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Nanotechnological approaches in seed science

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Abstract
Seed is the nature's Nano-gift to human beings. Engineered nano-materials like Carbon Nanotubes, Quantum dots, Nano Gold, Nano-Zinc, Nano-Aluminum and Nano Titanium, Zinc oxide and Nano-Sized Polymers have received greater attention in Seed Science. In global seed trade, tracking seed materials is difficult, which can be positively overcome by nano-barcoding of seeds and helps to download the information about the seed lot information like location, date of harvest, germination, purity *etc*. and also help in IPR protection for seed companies seeking to enter highly competitive market of GM seeds. Nano formulation of Silver metal was found to possess antibacterial and antifungal properties. Use of silver nanoparticles for seed coating has tremendous potential in effectively controlling the seed borne diseases and prevents the sporulation, penetration of fungus and the growth of bacteria which will help in saving the life of seeds. Seed hardening with Carbon nano tubes and nano polymers play an effective role in increasing the germination through improved water penetration. Accumulation of free radicals is a major cause for seed deterioration during storage and emission of certain volatile aldehides that determine the degree of ageing which can be prevented by use of TiO_2 nano particles (Spinach). Similarly, it promotes photosynthetic activity and in turn increased the assimilation of ammonium.

Keywords: Nanotechnology, nanoparticles, seed science biosenors, nano barcoding

Introduction

Nanotechnology is a broad and interdisciplinary science which encompasses research and development activity that has been growing at a rapid pace at worldwide in the past few years. It is one of the emerging fields of science interfacing science, material science and information technology. Nanotechnology is the manipulation or self – assembly of individual atoms, molecules or molecular clusters into structures to create materials and devices with new or vastly different properties (Roco *et al*., 1999) [21]. The application of nanotechnology.
to the agricultural and food industries was first addressed by a United States Department of Agriculture in September, 2000. Nanotechnology in the agricultural front has been more useful in improving the existing crop management techniques. Nano encapsulated agrochemicals usage in effective delivery of the chemicals at the needed place, utilization of nano capsulated herbicides for targeted delivery and control of parasitic weeds, nano coated gene delivery for effective transmission of gene segments, nano fertilizers for smart delivery of the required nutrients to the plant system, surface modified hydrophobic nano silica for thwarting the pests attack (Nair *et al*., 2010) [15]. Most of the application of nanotechnology spearhead around these technologies. Besides its utilization in seed hardening, seed invigouration and seed germination enhancement and plant establishment, its use will not be fulfilled.
Nanotechnology has the potential to revolutionize the scientific world by allowing scientists to manipulate mater at the atomic or molecular scale. It enables researches to understand the relationship between macroscopic properties and molecular structure in biological materials of plants. This generic technology offers better – built, safer long lasting, cheap and smart products that will find wide application in agriculture. Seed is the nature's Nano - gift to human beings. Engineered nanomaterial's such as Carbon Nanotubes, Quantum dots, Nano gold, Nano zinc, Nano aluminum and Titanium oxide, Zinc oxide and nano – sized polymers have received a particular attention for their positive impact in seed technology .Up scaling available technology has helped humans to overcome resolving Nano materials. Invention of Scanning Tunneling Microscope (STM) and Atomic Force Microscope (AFM) has made the Nano World more accessible to us. The ability to observe nano structures has led to manipulating or construing them to our needs.

Nano – barcoding of seed

Nano – barcodes are free standing , cylindrically shaped metal nano particles having dimensions of 20 – 500 nm in diameter and 0.04 – 15 mm in length, which are encodable, machine – readable (Nicewarner - Pena *et al*., 2001) [17]. The particles are manufactured in a semi automated highly scalable process by electro–plating using inert materials like gold, silver into templates defining particle diameter and then releasing the resulting stripped Nano – rods from the templates.

