts through enzymatic nanobioprocessing, disease prevention and treatment in plants using various nanocides [12]. The development of nanodevices and nanomaterials could open up novel applications in plant biotechnology and agriculture [13]. Nanoparticles and nanocapsules provide an efficient means to distribute pesticides and fertilizers in a controlled fashion with high site specificity thus reducing collateral damage [14]. Several studies have been undertaken to determine both positive and negative effects of nanoparticles on seed. Some investigations have studied the use of ZnO nanoparticles on some crops such as cucumber, peanuts, sweet basil, cabbage, cauliflower, tomato and chickpea [15-19]. Seed treatments improve the physiological quality of the seeds and protect the seeds from ageing and soil, seed borne infestation of insects and diseases.

In this study, the promotory or inhibitory effects of different concentrations of ZnO nanostructures, prepared by simple sol-gel route, on the growth of blackgram was investigated. In plants, micronutrients are transported by chelators [20]; however, the efficacy is low. Decreased particle size also increases the specific surface area of micronutrients, which could increase the dissolution rate of micronutrients with low solubility in water, such as ZnO [21]. Studies were carried out to assess the effect of ZnO nanostructures in improving the seed vigour and viability in blackgram (Vigna mungo (L) Hepper) cv. VBN 4. Blackgram seeds were treated with two different ZnO nanostructures each at 750, 1000 mg kg-1 and assessed for physiological characters namely germination, seedling length, root length and vigour index both immediately and after accelerated ageing. The nano ZnO treated seeds were compared with control (untreated seeds) in order to study the effect of ZnO treatment.

2 Experimental

2.1. Synthesis of Porous ZnO Nanostructures (Sample code: A)

* Corresponding author: e-mail murugakoothan03@yahoo.co.in,
Phone: +91 9444447586,
MRDL, PG & Research Department of Physics, Pachaiyappa's College, Chennai 600030, India.

Porous ZnO nanostructures were prepared by the decomposition of Zinc oxalate. Zinc oxalate was prepared by mixing equimolar (0.2 M) solutions of zinc acetate and oxalic acid. The mixture was continuously stirred for 1 hour at room temperature. The resultant white coloured solution was rinsed with double deionized water several times to free off the acetate ions. It was then centrifuged and the resulting white gel precipitate was heated at 80 °C for 3 hours. After that the precipitate was dried in air and calcined at 600 °C in air for 1 hour.

2.2. Synthesis of ZnO Nanochips (Sample code: B)

In a typical experiment, 0.9 g of $ZnCl_2$ was dissolved in 100 mL deionized water to form an aqueous solution of $ZnCl_2$. Similarly, 1.4 g of NaOH was dissolved in 20 mL deionized water to form the aqueous solution of NaOH. Later, the aqueous solution of NaOH was added to the aqueous solution of $ZnCl_2$ drop wise under continuous stirring for 1 hour. A milky white solution was obtained and the mixture was then heated for 3 hours at 90 °C. The resulting gel suspension was centrifuged to retrieve the precipitate and the precipitate was washed with distilled water and absolute ethanol. The precipitate was then dried in air and calcined at 600 °C in air for 1 hour.

The structural and phase formation of the synthesized ZnO samples were identified by Reich Seifert XRD 3003 diffractometer using Cu-Kα (λ=1.5406 Å) radiation. The morphology and size of the ZnO nanoparticles were evaluated by SEM, FEI-Quanta 250 and TEM, FEI-Technai Sprit.

2.3. Seed Treatment and Tests

Blackgram seeds were dry dressed with ZnO nanoparticles at 750 and 1000 mg kg^{-1} in screw capped glass bottles at room temperature. The glass bottles containing seed and nanoparticles were shaken gently for 3 min., 5 times at an interval of 3 hours. Seeds shaken without nanoparticles served as control.

