Journal of Plant Growth Regulation (2020) 39:1107–1118
https://doi.org/10.1007/s00344-019-10046-7

Effect of Titanium Dioxide Nanoparticles (TiO_2 NPs) on Faba bean (*Vicia faba* L.) and Induced Asynaptic Mutation: A Meiotic Study

Kalyan Singh Kushwah[1] · Sapan Patel[1]

Received: 21 May 2019 / Accepted: 17 October 2019 / Published online: 13 November 2019

Abstract

We have synthesized titanium dioxide (TiO_2) nanoparticles (NPs) and studied the cytogenotoxic effect of the synthesized NPs on plants. The synthesized NPs were characterized by XRD, SEM and PSA. The XRD results showed the formation of crystalline TiO_2 nanoparticles. The SEM analysis showed that the synthesized nanoparticles range from 60 to 300 nm. The effect of synthesized TiO_2 nanoparticles on *Vicia faba* (2n = 12) was studied. The seeds of *Vicia faba* were treated with different concentrations (15, 30, 60, 120 and 240 mg/L, or 1.5, 3.0, 6.0, 12.0 and 24.0 mg/100 mL) of TiO_2 nanoparticles. Seeds treated with the higher concentrations of TiO_2 showed a change in the meiotic activity which causes a significant increase of chromosomal abnormalities in the reproductive parts of the plant. Different types of meiotic abnormalities, such as stickiness and the separation of univalent and bivalent chromosomes at metaphase were recorded. It was found that the number of univalent chromosomes ranged from 2 to 12 in 95% of pollen mother cells in diakinesis/metaphase I, and that there was a significant decrease in the number of chiasmata in seeds which were treated with the synthesized nanoparticles as compared to the control.

Keywords Asynaptic mutant · Cytotoxicity · Faba bean · Meiosis · *Vicia faba* · Titanium dioxide · Univalent/bivalent chromosomes

Abbreviations

AS	Asynaptic
M	Generation of plants
NPs	Nanoparticles
PMCs	Pollen mother cells
TiO_2	Titanium dioxide
V. faba	*Vicia faba*

Introduction

It is believed by many scientists that nanotechnology can bring the next revolution in biology, physics, medical science and chemistry, etc. (Gottschalk et al. 2013; Sun et al. 2015). In our daily life, titanium dioxide (TiO_2) nanoparticles (NPs) are used in paint, glass, cosmetics, solar cells and water-treatment products, and, after the use of these products, TiO_2 NPs in large quantities reaches the air, soil and water. As a result, TiO_2 NPs come into contact with the environment, plants and living organisms, and enter into the cells that join the cellular elements. Hence, for this reason, nanotoxicity research is drawing attention to plants. TiO_2 works as a photocatalyst, and photocatalysts contribute to water treatment by oxidizing organic pollutants in harmless material. TiO_2 NPs are favored because of their high photostability and high photon activity, and because they are easily available and affordable. TiO_2 compounds can compensate for inadequacies of nitrogen and promote different growth in crops, such as plant biomass and the activity of various enzymes, and increase the chlorophyll content.

Titanium is an essential element for plant growth and the role of Ti in crops is through the encouragement of the activities of certain enzymes, increasing the chlorophyll content and photosynthesis, and improving crop morphology and growth. However, high concentrations of TiO_2 NPs in in vivo and in vitro systems have been considered toxic to plants (Rafique et al. 2015). TiO_2 NPs increased the adhesion of beneficial bacteria to the roots of oilseed plants and protected the plants from infection (Palmqvist et al. 2015). Raliya et al. (2015) observed the activity and increase of Rubisco activity in photosynthesis with TiO_2NPs

✉ Kalyan Singh Kushwah
kalyansinghkushwah85@gmail.com

[1] SOS Botany, Jiwaji University, Gwalior, MP 474011, India

leaf treatment as well as the improved growth of plants, increased fruit yield and increased chlorophyll concentrations in *Solanum lycopersicum*. Some of the large NPs have been reported to form large pores to facilitate their entry through plant cell walls (Rastogi et al. 2017).

In the last two decades, many scientists have studied the effect of different types of NPs on different plants. NPs of heavy metals (HMs) play important roles in the metabolism, growth and development of plants as micronutrients; however, excessive amounts of HM NPs in the soil are harmful to plants. Apart from NPs, the effect of HMs is also being studied in plants, as they play an important role in the metabolism and cooperate in plant growth and development. However, at levels above the threshold, these act as HM contaminants and pose a worldwide environmental threat (Sytar et al. 2019). The effect of NPs on chromosomes has been observed in mitosis in different parts of plants, such as roots, shoots, leaves, stems, etc. The variable size of the silver NPs showed a clear correlation between the size and toxic relationship of NPs to the plants (Rico et al. 2011). NPs with a smaller size showed a higher toxicity to plants as compared to larger-sized NPs (Mehta et al. 2016; Jiang et al. 2014; Cvjetko et al. 2017). TiO_2 NPs promote nitrogen metabolism, photosynthesis and improved growth of plants (Yang and Watts 2005). The meiotic root meristems of *Vicia faba* have been used as cytogenetic material for the study of chromosomal aberrations and mutations (Ma 1982; Kanaya et al. 1994). TiO_2 NPs are reported to be potentially hazardous to the Rhizobium–Legume symbiotic system (Fan et al. 2014). In lettuce plants, TiO_2 NPs help in phosphorus uptake (Hanif et al. 2015) and nitrogen assimilation in spinach (Yang et al. 2007). There have been a number of studies of cytotoxicities, such as chromosomal aberration, alternation mitotic, DNA damage and micronucleus formation (Ghosh et al. 2010; Castiglione et al. 2011; Pakrashi et al. 2014).