The barcodes are so small (20 - 500 nm) in diameter that they can be affixed on the seeds. If the company wants to known the where abouts of the seed, the seed can be agitated in a solution to washout the barcodes and can be read using a barcode reader. Seed samples for the different seed lots can be nano bar coded differently. Information like the location, date of harvest, germination, purity *etc*, can be downloaded easily and used. This will also help in IPR protection for seed companies seeking to enter highly competitive market of GM (Genetically modified) seeds. As far as legal issues are concerned, tracking seeds will help resolving disputes arising from use of IPR (Intellectual Property Rights) protected seeds.

Seed protection

Nano formulation of Silver metal was found to possess antibacterial and antifungal properties (Singh *et al*., 2008; Jo and Kim, 2009; Panacek *et al*., 2009) [22, 9, 18] Silver nanoparticles were found to possess broad spectrum of antimicrobial activity which can reduce various plant diseases by controlling the spore producing fungal pathogens (Jo and Kim, 2009) [9]. The effectiveness of nanoparticles can be improved by applying them well before the penetration and colonization of fungal spores within the plant tissues. Powdery mildew disease of pumpkin was effectively controlled by using small size of the active ingredient (diameter of 1 -5nm) (Park *et al*., 2006) [20]. However, it was also found that a very high concentration of nanosilica – silver produced some chemical injuries on the tested plants (cucumber leaves and pansy flowers). The use of Silver nanoparticles as an alternative to pesticides for the control of sclerotium forming Phytopathogenic fungi was also investigated (Min, 2009) [14]. Exposure of fungal hyphae to Silver nanoparticles caused severe damage by the separation of hypha wall and collapse of hyphae.

Thus the use of silver nanoparticles as a coat in the seed has tremendous potential in effectively controlling the seeds borne diseases. If the selected seeds are embedded / coated with silver nanoparcticles, these particles can prevent the sporulation.

Seed hardening

Seed hardening techniques have been standardized for ma wide array of crops and strategies were evolved ensure germination utilizing the available moisture optimally. This Process will make the seed hardened and emerge faster besides with standing early drought. Through it is a useful strategy rarely adopted by farmers due to impractical feasibility (Vanangamudi *et al.*, 2006) [24].

i. Carbon nanotube fast forward seed germination

Carbon Nano Tubes (CNT) which could play an effective role in increasing the germination of the seeds through improved water penetration was testified by the research carried out by the scientists of University of Arkansas. Khodakovskaya *et al*. (2009) [12] observed that addition of carbon nanotubes to the MS medium where the tomato seeds were placed for germination had accelerated the process of seed germination and significantly shortened the germination time. The germination percentage for seeds that were placed on regular medium, averaged 32% in 12 days and 71% in 20 days while the germination percentage had a spurt for the seeds placed in MS medium with CNTs (74 – 82 % in 12 days ; 90 % in 20 days). It was also reported by them that seeds exposed to CNTs had a significantly higher level of moisture compared to the seeds that were not treated. With a start level moisture of 18.4% content, CNT treated seeds accumulated 57.6% while the non treated one had 38.9% only.

Possible explanation for this phenomenon would be the role of CNT in penetrating the seed coat while supporting and allowing water inside the seeds. Thus the ability of CNT to penetrate thick seed coat of tomato and supporting its germination in a hastened in a manner leaves a hope on its utilization in other crop seeds as well.

Mature seeds are relatively dry and need to take significant amounts of water before cellular metabolism and growth can start. So the researches hypothesized that the observed activation of germination is based on the role of CNTs in the process of water uptake inside the seed embryo. Since this proven technology makes the seeds to emerge faster, it can be modified to coat the seeds with CNTs so that the treated seeds will germinate faster utilizing the available moisture once the seeds are sown in condition. Thus, these experiments pave a way for its utilization on test verification of the seeds treated with varied forms of CNTs in varied soil / climatic conditions for improving the germination.