2.3.1. Accelerated Ageing of Seeds

The ZnO nanoparticles treated black gram seeds were placed in butter papper bags and subjected to a relative humidity (RH) of 95±1% and temperature of 40±1°C for 8 days [22]. During this period, the seeds were shuffled daily. The relative humidity, temperature and duration in respect of ageing test were standardized through pilot studies using progressively decreasing RH from 100 to 90 %, temperature 40-35 °C. The treated seeds along with the control were tested for vigour and viability parameters, immediately and after accelerated ageing. The following observations were made.

2.3.2. Germination

The germination test was carried out by inclined plate method using 4x100 seeds [23] in a germination room maintained at 25±1°C temperature and 95±3% RH. After seven days, the seedlings were evaluated and the normal seedlings were counted and expressed in percentage.

2.3.3. Root Length

At the time of germination count, all normal seedlings from each treatment and replication were used for measuring the root length. Root length was measured from the collar region to the tip of primary root and the mean is expressed in centimetre.

2.3.4. Shoot Length

The seedling used for measuring root length was also used for measuring shoot length. The shoot length was measured from the collar region to shoot apex and the mean is expressed in centimetre.

2.3.5. Vigour Index

Vigour index (VI) was calculated using the formula suggested by Abdul-Baki and Anderson [24] and expressed in whole number. VI = Germination percentage x [Root length (cm) + shoot length (cm)].

2.3.6. Statistical Analysis

The results obtained from different laboratory experiments and were analyzed statistically adopting techniques described by Panse and Sukhatme [25] and presented as mean standard deviation (SEd). Each experimental value was compared to its corresponding control. The critical differences (CD) were calculated at 5 percent probability level. Statistical significance was accepted when the probability of the result assuming the null hypothesis (p) is less than 0.05 (level of probability).

3 Results and Discussion

3.1. Structural Characterization of ZnO Samples

The XRD patterns of the synthesized ZnO samples are shown in Fig. 1. The XRD patterns of the samples reveal that all peaks correspond to the characteristic peaks of the hexagonal *wurtzite* type structure of ZnO with space group $P6_3mc$ and lattice parameters of $a = b = 0.3250$ nm and $c = 0.5207$ nm according to the JCPDS database 36-1451. The average crystallite size of ZnO samples is determined by Debye-Scherrer's formula, $D = K\lambda / \beta \cos\theta$, where D is the average crystallite size, K is a constant = 0.89, λ is the wavelength of the X-ray used, β is a line width in radians at half maximum intensity of the observed peaks and θ is the Bragg angle. The calculated crystallite size of ZnO samples A and B are 53 nm and 31 nm respectively. Compared with the standard diffraction pattern, no characteristics peaks of any other phase are detected, indicating that the ZnO samples obtained by the current sol-gel route are highly pure. Furthermore, clear and sharp peaks confirmed that the as-synthesized ZnO nanostructures possess a high crystalline quality [26].

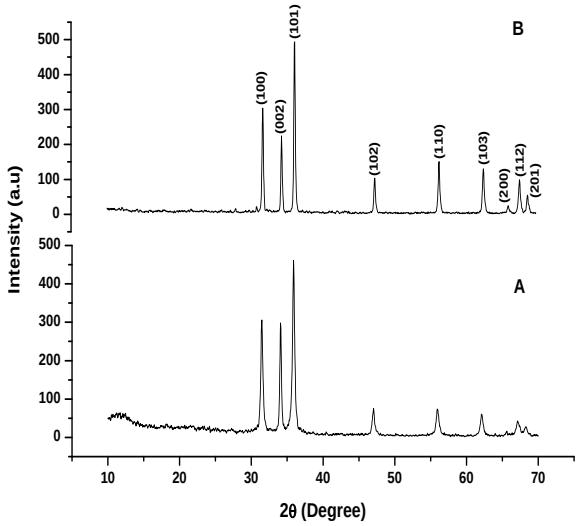

Fig. 1. XRD patterns of ZnO nanostructures

3.2 Morphology and Size of ZnO Nanostructures

The SEM and TEM micrographs of sample A are shown in Fig. 2. The SEM micrograph of sample A demonstrates the existence of porous ZnO nanostructures. The porous structure is further confirmed by TEM micrograph. The average size of sample A is around 55 nm. The SEM and TEM micrographs of sample B are shown in Fig. 3.