Vicia faba, known in the culinary sense as the broad bean, fava Bean or faba bean, is a species of flowering plant belonging to the Fabaceae family. It is widely cultivated as a crop for human consumption in India, China, Pakistan, Europe, Australia and North Africa. It is grown in cold climates in soil having a high salinity. Faba beans are said to be used as a diuretic, tonic and expectorant. It is cultivated as a vegetable and is consumed green or dried, fresh or canned. It is also used as a stock feed. In the Middle East, faba beans are one of the most important winter crops for human consumption. *V. faba* seeds are rich in vitamins, carbohydrates, starch, fiber and proteins. The most important characteristics for improving the seed yield of *V. faba* are considered the plant height, number of stems and number of pods, the organic yield, crop index, 100-seed weight and the number of days needed for flowering and maturity (Li and Yang 2014; Loss and Siddique 1997). *V. faba* has the ability to fix atmospheric nitrogen by a symbiotic relationship with bacteria in the root nodules of the plants (Verma 2004). A large genetic variability has already been identified in *V. faba* in terms of flowers, seed size and structure, and also tolerance to major biological and abiotic stresses (Singh et al. 2013). *V. faba* has a diploid (2n) chromosome range of 12 (six homologous) pairs.

Materials and Methods

Synthesis of TiO_2 NPs by a Precipitation Method

TiO_2 particles were suspended in 10 M of NaOH solution and heated at 100 °C for 3 h under reflux with constant stirring using a magnetic stirrer. The pH value of the suspension was maintained at 5 by adding 1 M HCl with continuous stirring for 5 h, so that ion exchange took place completely, forming NaCl. The solution was allowed to rest for 8 h while the suspended TiO_2 NPs settled. After filtering and washing with distilled water and drying at 90 °C for 6 h, further heat treatment was given at 500 °C to form the anatase phase of TiO_2 NPs (Tiwari et al. 2017).

Biological Material

The seeds in the present investigation were of the plants (a local variety of *V. faba*) maintained at the Botanic Garden of the School of Studies in Botany, and used for the cytogenetic and morphology studies, and the studies of the effect of TiO_2 NPs on *V. faba*.

Treatment of Seeds and Collection

Dry seeds of *V. faba* (6.64% moisture) were sterilized with 4% sodium hypochlorite solution and wash with distilled water. Different concentrations (15, 30, 60, 120 and 240 mg/L) of the TiO_2 NPs were prepared and sonicated for 3 h. The seeds were treated with the different concentrations and kept for 24 h. They were then sown (10 seeds) in separate Petri dishes and transferred to pots in the first week of November at 22 ± 2 °C. The treated plants were observed for the various morphological changes, such as roots, shoots, leaves, and the size of TiO_2flowers and seeds. For the meiotic studies, young flower buds were fixed in Corney's fluid (ethanol:acetic acid 3:1) in the morning between 0800 and 1000 hours. The flower buds were collected in different collection bottles from each individual plant, while the anthers were stained and squashed in 2% iron acetocarmine (Kushwah et al. 2018).

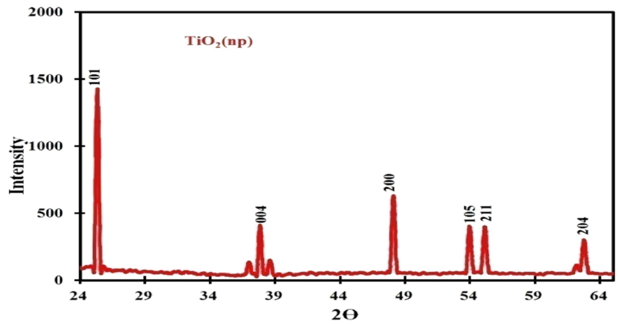


Fig. 1 XRD pattern of TiO_2 NPs synthesized by TiO_2 and NaOH aqueous solution

Statistical Analysis

The significance of the differences between the treated TiO_2 NPs and control groups of *V. faba* were tested by one-way analysis of variance using SPSS statistical software (SPSS, IBM, USA). All the data were represented as arithmetic means ± SE (standard error) of seed germination, plant survival, and root and shoot growth.

Characterization of TiO_2NPs

Various techniques were used to characterize the synthesized samples (TiO_2) such as X-ray diffraction (XRD), UV–visible spectroscopy (UV–vis), particle size analysis (PSA) and scanning electron microscopy (SEM).

X-ray Diffraction (XRD)

XRD (Modal no Mini Flex 600) was the most commonly used technique for the characterization of the NPs, and was used in powder samples to provide information about their crystalline structure, to estimate the average particle size, the nature of the phases, the lattice parameters, and the crystalline grain sizes. The XRD analysis of the prepared samples of TiO_2 NPs was taken for the 2θ range of 24°–65°, and the peaks obtained confirmed the TiO_2, as he peaks at 25.28, 37.8, 48.04, 53.89, 55.06 and 62.68 were the corresponding the (101) (004) (200) (105) (211) and (204) planes of TiO_2, respectively (Fig. 1). The obtained data were matched with the Joint Committee on Powder Diffraction Standards (JCPDS card no 21-1272).

UV–vis Spectroscopy

UV–vis spectroscopy (UV-1280 Multipurpose) of the TiO_2 NPs was carried out using a Cary 60 UV–Vis Spectrometer. The synthesized TiO_2 NPs were represented by the UV–vis transmission spectra, and the formation of milky-white colloidal solutions indicated the conversion of titanium nitrate into TiO_2 NP colloidal particles. The physical properties of the TiO_2 NPs were also investigated using UV–vis spectroscopy, and the synthesis of the TiO_2 colloidal particles was confirmed with transmittance spectra between 150 and 750 nm. Figure 2 and 3 show the graphs of the transmission spectra of the TiO_2 NPs, revealing that, above the wavelength of 250 nm, all the incident rays were transmitted.

Figures 2 and 3. UV-vis transmitted spectra of TiO_2 NPs recorded in 250 nm wavelength and energy bandgap of TiO_2 NPs.

The energy bandgap of the TiO_2 NPs is shown in Fig. 3 and was determined by plotting the graph and found to be 3.4 eV which is the same as previously reported data.