CNTs also enhanced root elongation in onion and cucumber and nanotubes sheets were formed on cucumber root surface due to their interaction with root surface (Canas *et al*., 2008) [4]. However none entered into the roots. Some other studies also support the positive effects of suspension of Multiwalled CNTs (MWCNTs) on seed germination and root growth of six different crop species namely radish (*Raphanus sativus*), rape (*Brassica napus*), rye grass (*Lolium perenne*) lettuce (*Lactuca sativa*), corn (*Zea may*) and cucumber (*Cucumis sativus*) (Lin and Xing, 2007) [13]. The works conducted by Nair *et al*. (2010) [15] also supported the positive effects of carbon nanotubes on germination and growth of plants. They studied the effects of both single walled CNT and multi wall CNTs on the germination of rice seeds and observed an enhanced germination for seeds germinated in the presence of nanotubes. They concluded that the response of plants to nano materials with the type of plant species, their growth stages and the nature of nano materials the studies showed contradictory effects of even the same nano material in different plants at different developmental stages.

ii. Nano polymer coating

Several polymers are available in the market of which water soluble polymers are of immense use for coating the seeds. These water soluble polymers at a threshold of moisture gets dissolved allowing the coated seed to absorb the moisture and to germinate and withstand. This technology, if employed in the pre monsoon condition will help to ensure successful establishment of the treated seeds. When the seeds are coated with a specific water soluble nano polymer, they will break open only when the moisture content of the soil is above 45-50%. This will ensure continued growth of the germinating seedling for successful establishment under dry land conditions. Another type of polymer utilized for enhancing / delaying the seed germination is temperature responsive. This polymers exhibit temperature induced phase transitions that change the permeability properties of the polymer. Seeds treated with Intelimer (Polymer) were found to take less water at 10º C compared to 25 ºC. This facilitates the sowing of seeds coated with polymer during the cool months which will germinate once time reaps up.

Southeast Iowa is often dry enough to plant during the second half of March or the first half of April, but producers usually delay planting during this period because the soil is still too cool. Unfortunately, when sails do warm later in the spring, wet soil conditions may occur resulting in planting delays. The polymer coating technology allows the opportunity to plant when soils are cool but otherwise fit. Polymer - coated seed emerged slowly and had less than ideal stands (Johnson and Hicks, 1999)[10]. This observation suggests that the polymer coating protected the seeds early in the growing season.

Landec Agriculture Company has laughed three commercial product families utilizing the application of polymers. They are: 1) Early plant ® Corn coating which allow the formers to plant 3 -4 weeks earlier than normal in the spring broadening the planting window and serving as a planting management tool. The seed coating protects the seed from imbibing moisture until soil temperatures are ideal for germination. This helps farmers plant all their crops on time maximize yield potential, avoid late planting losses and lower post – harvest dry down costs, 2) Pollinator plus® coating which eliminate spilt planting operations and reduce weather related risks in seed production of hybrid corn and 3) Relay crop soybean coating is a double cropping system that allows farmers to grow two crops in one season in a single field.

Most of these technologies were developed and utilized in the temperate countries especially in corn seed production and hybrid development. However, for the tropical countries like India, these technologies are to be tailored to suit our requirements where in temperature depended, water loving polymers to be developed and employed in coating with the seeds to make them germinate under favorable conditions. For example, thermosensitive nano polymer coated seeds if sown in high temperature regimes will not germinate and desiccate until the favorable condition for the germination set in owing to the lowered temperature. Similarly, hydrophilic nanopolymers can help in getting a better crops stand through uniform germination of the coated seed whenever the soil moisture attains an appreciable level.

Extension of this polymer coating technology includes polymers may be hydrophilic or hydrophobic, thermostable or thermo mobile, nutrient rich, *etc*. Coating of multi layers of polymers will help in ensuring the uniform stand of the seed crop by allowing them to germinate under favourable condition, ensuring optimum growth and vigour.

Seed Physiology

Seed invigouration implies an improvement in performance of seed by any post – harvest treatment resulting in good germinability, greater storability and better field performance, pre-storage treatment of harvest fresh seeds is mainly given towards protection against deteriorative senescence (Basu, 1990) [2].