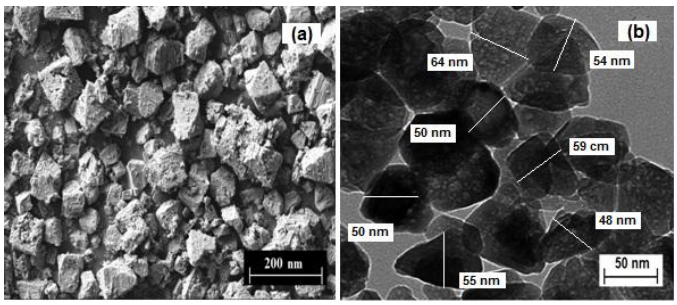

Fig.2(a).SEM micrograph **(b).**TEM micrograph of sample A

The SEM micrograph of sample B apparently exhibits ZnO nanochip structure. The average size of the ZnO nanochips is around 33 nm and is revealed by the TEM micrograph. It may be noted that the average size of the ZnO samples estimated from TEM micrographs is in good agreement with the particle size calculated from XRD analysis.

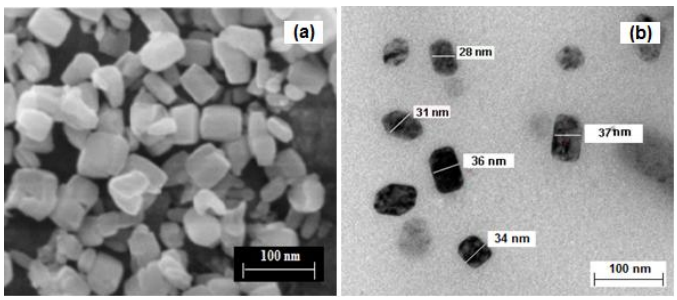

Fig.3(a).SEM micrograph **(b).**TEM micrograph of sample B

3.3. Effect of ZnO Nanostructures in Blackgram

Highly significant differences were obtained due to ZnO nanoparticles treatments in fresh and aged seeds for all the evaluated seeds and seedling quality parameters as can be observed from the photographs and tables. Photographs of seeds showing differences in germination root and shoot growth for both fresh and aged seeds treated with ZnO samples A and B at two different concentrations (750 mg and 1000 mg) and the control photographs for both fresh aged seeds are shown in Fig. 4.

3.3.1. Seed Germination

Effect of dry treatment of ZnO nanoparticles on germination in fresh and aged seeds of blackgram is given in Table. 1. The germination percentage was maximum in seeds treated with sample B 1000 mg (94%), while minimum with control (86%) in fresh seeds. In aged seeds maximum of 80 percent was recorded with sample B 1000 mg and minimum of 58 percent was recorded in control. When compared with the control sample B 1000 mg has shown significant influence in increasing the viability both in fresh and accelerated aged seeds. Among fresh seeds

Table. 1. Effect of dry treatment of ZnO samples A and B on germination (%) in fresh and aged seeds of blackgram different treatments resulted in limited improvement only when compared with control. With reference to the aged seeds the ZnO nanoparticles treatments show significant improvement when compared with control.

Treatments	Age of seed		Mean
	Fresh	Aged	
CONTROL	86	58	72
A 750 mg	89	73	81
A 1000 mg	92	76	84
B 750 mg	92	77	85
B 1000 mg	94	80	87
	Ageing	Dosage	Ageing ×Dosage
SEd	1.00	1.58	2.23
CD(p=0.05)	2.08**	3.29**	4.66**

**Highly Significant at p (level of probability) less than 0.05.

3.3.2. Shoot length

Effect of dry treatment of ZnO nanoparticles on shoot length in fresh and aged seeds of blackgram is given in Table. 2. It is observed from the treatments that the influence of ZnO nanoparticles on shoot length of fresh seeds is less significant. In both fresh and aged seeds the influence of B 1000 mg treatment is noticeable and it is significant in aged seeds (18.5 cm) when compared with control (16.7 cm). With reference to control the influence of ZnO nanoparticles on shoot length is significantly more on aged seeds than fresh seeds.