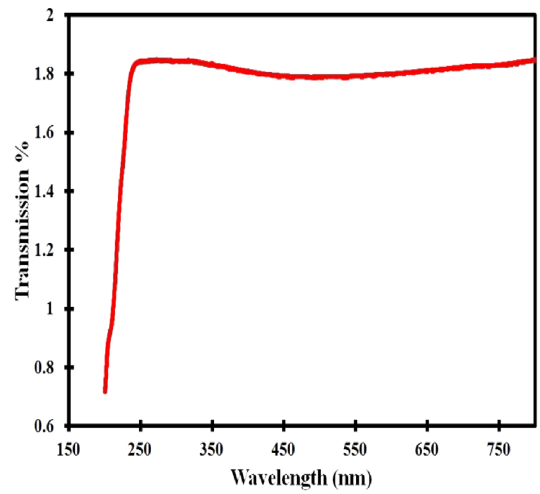


Fig. 2 UV–vis transmitted spectra of TiO_2NPs recorded at 250 nm wavelength

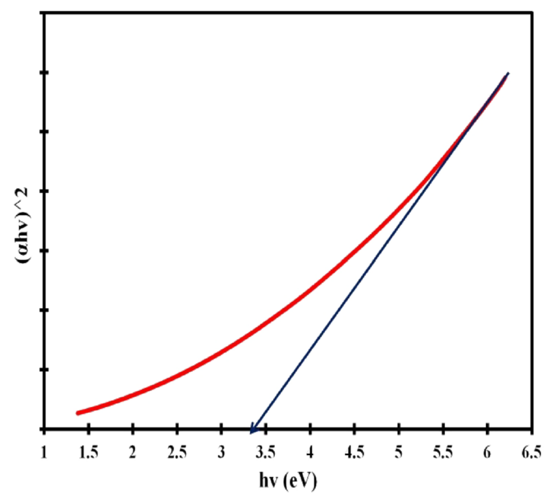


Fig. 3 UV–vis energy gap of TiO_2 NPs

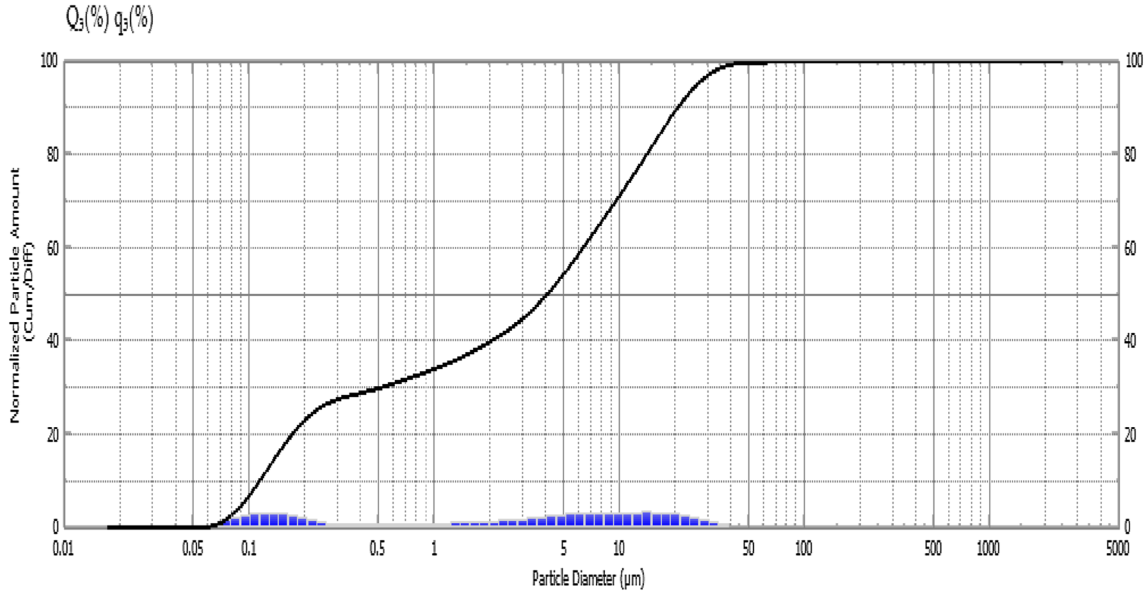


Fig. 4 TiO_2 NPs image from the PSA

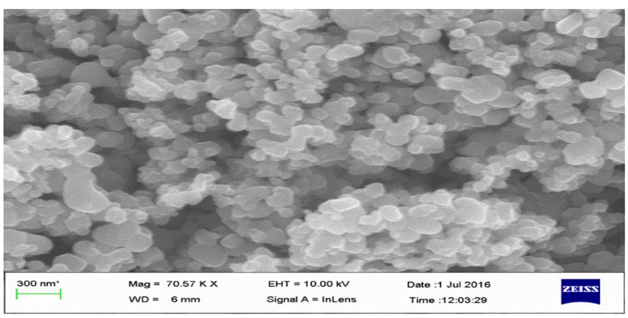


Fig. 5 SEM micrograph of TiO_2NPs

Particles Size Analyzer (PSA)

In the PSA (Shimadzu Modal no. SALD-2300), a gas laser is used as a coherent light source, and the particle size distribution is calculated from intensity variations within the diffraction pattern at infinity. The average particle diameter ranged from 60 to 300 nm (Fig. 4), which is almost same as the SEM analysis below.

Scanning Electron Microscope (SEM)

The SEM (Zeiss EVO MA10) micrograph in Fig. 5 suggests that the TiO_2 NPs are more or less spherical in shape or have a core–shell structure. The chemical composition of the specimens can be determined by analyzing the secondary electrons, while the TiO_2 NPs size ranged from 60 to 300 nm, as shown in the figure.

Results

Seed Germination of *Vicia faba*

The control and the seeds in the different concentrations (10 seeds/dose) were prepared before treatment, and the average weight of three replicates ($n=3$ or $10 \times 3=30$) of each dose was found. The seeds were soaked in TiO_2 NP solutions with concentrations of 15, 30, 60, 120 and 240 mg/L. The weight of the control seeds was 1.88 g (average/dose) and this weight was higher than the seed doses of the different concentrations. The highest average weight in the seed doses in the different concentrations was 1.86 g in 30 mg/L and the lowest average weight was 1.71 g in 120 mg/L (Table 1).Ten seeds were included in each Petri dish at room temperature, and the germination rate of the seeds was checked every 5 days.