Basu (1994) [3] in several agricultural crops observed that treatment of high vigour (harvest fresh) seeds with crude plant materials *viz*., Red chill fruit powder, Tamarind powder and *Trigonella* seed powder@ 2g kg^{-1} significantly slowed down the deterioration of seeds under various ageing conditions.

The entry of the crude powder in the dry state into the dry seeds is through the cracks in the seed coat. Normally, these crude powders are of macro size and irrespective of its size; it gets entry into the seed. Under this circumstance, if nano sized plant powder is used; its efficiency of penetration and action will be more. Besides that, the quantum of Nano particle to be used for this treatment will be also very minimal. Macro – sized particles can be reduced into Nano size through ball milling procedure, which ultimately invigorate the seeds with consequent reduction in loss of vigour and viability during storage.

It is known that the vigour or strength of seed decreases as a function of the storage time.The decrease in seed vigour is results of the accumulation of free radicals (Wilson and McDonald, 1986) [25]. It has been shown that pretreatment of seeds before sowing with nanomaterial's could significantly reverse the effects of ageing. Studies of Yang *et al.* (2005) [26] had conclusively proved that nano – anatase TiO_2 (nano – anatase titanium Oxide) treatment could promote spinach to absorb nitrate, accelerate inorganic nitrogen to be translated into organic nitrogen and enhance fresh and dry weight. Their earlier study also indicated that nano – anatase TiO_2 treatment could promote the photosynthesis of spinach which also provided much energy, a carbon source, and a proton for the reduction of nitrate and the assimilation of ammonium.

Utilization of nano TiO_2 by Zheng *et al*., (2005) [28] on spinach for studying the germination and growth of naturally aged seeds and indicated that the nano TiO_2 treatment in proper concentration accelerates germination of aged seeds and increases its vigour. It also improves the growth of spinach and information of chlorophyll and enhances the rubisco activity and the photosynthetic rate during the growth stage of spinach. This effect was found to be inversely proportional to the size of TiO_2 Particles. Scientists also found that nano – anatase titanium Oxide (TiO2) promoted antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, malonydialdeyhde content and enhance the activities of superoxide dismutase, catalase, ascorbate peroxidase. In addition to TiO_2 nano particles of Zinc oxides, iron oxide, platinum, Manganese etc are also found to have anti oxidant properties. If these particles are used to treat the seed, it can slow down the ageing process.

Investigation were also made at the Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore to study the effect of different dosage of ZnO (Zinc oxide), TiO_2 nanoparticles for the maintenance of vigour and viability of black gram seeds. Upon accelerated ageing, the seed treated with nanoparticles (400 mg kg^{-1} of seeds) maintained higher the vigour and viability than the untreated check (Natarajan, 2020) [16].

Biosensors

Bio- analytical nano sensors are currently being manufactured. Electronic nose (E – nose) is a gadget that mimics the operation of human nose in detecting an assemblage of gases. This device contains several gas sensors to detect different types of the odor. The main component of e–nose is gas sensor composed of Nano - particles (zinc oxide nanowires) whose resistance changes when a certain gas passes over it (Suguna *et al*., 2004; Hossain *et al*., 2005) [23, 8]. The change in resistance causes a change in electrical signal that forms the fingerprint for gas discernment. The advantage of using nanoparticles is improved uncontaminated surface area for better gas adsorption.

Seeds during storage emit several volatile aldehydes that determine the degree of ageing. These gases are harmful to even other seeds. Such volatile aledehydes can be detected and seeds showing signs of deterioration can be separated and invigorated prior to their use. Hence, this technique can be effectively employed in storage decision making.