Fig. 4. Photographs of the seeds showing differences in germination, root and shoot growth for fresh, control and aged seeds.

Table. 2. Effect of dry treatment of ZnO samples A and B on shoot length (cm) in fresh and aged seeds of blackgram

Treatments	Age of seed		Mean
	Fresh	Aged	
CONTROL	21.7	16.7	19.2
A 750 mg	22.0	17.8	20.0
A 1000 mg	22.3	18.2	20.3
B 750 mg	22.5	18.2	20.4
B 1000 mg	22.7	18.5	20.6
	Ageing	Dosage	Ageing × Dosage
SEd	0.19	0.30	0.43
CD(p=0.05)	0.40**	0.63*	NS

*Significant at p (level of probability) less than 0.05.
**Highly Significant at p (level of probability) less than 0.05.
NS – Non Significant at p (level of probability) less than 0.05.

3.3.3. Root length

Table. 3. Effect of dry treatment of ZnO samples A and B on root length (cm) in fresh and aged seeds of blackgram

Treatments	Age of seed		Mean
	Fresh	Aged	
CONTROL	16.6	13.0	14.8
A 750 mg	16.8	13.6	15.2
A 1000 mg	17.2	14.1	15.7
B 750 mg	17.1	14.5	15.8
B 1000 mg	17.3	14.7	16.0
	Ageing	Dosage	Ageing ×Dosage
SEd	0.14	0.22	0.32
CD(p=0.05)	0.30**	0.47*	NS

*Significant at p (level of probability) less than 0.05.
**Highly Significant at p (level of probability) less than 0.05.
NS – Non Significant at p (level of probability) less than 0.05.

The effect of dry treatment of ZnO nanoparticles on root length in fresh and aged seeds of blackgram is given in Table 3. From this table it is observed that the treatment of B 1000 mg has high influence on the root length both in the fresh and aged seeds. The effect of nano ZnO on root length is significant in aged seeds with reference to control.

3.3.4. Vigour Index

The effect of ZnO nanoparticles on vigour index in fresh and aged seeds of blackgram are given in Table. 4. The computed vigour index values are the maximum in seeds treated with B 1000 mg (3760), while minimum in control (3294) in fresh seeds. In aged seeds the maximum was recorded in B 1000 mg (2656) and it was minimum in control (1723). The effect of ZnO nanostructures is more in aged seeds compared to fresh seeds.

Table. 4. Effect of ZnO samples A and B on vigour index in fresh and aged seeds of blackgram

Treatments	Age of seed		Mean
	Fresh	Aged	
CONTROL	3294	1723	2508
A 750 mg	3453	2029	2813
A 1000 mg	3634	2455	3044
B 750 mg	3643	2518	3081
B 1000 mg	3760	2656	3208
	Ageing	Dosage	Ageing × Dosage
SEd	27.67	43.75	61.88
CD(p=0.05)	57.73**	91.28**	129.09*

*Significant at p (level of probability) less than 0.05.
**Highly Significant at p (level of probability) less than 0.05.

Zinc plays vital role in protecting and maintaining structural stability of cell membranes [27]. Zn is used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses [28]. Plants emerging from seeds with low Zn have poor seedling vigor [29]. Rengel and Graham, [30] reported from pot culture experiments on wheat plants that increasing seed zinc content from 0.25 μg per seed to 0.70 μg per seed significantly improved the root and shoot growth. Ajouri et al., [31] reported that seed priming with Zn is very effective in improving seed germination and seedling development in barley. It is explicit from these results that high Zn concentration in seeds has very important physiological roles during seed germination and early seedling growth.

In the present study, enhancement of seed vigour and viability in blackgram seeds is observed when the seeds are treated with nano ZnO. Zinc oxide sample B at a concentration of 1000 mg has shown significant improvement in germination, shoot length, root length and vigor index over other treatments. The reason for these effects is likely to be due to the smaller particle size and higher concentration of zinc in the seed when treated with ZnO nanoparticles.