Germination percentage was lower for higher concentrations than for lower concentrations: 100% seeds germinated at 15 mg/L, 90% at 30 mg/L, 50% at 60 mg/L, 70% at 120 mg/L, 60% at 240 mg/L distilled water and 100% seeds germinated in the control 15 days after the seeds were sown (Fig. 6). The first control seeds germinated after 8 days but all control seeds germinated within 8 to 15 days and the average time was 12.1 days while the treated seeds started to germinate within 5 to 11 days. Except for seeds treated with 60 mg/L, the germination time of the first seed of all other treated seeds was less than the germination time of the control seeds. The germination time of all seeds treated with 15 and 30 mg/L was 6 to 11 days and the average time was 8.9 days, the maximum time for germination of all

Table 1 The weight and effect of different concentrations of TiO_2 NPs on the seed germination and first seedling time

Sample no.	Concentration (mg/L)	Seed weight (average) in g/dose	First seed germination time from sowing (days)	First seedling time from sowing (days)
1	Control	1.88	8	13
2	15	1.84	6	11
3	30	1.86	6	10
4	60	1.84	11	15
5	120	1.73	7	10
6	240	1.71	5	9

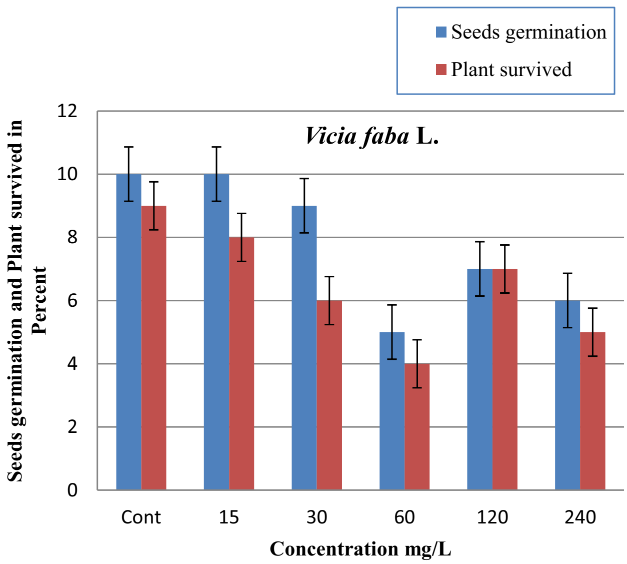


Fig. 6 Percentages of *V. faba* seed germination and plant survival with standard errors (0.86 and 0.76); the level of significance was $p<5$

seeds treated with 60 mg/L was 11 to 20 days and the average time was 18.7 days. The germination time of all seeds treated 120 mg/L was 7 to 16 days and the average time was 13.5 days and the germination time of all seeds treated with 240 mg/L was 5 to 8 days and the average time was 6.7 days. This was less than the time taken for germination of treated seeds from all other concentrations (Fig. 7; Table 1).

The time from seed sowing to the first seedling was measured for the control and the treated seeds. It took 13 days for the first seedling in the control seeds, while seeds treated with different concentrations of TiO_2 NPs took different times. The maximum time was 15 days in 60 mg/L concentration and the shortest time was 10 days in the 30 mg/L and 240 mg/L concentrations. According to the results, the 60 mg/L concentration of TiO_2 NPs increases seed germination and seedling times, while all other concentrations reduce them (Table 1).

Plants Survival of *Vicia faba*

Only 80% plants survived in 15 mg/L, 60% in 30 mg/L, 40% in 60 mg/L, 70% in 120 mg/L, 50% in 240 mg/L of

Fig. 7 Seed germination of *V. faba* for seeds in the control and treated with different concentrations (mg/L) of TiO_2 NPs

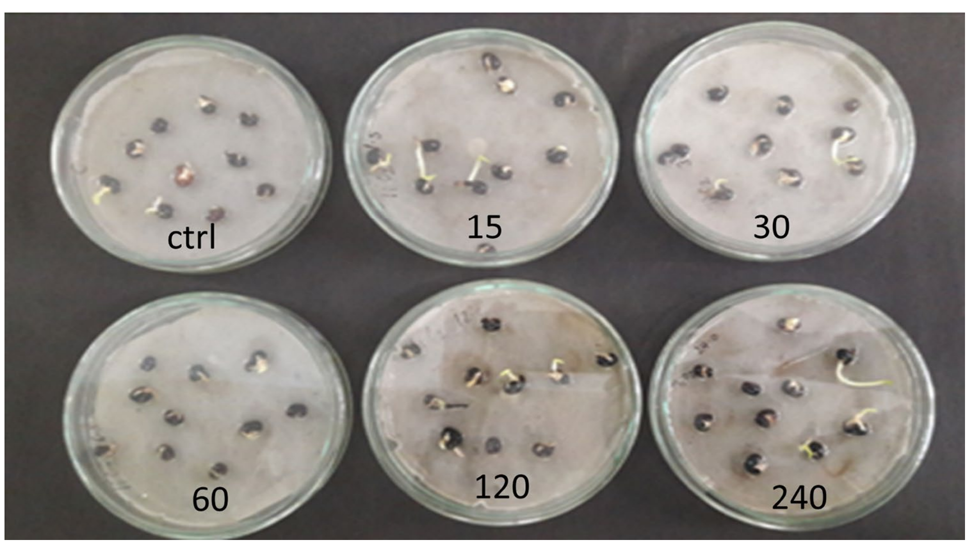


Fig. 8 Root and shoot growth of control and treated plants with TiO_2 concentrations of mg/100 mL or mg/L distilled water 20 days after seed sowing

Table 2 Root and shoot growth mean and ± SE, stem height and first flower time of control and treated plants

Sample no.	Concentration (mg/L)	Root growth (in cm) after 20 days (mean ± SE)	Shoot growth (cm) after 20 days (mean ± SE)	Stem height at first flower (cm)	Time of first flower (days)
1	Control	0.97 ± 0.21	2.95 ± 0.12	79	82
2	15	0.38 ± 0.04	1.73 ± 0.13	64	74
3	30	0.24 ± 0.03	1.64 ± 0.11	73	69
4	60	0.84 ± 0.15	2.76 ± 0.17	78	76
5	120	0.72 ± 0.09	2.82 ± 0.11	96	56
6	240	0.51 ± 0.07	3.05 ± 0.12	67	71

TiO_2 NPs, and 90% plants survived as control 30 days after the seeds were sown (Fig. 6). Some plants did not survive because of the regime of physical care.