Seed production generally requires of selected genotypes in isolation. Particularly for wind pollinated crops, isolation distance may run to several meters to avoid contamination of foreign pollen. Even some time after providing the required isolation, contamination of the seed crop due to invasion of foreign pollen occurs. Hence, detecting pollen load that will cause contamination is a sure method to ensure genetic purity. Usually pollen flight is determined by its weight, air temperature, humidity, and wind velocity and pollen production of the crop. Provision of micro electronic sensors specific sensors specific to the seed crop pollen placed along the perimeter of the seed field at varied heights can help to detect the possible contamination. This technique has its own advantage like fixing up the isolation distance for the seed crop in lieu of the regular method which is now followed being tedious, for assessing the purity of the seed crop and to quantify the contaminants thereby taking steps to reduce the contamination. The same method can also be used to prevent pollen from Genetically Modified crop from contaminating natural crop.

Seed Dormancy

Several seeds exhibit dormancy due to the presence of inhibitors like ABA, dormin, phenols, etc. To overcome this type of dormancy seeds are being soaked in growth promoters before sowing requiring high volume. In contrast, if nanoparticles of growth regulators are used, required quantity of seed can be treated with very low doses over the surface of the seeds. These Nano – particles will enter into the seed during the imbibition and release dormancy and ensure continued growth.

Lin and Xing (2002) [13] reported that a mixture of nano SiO_2 and nano TiO_2 could increase the nitrate reductase in soybean, enhance its abilities of absorbing and utilizing water and fertilizers stimulate its antioxidant system and apparently accelerate its germination and growth. Study undertaken by Pandy (2010) [19] utilizing ZnO nano particles on growth rate of *Cicer arietinum* indicated the presence of IAA in seeds which had a spurt in its concentration in the roots owing to the treatment of ZnO nanoparticles.

Thus either by utilizing the nano sized particles of growth regulators or by treating the seeds with ZnO nanoparticles, the level of growth regulator inside the seed can be altered to enhance its germination.

Seed moisture detection

Seed moisture has definite role in seed storage and longevity. High moisture seeds are poor storers. Seed men try to reduce the moisture content of stored seeds to a safe limit prior to storage. Lithium chloride is currently used for doping in silica gel to indicate the hygroscopicity of silicon colourless. Lithium chloride is blue when it dry and upon hydration turns pink colour. The degree of pink colour is an indicator of its hydration.

Seeds can be coated with highly purified lithium chloride quantum dots and sprayed on seed so as to serve as indicator for monitoring content during storage. Seed store managers can then use driers to dry the seed or plan to reduce seed moisture just by observing the colour changes in the quantum dots. This facilitates real time monitoring of moisture content in charge. This technology will be of immense use in germplasm conservation.

Seeds image analysis

Seeds, to be qualified as a silent living legend, had so many variations in its surface among he different genotypes of a particular species and innumerable variations among the different genus. Seed coat is a specific feature for each crop variety a part from protecting the embryo and plays a key role in the maintenance of viability particularly in *Leguminacae*. Hard seed coat aids in extending the seed longevity by preventing the moisture absorption from atmosphere (Yasseen *et al*., 1994) [27].

The emerging Nano world has options of studying these variations through new and novel means to investigate structure and systems, besides exploiting the well – known microscopic diffraction and spectroscopic methods.

Examination of seed coat under Scanning Electron Microscope (SEM) helps in-depth understanding of its structure for the following purpose.

i. Temporal change in seed coat structure and its pattern during seed development and maturation will aid in devising suitable methods to protect the seeds.
ii. Varietal / species confirmation based on seed coat pattern and also confirmation of inter specific and inter – generic hybrids.

Utilization of the image taken through scanning Electron Microscope helps in understanding the mode of action of scarification treatment on seed coat. Since most of the times, effectiveness of pretreatments depends on seed characteristics their application should be modulated with different period concentration or degree combinations for achieving maximum percent germination.

A Scanning electron microscopy study of the seed development in *Angelonia salicariifolia* made by (Fabiola *et al*., 2001) [5] allowed species identification at different stages of development under field conditions. It also facilitated the differentiation at different stages of development under field conditions. It also facilitated the differentiation between very similar taxonomic characters, SEM images more helpful.