4 Conclusions

Effects of as-synthesized ZnO nanostructures on blackgram seeds were studied by measuring germination percentage, shoot length, root length and vigour index. The results indicated that the nanoscale ZnO treatments in proper concentration accelerates the germination of blackgram seeds and increases the vigour. The results suggest that the micronutriuent, Zn can be delivered into blackgram seeds through ZnO nanoparticles. The inherent small size and the associated large surface area of nanoscale ZnO micronutrients may enhance the Zn uptake. This improves the germination, shoot length, root length and vigour index of the treated seeds. The results emphasize that nanoscale nutrients can be supplied to the crops through seed treatment with much decreased doses to get the desired results. These results will help to further understand the effects of nanosized materials and are important in terms of the relationship between size and concentration of materials and their effects on germination and plant growth.

References

[1] K. S. Subramanian, C. Tarafdar, Prospects of nanotechnology in Indian farming, The Indian Journal of Agricultural sciences, 81 (2011) 887-893.

[2] L. Yan, Y. B. Zheng, F. Zhao, S. Li, X. Gao, B. Xu, P. S. Weiss, Y. Zhao, Chemistry and physics of a single atomic layer: Strategies and challenges for functionalization of graphene and graphene-based materials, Chemical Society Reviews, 41 (2012) 97–114.

[3] M. Amelia, C. Lincheneau, S. Silvi, A. Credi, Electrochemical properties of CdSe and CdTe quantum dots, Chemical Society Reviews, 41 (2012) 5728–5743.

[4] D. Y. Joh, J. Kinder, L. H. Herman, S. Y. Ju, M. A. Segal, J. N. Johnson, K. L. ChanGarnet, J. Park, Single-walled carbon nanotubes as excitonic optical wires, Nature nanotechnology, 6 (2011) 51–56.

[5] F. Tang, L. Li, D. Chen, Mesoporous silica nanoparticles: Synthesis, biocompatibility and drug delivery, Advanced Materials, 24 (2012) 1504–1534.

[6] S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. van der Elst, R. N. Muller, Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations and biological applications, Chemical Reviews, 108 (2008) 2064–2110.

[7] Z. Liu, J. T. Robinson, X. Sun, H. Dai, PEGylated aanographene oxide for delivery of water-insoluble cancer drugs, Journal of the American Chemical Society, 130 (2008) 10876–10877.

[8] X. Sun, Z. Liu, K. Welsher,J. Robinson, A. Goodwin, S. Zaric, H. Dai, Nano-graphene oxide for cellular imaging and drug delivery, Nano Research, 1 (2008) 203–212.

[9] K. Yang, S. Zhang, G. Zhang, X. Sun, S. T. Lee, Z. Liu, Graphene in mice: Ultrahigh *in vivo* tumor uptake and efficient photothermal therapy, Nano letters, 10 (2010) 3318–3323.

[10] Z. Liu, K. Chen, C. Davis, S. Sherlock, Q. Cao, X. Chen, H. Dai, Drug delivery with carbon nanotubes for *in vivo* cancer treatment, Cancer research, 68 (2008) 6652–6660.

[11] L. Harhaji, A. Isakovic, N. Raicevic, Z. Markovic, B. Todorovic-Markovic, N. Nikolic, S. Vranjes-Djuric, I. Markovic, V. Trajkovic, Multiple mechanisms underlying the anticancer action of nanocrystalline fullerene, European Journal of Pharmacology, 568 (2007) 89–98.

[12] F. Patolsky, G. Zheng, C. M. Lieber, Nanowire sensors for medicine and life sciences, Nanomedicine, 1 (2006) 51-65.

[13] G. Scrinis, K. Lyons, The emerging nano-corporate paradigm: nanotechnology and the transformation of nature, food and agri-food systems, International Journal for the Sociology of Food and Agriculture, 15 (2007) 22-44.