Plants Growth

Rate of Success

Following the seed treatment method (120 mg/L), one mutant plant was produced. The rate of success was 14.28%.

Growth Rate

Different concentrations of TiO_2 NPs treatment had a significant effect on root and shoot growth ($p < 5$). Thus, for root elongation, root and shoot growth was a mean 0.97 cm and 2.95 cm, respectively, of control plants while there was higher root growth with a mean 0.84 cm at 60 mg/L and higher shoot growth with a mean 3.05 cm at 120 mg/L after 20 days (Fig. 8; Table 2).

A typical slower rate of growth was observed in mutant plants compared the control plants (Fig. 9, {5}). While it took 56 days for the first flowering with a 79-cm plant height for the control, it took 82 days with a 96-cm height in the mutant plant. The flowering was delayed by 26 days and there was a 17-cm height increase in the mutant plant compared with the control plant (Fig. 9; Table 2).

Meiosis in Control Plants of *Vicia faba*

The major aspect undertaken in the present investigation has been cytogenetics (meiotic) studies of *V. faba*. A total of 100 Pollen mother cells (PMCs) were analyzed at the diakinesis/metaphase-I in the control. *V. faba* has a diploid (2n) chromosome range of 12 (six homologous pairs) of which five pairs are acrocentric chromosomes and one pair is metacentric. The control plant had 6II with 20 chiasmata at the diakinesis/metaphase-I (Fig. 10a), and anaphase-I and II had a normal distribution of chromosomes, 6:6, (Fig. 10b, c) of meiosis. In the diploids, pollen stainability was 100%.

Meiosis in the Mutant Plant of *Vicia faba*

The present research work indicates the effect of TiO_2 NPs on *V. faba* demonstrating that they induce chromosomes aberrations. Asynaptis in meiotic cells is significant, as the persistence of such changes can bring heritable alteration in the genotype. The mutant was identified during the meiosis

Fig. 9 Control and treated plants of *V. faba* with TiO_2 concentration in mg/L distilled water

of a plant (M2 generation) from the 120-mg/L treated seeds. The chromosome's behavior was completely different from that of the control (Fig. 10d–l). At diakinesis/metaphase-I in the asynaptic mutant out of 178 PMCs observed in M2, 30 had normal 6II bivalents, 36 had 5II + 2I, 22 had 4II + 4I, 34 had 3II + 6I, 32 had 2II + 8I, 14 had 1II + 10I and 10 had 12I (Fig. 10d–f). Of the 138 PMCs observed in M3, 55 had normal 6II bivalents, 25 had 5II + 1I, 18 had 4II + 4I, 23 had 3II + 6I and 17 had 2II + 8I (Fig. 10g–i). And of the 104 PMCs observed in M4, 68 had normal 6II bivalents, 14 had 5II + 2I, 13 had 4II + 4I and 9 had 3II + 6I (Fig. 10j–l) (Table 3).

In some plant PMCs with more univalent chromosomes, these were in polar orientation (Fig. 10d, e), and the univalent appeared more scattered (Fig. 10f–i). In the PMCs within 3–5 bivalents, the univalent chromosomes were closer to the equator of the cell (Fig. 10 j–l).

Chiasmata were randomly present in nature and always terminal in position (Fig. 10b–l). The mean number of chiasmata /PMCs at diakinesis/metaphase-I was only 3.5 as compared to 20 of the control diploid, and of these 1.9 were terminalized and 1.6 unterminalized, giving a terminalization coefficient of 0.59 in the M2, 0.48 in the M3 and 0.52 in the M4 generations (Table 4; Fig. 10b–l) in the partial asynaptic mutant. The pollen fertility was 55%.

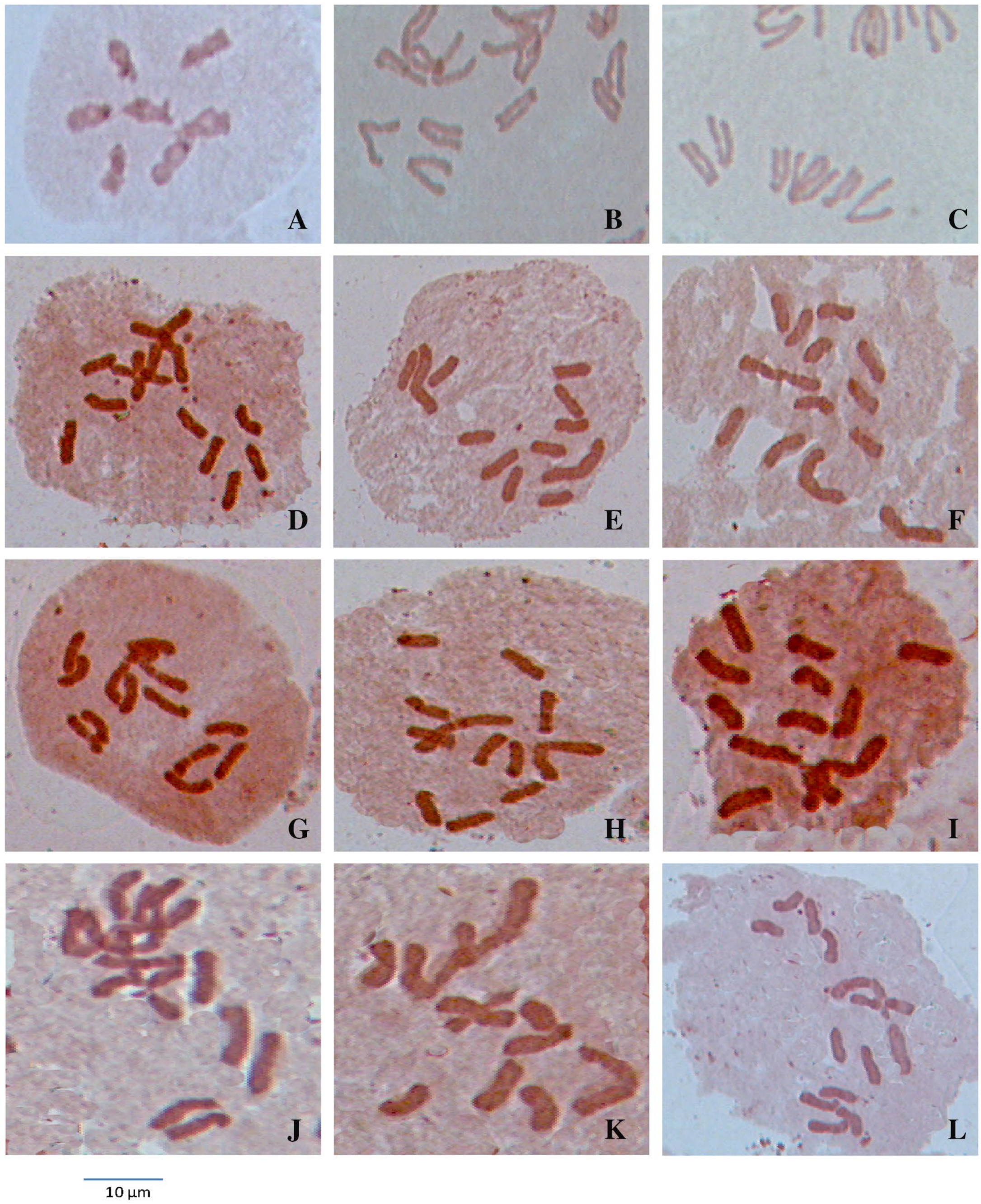
The asynaptic mutation isolated in M2 produced 24 seeds, whereas 2 plants in M3 again exhibited partial asynaptic behavior and produced 7 seeds. Similarly, in M4, one plant again exhibited partial asynaptic behavior and 4 seeds were collected from this plant. The collected mutant seeds will be used to cross the next generation with the control plants.