Ultrastructural analysis of seed testa in developing *Lupinus pilosus* seeds helped in characterizing the development of 'rough – seed' during seed maturation. Roughness of seed testa surface starts from 26 days after flowering. At this point seed dehydration starts quickly macrosclereid layer is basically responsible for seed testa roughness trait in *Lupinus pilosus* (Agnieszka *et al*., 2008) [1].

Effects of ageing on amylase activity and scutellar cell structure during imbibition in wheat seed was well studied by Ganguli and Swati Sen (1993) [6]. In fresh seeds during later stages of germination, the scutellar cells develop fingers like projections. These cells serve to absorb endospermic reserves hydrolyzed by aleuronic amylase. Further, non – germinating seed showed no fingers like projection even after prolonged imbibition particularly in aged seeds. Observation on finger like projections appearing on the seeds helps in understanding the seed viability.

SEM studies helped in understanding the onion seed ageing phenomenon as described by Gorinstein *et al*., (2004) [7]. Microscopic studies help in comprehending and visualizing structural changes and textural differences in protein fractions. This study helped to correlate electrophoretic pattern and microstructure of proteins which can exhibit close identity with each other. This method serves as an efficient alternate for studying the seed coat structural and textural changes. In general, seed coat micromorphology helps in classifying *Solanum* species. Studies undertaken by (Junlakitjawat *et al*., 2010) [11] had resulted in classifying eight species or into three groups based on presences of the spiral secondary thickening and fibrils or hair on the surface. The phylogenetic relationship among members of the genus indicated that possibilities of having variation in the seed surface in the genus can be elucidated.