[14] Remya Nair, Saino Hanna Varghese, Baiju G. Nair, T. Maekawa, Y. Yoshida, D. SakthiKumar, (Review) Nanoparticulate material delivery to plants, Plant Science, 179 (2010) 154-163.

[15] L. Zhao, Y. Sun, J. A. Hernandez-Viezcas, A. D. Servin, J. Hong, G. Niu, J. R. Peralta-Videa, M. Duarte- Gardea, J. L. Gardea-Torresdey, Influence of CeO2 and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: a life cycle study, Journal of Agricultural and Food Chemistry, 61 (2013) 11945–11951.

[16] T. N. V. K. V. Prasad, P. Sudhakar, Y. Sreenivasulu, P. Latha, V. Munaswamy, K. Raja Reddy, T. S. Sreeprasad, P. R. Sajanlal & T. Pradeep, Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut, Journal of Plant Nutrition, 35 (2012) 905-927.

[17] M. A. El-Kereti, S. A. El-Feky, M. S. Khater, Y. A. Osman, S. A. El-Sherbini, ZnO nanofertilizer and He Ne laser irradiation for promoting growth and yield of sweet basil plant, Recent Patents on Food, Nutrition and Agriculture, 5 (2014) 69–81.

[18] N. B. Singh, N. Amist, K. Yadav, D. Singh, J. K. Pandey, S. C. Singh, Zinc oxide nanoparticles as fertilizer for the germination, growth and metabolism of vegetable crops. Journal of Nanoengineering and Nanomanufacturing, 3 (2013) 353–364.

[19] A. C. Pandey, S. S. Sanjay, R. S. Yadav, Application of ZnO nanoparticles in influencing the growth rate of *Cicer arietinum*, Journal of Experimental Nanoscience, 5 (2010) 488–497.

[20] B. M. Waters, and R.P. Sankaran, Moving micronutrients from the soil to the seeds: Genes and physiological processes from a biofortification perspective, Plant Science, 180 (2011) 562-574.

[21] J. J. Mortvedt, Crop response to level of water soluble zinc in granular zinc fertilizers, Fertilizer Research 33 (1992) 249-255.

[22] J. C. Delouche, Baskin, Precepts of seed storage (revised), Proc. Mississippi State Seed Processors Short Course, (1973) 97-122.

[23] ISTA (International Rules of Seed Testing), Seed Science and Technology, 27 (1999)27-32.

[24] A. A. Abdul-Baki, J. D. Anderson, Vigour deterioration of soybean seeds by multiple criteria, Crop Science, 13 (1973) 630-633.

[25] V. G. Panse, P. V. Sukhatme, Statistical methods for agricultural workers, fourth ed., ICAR Publication, New Delhi, (1985) 359.

[26] L. Feng, A. Liu, Y. Ma, M. Liu, B. Man, Fabrication, Structural Characterization and Optical Properties of the Flower-Like ZnO Nanowires, Acta Physica Polonica A, 117 (2010) 512-517.

[27] R. M. Welch, M. J. Webb, J. F. Loneragan, Zinc in membrane function and its role in phosphorus toxicity, In: Proceedings of the Ninth Plant Nutrition Colloquium, ed. A. Scaife, (1982) 710-715.

[28] I. Cakmak, Role of zinc in protecting plant cells from reactive oxygen species, New Phytologist, 146 (2000) 185-205.

[29] A. Yilmaz, H. Ekiz, I. Gultekin, B. Torun, H. Barut, S. Karanlik, I. Cakmak, Effect of seed zinc content on grain yield and zinc concentration of wheat growth in zinc-deficient calcareous soils, Journal of Plant Nutrition, 21 (1998) 2257-2264.

[30] Z. Rengel, R. D. Graham, Importance of seed zinc content for wheat growth on zinc-deficient soil, I. Vegetative growth. Plant and Soil, 173 (1995) 259-266.

[31] A. Ajouri, H. Asgedom, M. Becker, Seed priming enhances germination and seedling growth of barley under conditions of P and Zn deficiency, Journal of Plant Nutrition and Soil Science, 167 (2004) 630-636.