Discussion

The three main objectives were studied concerning the genotoxicity of TiO_2 NPs on *V. faba* L.: (1) the synthesis of TiO_2 NPs, (2) characterizations of synthesized NPs, and (3) the effect of NPs on *V. faba*.

XRD analysis was used extensively to examine the shape of the crystalline NPs. The incident angle relative to a crystal that has constant internuclear spacing is related to the wavelength of the mediated radiation. The TiO_2 NPs were synthesized by a chemical route in the laboratory. The average particle size of the NPs was found to be 60–300 nm. The crystalline nature of the NPs was confirmed by XRD. The synthesized NPs had an energy band gap of 3.4 eV. The physical properties of the TiO_2 were examined using UV–vis spectroscopy. The synthesis of TiO_2 NPs was confirmed by the transmission spectra between 150 and 750 nm. The SEM micrograph suggested that TiO_2NPs were more or less crystalline and spherical in shape (Figs. 1, 2, 3, 4, 5).

To our knowledge, this is the first report on absorbed TiO_2 NPs in seed tissue showing the phytotoxicity and genotoxicity effects of nano-TiO_2 on plants. Ionic TI (T1 = 0, T2 = 1.25, of five different levels on morphology, growth, biomass distribution, chlorophyll fluorescence performance and Rubisco activity of *Glyacine max* L. soybean under normal light and shade conditions, T3 = 2.5, T4 = 5



Meiotic stages of control and Asnaptic mutant plants Diakinesis/Metaphase I - Control Fig.-A,B and C . AS(M2) Fig.- D,E and F AS(M3) Fig.- G,H and I AS(M4) Fig.- J,K and L.

Fig. 10 The distribution of univalent and bivalent chromosomes in pollen mother cells diakinesis/metaphase-I control and asynaptic mutant plant (AS). *AS* asynaptic mutant, *M* generation of plants

and T5 = 10 mg Ti plant—1) haas been studied (Hussain et al. 2019). The germination rates of *Vicia faba* species were different between treatments. Table 1 shows that TiO_2 NPs were absorbed by the seeds. Germination rates of *V. faba* species have been shown by each treatment group separately. The high treatment group with 24-h treatment showed a negative effect of TiO_2 NPs on germination. In this study, seeds were treated for a shorter time (24 h) than previous studies (Movafeghi et al. 2018). The seeds were tested for germination at room temperature. Ten seeds were included in each Petri plate ($n = 3$) and the germination rate was checked every 5 days. The seeds were soaked in TiO_2

Table 3 Distribution of bivalents and univalents at diakinesis/metaphase I in control and asynaptic mutant (*AS*) of *V. faba*

Plant type	No. of cells observed	6II	5II + 2I	4II + 4I	3II + 6I	2II + 8I	1II + 10I	12I
Control	100	100	—	—	—	—	—	–
AS1 (M2)	178	30	36	22	34	32	14	10
AS2 (M3)	138	55	25	18	23	17	–	–
AS3 (M4)	104	68	14	13	9	–	–	–

Table 4 Total number of chiasmata terminalized, unterminalized and the terminalization coefficient control and TiO_2 NPs induced asynaptic mutation

Plant type	No. of chiasmata/cell	Chiasmata terminalized	Chiasmata unterminalized	Termilization coefficient
Control	20 ± 1.8	9.6 ± 0.8	10.4 ± 1.2	0.47 ± 0.02
AS1 (M2)	3.50 ± 1.1	1.9 ± 0.7	1.6 ± 0.8	0.59 ± 0.25
AS2 (M3)	5.23 ± 2.06	2.6 ± 1.3	2.5 ± 1.1	0.48 ± 0.21
AS3 (M4)	6.40 ± 2.2	3.3 ± 1.4	3.3 ± 1.3	0.52 ± 0.17

NP solutions with concentrations of 15, 30, 60, 120, and 240 mg/L. Because a previous study on nano-TiO_2 solutions used concentrations up to 5000 mg/L and found significant effects on germination and growth of *V. faba* in 5000 mg/L (Song et al. 2013), 120 mg/L was considered sufficient to test for toxicity. Another previous study on TiO_2 NP solutions used concentrations up to 6000 mg/kg and did not report any negative effects (Zheng et al. 2005). The concentrations above 6000 mg/kg have been considered unrealistic in natural systems, as concentrations of 500 mg/kg or less have been used in other studies on TiO_2 NPs (Trouiller et al. 2009; Nohynek et al. 2008; Newman et al. 2009).