Reference

1. Agnieszka I, Piotrowicz–Cieslak, Barbara Adomas, Dariusz Michalczyk J, Kamilla Groska. Ultrasturctural analysis of seed testa in developing *Lupinus piolsus* seeds. Proceedings of the 12th International *Lupin* Conference**,** 14 – 18 Sept., Fremantle, Western Australia, New Zealand, 2008.
2. Basu, RN. Seed invigouration for extended storability. Proc. of the International Conference on Seed Science and Technology**,** New Delhi, 1990, 88.
3. Basu RN. An appraisal of research on wet and dry physiological seed treatments and their applicability with special reference to tropical and sub – tropical countries. Seed Sci. and Technology. 1994; 22:107-126.
4. Canas JE, Long M, Nations S, Vadan R, Dai L, Luo M *et al*. Effects of functionalized and non – functionalized single – walled carbon nanotubes on root elongation of select crop species**.** Environmetal Toxicology. 2008; 27:1922-1931.
5. Fabiola Moro V, Ana Pinto CR, Jamime Dos Santos M, Carlos Damiao Filho F. A scanning electron microcopy study of the seed and post – seminal development in *Angelonia salicariifolia* Bonpl. (Scrophulariaceae). Annals of Botany. 2001; 88:499-506.
6. Ganguli S, Swati Sen – Mandi. Effects of ageing on amylase and scutellum cell structure during imbibition in wheat seed. Annals of Botany. 1992; 71:411-416.
7. Gorinstein S, Elke Pawelzik, Efren Delgado–Licon, Kazutaka Yamamoto, Shoichi Kobayaashi, Hajiime Taniguchi *et al*. Use of Scanning Electron Microscopy to indicate the similoaities and differences in psedocereal and cereal proteins. International Journal of Food Science and Technology. 2004; 39:183-189.
8. Hossain MK, Ghosh SC, Boontongkong Y, Thanachayanont C, Dutta J. Growth of zinc oxide nanowires and nanobelts for gas sensing application. Journal of Metastable and Nanocrystlline Materials. 2005; 23:27-30.
9. Jo YK, Kim BH. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease. 2009; 93:1037-1043.
10. Johnson GA, Hicks DH. Use of temperature – responsive polymer seed coating to control seed germination. Proc. of the Int. Symp. Stand Establishment seed. (Eds. Liptay, Vavrina Welbanum), Acta Horticulture, ISHS. 1999; 504:229-236.
11. Junlakitjawat A, Thongpukdee A, Thepsithar C. Seed coat micromorphology of some solanum species in Thailand. Journal of the microscopy society of Thailand. 2010; 24(1):13-16.
12. Khodakovskaya Mariya, Enkeleda Dervishi, Meena Mahamood, Yang Xu, Zhongrui Li, Frimiya Watanabe *et al*. Biris. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth**.** American Chemical Society. 2009; 10(3):3221-3227.
13. Lin D, Xing L. Phytotoxicity of nanoparticles: inhibition of seed germination and root growth. Environment Pollution. 2007; 150:243-250.
14. Min JS. Effects of colloidal silver nanoparticles on sclerotium–forming phytopathogenic fungi. Plant Pathology Journal. 2009; 25:376-380.
15. Nair Remya, Saino Hanna Varghese, Baiju Nair G, Maekawa T, Yoshida Y, Sakthi Kumar D. Nanoparticles material delivery to plants. Plant science. 2010; 179:1154-1623.
16. Natarajan N. Effect of ZnO nanoparticles on vigour and viability blackgram. Unbulished data. (In press), 2020.
17. Nicewarner–Pena SR, Freeman RG, Reiss BD, He L, Pena LD, Walton ID *et al*. Submicrometer metallic barcodes. Science. 2001; 294(5540):137-141.
18. Panacek AM, Kolar R, Vecerova R, Prucek J, Soukupova VP, Hamal Zboril R *et al*. Antifungal activity of silver nanoparticles against spp. Biometals. 2009; 30:6333-6340.
19. Pandey Avinash C, Sharda Sanjay S, Raghvendra Yadav S. Application of ZnO nanoparticles in influencing the growth rate of *Cicer arietinum*. Journal of Experimental Nanoscience. 2010; 5(6):488-497.
20. Park HJ, Kim SH, Kim KH, Choi SH. A new composition of nanosized silica – silver of control of various plant diseases. Plant Pathology Journal. 2006; 22:295-302.
21. Roco MC, Williams RS, Alivasatos P. Nanotechnology Research Directions; IWGN Workshop Report, Kluwer Academy Polishers: Norwell, MA, 1999.
22. Singh M, Singh S, Prasad S, Gambhir IS. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest Journal Nanometer. Biostructure. 2008; 3:115-122.
23. Suguna A, Warad C, Thanachayanont C, Dutta J, Hofmann H. Zinc oxide nanowires on non – epitaxial substrates from colloidal processing for gas sensing application, Nanostructured and Advanced Materials for Application in Senson, Optoelectronic and Photovoltaic Technology, NATO – Advance Study Institute, Sozopol, Bulgaraia, 6- 17th September, 2004.
24. Vanangamudi K, Natarajan K, Saravanan T, Renuka Natarajan R, Umaraani R *et al*. Seed Hardening, Pellecting and coating. In: Principles and practices. (Eds: K. Vanangamudi *et al*.) Satish Serials Pub, 2006, 418-425.
25. Willson DO, McDonald MB. The lipid peroxidation model of seed deterioration. Seed Sci. and Technol. 1986; 14:296-300.
26. Yang Fang, Fashui Hong, Wenjuan You, Chao Liu, Fengqing Gao, Cheng Wu *et al*. Influence of nano – anatase TiO_2 on the nitrogen metabolism of growing spinach. Biological Trace Element Research. 2005; 110:179-189.
27. Zheng Lei, Yaseen Mohamed Y, Sheryl Barringer A, Water Splittstosser E, Suzanne Costanza. The role of seed coat in seed viability. The Botanical Review. 1994; 60:426-439.
28. Fashui Hong, Shipeng Lu, Chao Liu. Effects of nano–TiO_2 on strength of naturally aged seeds and growth of spinach. Biological Trace Element Research. 2005; 104:83-91.