We placed ten seeds in each Petri dish, with three replicates and measured the germination rate every 5 days. However, 5 days later, germination had only started at 240 mg/L. Except for concentrations of 60 mg/L, all the other concentrations had a positive effect on the seed germination rate compared to control. This study reported positive and negative effects of TiO_2 NPs on the germination rate. The results of seed germination and root elongation from previous studies suggest that the effect of TiO_2 NPs was possible with the previous two opposing results: positive effects and negative effects of nano-TiO_2 (Ghosh et al. 2010, 2016).

Cytotoxicity of NPs also depends on the cell type, and the different types of cells (epithelium, connective, nerve, macrophages, etc.) can affect all factors (Vevers and Jha 2008). Earlier studies showed that TiO_2 NPs had an effect on the response of plant chromosomes with few reported mitosis studies of chromosomes, but this current research includes meiosis studies of plant chromosomes. This is a special kind of research, in which the synthesis of TiO_2 NPs showed the effects of TiO_2 NPs on plants, with meiosis studies and chromosome aberrations occurring in the next generations, in which the phytotoxicity and genotoxicity effects of TiO_2 NPs were observed on *V. faba*. This research result was different from results of other research. For the last two decades, many kinds of research have been carried out in plant nanotechnology, in which physiological and toxicological effects of various types of NPs (Ag, TiO_2, ZnO, Au, Fe, Cd, etc.) were observed in plants. Ag NPs caused oxidative stress in the onion root and demonstrated toxicity when applied in high concentrations of Ag NPs (Cvjetko et al. 2017). Patlolla et al. (2012) have reported the use of *V. faba* root-tip meristem to examine the genotoxicity of Ag NPs under modified Gene-TOX test conditions. Root-tip cells of *V. faba* were treated with four different concentrations of engineered Ag NP dispersions to study toxic endpoints, such as the mitotic index, chromosomal aberrations and micronucleus induction. Three different concentrations (0, 50 and 75 ppm) of ZnO NPs were applied to three different crop species (wheat, cowpea and brassica) via foliar spray. After harvesting, shoot and root parameters were compared, and it was observed that root elongation was observed at 50-ppm treatment in cowpea, while improved shooting parameters were recorded at 75 ppm in brassica (Mehta et al. 2016). Ag NPs were able to cause oxidative stress in plants and affect the chloroplast structure and function of *S. polyrhiza* (Jiang et al. 2014). Our research is based on TiO_2 NPs, which had a dramatic effect on cellular response due to size, surface charge, and dissimilarity, which were caused by altered cellular uptake, bioavailability, and toxic reaction (Magdolenova et al. 2014). Genotoxicity studies at the cytological level in the root meristem through traditional cytogenetic approaches evidenced possible changes in mitotic activity, chromosomal aberration and micronuclei release. The emergence of weather as a nano-TiO_2-induced genotoxic effect resulted in a decrease and an increase in reduction dependence (Castiglione et al. 2011). In our research, the genotoxicity effect of TiO_2 NPs studied meiosis in the reproductive part of plants. Studies of the asynaptic genotoxicity effect of NPs in international journals reporting on plants were very difficult to find. The number of diploid (2n) chromosomes in *V. faba* is 12 (six homologous pairs), of which there are five pairs of acrocentric chromosomes while one pair is metacentric. In the present study, one asynaptic mutant was isolated from the M2 population showing the cytogenetical consequences (using meiotic attributes including primary morphological parameters like seed germination, root and shoot growth, flowering) of TiO_2 NPs treatment in *V. faba*. It has been previously reported that EMS induced asynaptic mutants in *V. faba* (Joshi and Verma 2005). The chromosome aberrations are changes in chromosome structures resulting from a break or exchange of chromosomal material. In the present study, different kinds of chromosomal aberrations in meiosis were observed with the 120 mg/L TiO_2 concentration. The cytological chromosomes aberrations in mitosis like breaks, gaps, bridges, disturbed metaphase and cell wall disintegration were notable in the treated cells. Mitosis chromosomal stickiness was observed in the metaphase and anaphase (Kumari et al. 2009). Rico et al. (2011) studied the effect of Ag NPs on *Arabidopsis thaliana* and ryegrass, including the effect of nanomaterials on seed germination or on 15-day-old seedlings. According to these studies, the biotransformation of nanomaterials in a very few reference food crops, the potential transmission of next-generation in nanomaterials and the possible biomagnification of NPs in the food chain is also unknown. In the current research, the effects of TiO_2 NPs have been known for up to three generations. According to the results, a mutant plant was identified in the AS1 M2 generation and 24 mutant seeds were collected. A total of 178 PMCs of the mutant plant and 148 PMCs of the univalent chromosomes have been observed, out of which 10 PMCs were of higher univalent chromosomes (12 I), in which all the chromosomes were univalent. In the next generations, a decrease in the number of univalent chromosomes was demonstrated.

Conclusion and Future Perspectives

Many scientists have demonstrated the effectiveness of various NPs on plants, including Ag, TiO_2, ZnO, Au, Fe, Cd, etc., and have reported different types of results. International journal reporting studies have included research on Ag NPs and their effects on plants have been shown to be root elongation, shoot elongation, genotoxicity and plant development. Some scientists have shown both negative and positive expressions of NPs on plants. Toxicity on plants has been demonstrated by NPs of small size and low concentrations. In general, the genotoxicity effects of NPs have been reported by studying mitosis in plants. In the current research, TiO_2 NPs were synthesized by the chemical precipitation method, and characterization was performed by XRD, PSA, UV–vis and SEM, including NPs shape, size and experimental techniques. The toxicity of TiO_2 NPs occurs at several levels including cytotoxicity, genotoxicity, germination rate, root elongation, shoot elongation and development. Seed treatments and germination were studied with soaked filter papers set in Petri dishes and the negative and positive effects of different concentrations (15, 30, 60,120 and 240 mg/L) of TiO_2 NPs on *V. faba* plants. All the *V. faba* plants were transferred to pots for meiosis studies. Cytogenotoxicity and chromosome aberrations (synaptic mutation) were reported in *V. faba* meiosis studies affected by 120 mg/L concentrations of TiO_2 NPs. In this study, bivalent chromosome deficiency and univalent chromosome excess were observed. In diakinesis/metaphase-I of PMCs in asynaptic mutations, univalent chromosome numbers, bivalent chromosome numbers, chiasma frequency and termilization coefficients were observed. The number of univalent chromosomes and seed production was reduced in AS2 (M3) and AS3 (M4) mutant plants, respectively, compared to AS1 (M2) mutant plants. Nowadays in the field of plant research there is a need for meiotic studies of the genotoxicity and cytotoxicity effects of various types of NPs. The increases in the seed yield of *V. faba* will result in greater economic profits and larger food supply in the whole world. Moreover, we propose studies of the toxicity of NPs on the plants, and their meiotic studies, between cross-mutant and control plants, and the change of chromosome ploidy number in plants, which will produce increased production, and hybridized and disease-resistant seeds.

Acknowledgements The authors are grateful to the Head of Department, School of Studies in Botany, Jiwaji University, Gwalior, for providing support. We are also grateful to the central instrumentation facility and the physics department of Jiwaji University for providing all the required instruments.

Author Contributions KSK designed the plan of work, performed the experiments, prepared the manuscript, and the characterization of TiO_2 NPs. SP co-wrote the manuscript together with KSK, supervised the study and provided valuable suggestions to improve the study.

Compliance with Ethical Standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Research Involving Human and Animal Rights The authors declare that the current work was done on plants and there was no involvement of animals. We declare that this work has no harmful effects on any animals or human beings.

References

Castiglione MR, Giorgetti L, Geri C, Cremonini R (2011) The effects of nano-TiO_2 on seed germination, development and mitosis of root tip cells of *Vicia narbonensis* L. and *Zea mays* L. J Nanopart Res 13(6):2443–2449

Cvjetko P, Milošić A, Domijan AM, Vrček IV, Tolić S, Štefanić PP, Balen B (2017) Toxicity of silver ions and differently coated silver nanoparticles in *Allium cepa* roots. Ecotoxicol Environ Saf 137:18–28

Fan R, Huang YC, Grusak MA, Huang CP, Sherrier DJ (2014) Effects of nano-TiO_2 on the agronomically-relevant Rhizobium–legume symbiosis. Sci Total Environ 466:503–512

Ghosh M, Bandyopadhyay M, Mukherjee A (2010) Genotoxicity of titanium dioxide (TiO2) nanoparticles at two trophic levels: plant and human lymphocytes. Chemosphere 81(10):1253–1262

Ghosh M, Jana A, Sinha S, Jothiramajayam M, Nag A, Chakraborty A, Mukherjee A (2016) Effects of ZnO nanoparticles in plants: cytotoxicity, genotoxicity, deregulation of antioxidant defenses, and cell-cycle arrest. Mutat Res/Genet Toxicol Environ Mutagenesis 807:25–32

Gottschalk F, Sun T, Nowack B (2013) Environmental concentrations of engineered nanomaterials: review of modeling and analytical studies. Environ Pollut 181:287–300

Hanif HU, Arshad M, Ali MA, Ahmed N, Qazi IA (2015) Phyto-availability of phosphorus to *Lactuca sativa* in response to soil applied TiO_2 nanoparticles. Pak J Agric Sci 52(1):177–182

Hussain S, Iqbal N, Brestic M, Raza MA, Pang T, Langham DR, Liu W (2019) Changes in morphology, chlorophyll fluorescence performance and Rubisco activity of soybean in response to foliar application of ionic titanium under normal light and shade environment. Sci Total Environ 658:626–637

Jiang HS, Qiu XN, Li GB, Li W, Yin LY (2014) Silver nanoparticles induced accumulation of reactive oxygen species and alteration of antioxidant systems in the aquatic plant *Spirodela polyrhiza*. Environ Toxicol Chem 33(6):1398–1405

Joshi P, Verma RC (2005) Ethyl methane sulphonate (EMS) induced (partial) asynaptic mutant in faba bean (*Vicia faba* L.). Cytologia 70(2):143–147

Kanaya N, Gill BS, Grover IS, Murin A, Osiecka R, Sandhu SS, Andersson HC (1994) *Vicia faba* chromosomal aberration assay. Mutat Res/Fund Mol Mech Mutagenesis 310(2):231–247

Kumari M, Mukherjee A, Chandrasekaran N (2009) Genotoxicity of silver nanoparticles in *Allium cepa*. Sci Total Environ 407(19):5243–5246

Kushwah KS, Verma RC, Patel S, Jain NK (2018) Colchicine induced polyploidy in *Chrysanthemum carinatum* L. J Phylogenetics Evol Biol 6(193):2

Li X, Yang Y (2014) A novel perspective on seed yield of broad bean (*Vicia faba* L.): differences resulting from pod characteristics. Sci Rep 4:6859

Loss SP, Siddique KHM (1997) Adaptation of faba bean (*Vicia faba* L.) to dryland Mediterranean-type environments I. Seed yield and yield components. Field Crops Res. 52(1–2):17–28

Ma TH (1982) Vicia cytogenetic tests for environmental mutagens: a report of the US environmental protection agency gene-tox program. Mutat Res/Rev Genet Toxicol 99(3):257–271

Magdolenova Z, Collins A, Kumar A, Dhawan A, Stone V, Dusinska M (2014) Mechanisms of genotoxicity A review of in vitro and in vivo studies with engineered nanoparticles. Nanotoxicology 8(3):233–278

Mehta CM, Srivastava R, Arora S, Sharma AK (2016) Impact assessment of silver nanoparticles on plant growth and soil bacterial diversity. 3 Biotech 6(2):254.

Movafeghi A, Khataee A, Abedi M, Tarrahi R, Dadpour M, Vafaei F (2018) Effects of TiO2 nanoparticles on the aquatic plant *Spirodela polyrrhiza*: evaluation of growth parameters, pigment contents and antioxidant enzyme activities. J Environ Sci 64:130–138

Newman MD, Stotland M, Ellis JI (2009) The safety of nanosized particles in titanium dioxide-and zinc oxide-based sunscreens. J Am Acad Dermatol 61(4):685–692

Nohynek GJ, Dufour EK, Roberts MS (2008) Nanotechnology, cosmetics and the skin: is there a health risk? Skin Pharmacol Physiol 21(3):136–149

Pakrashi S, Jain N, Dalai S, Jayakumar J, Chandrasekaran PT, Raichur AM, Mukherjee A (2014) In vivo genotoxicity assessment of titanium dioxide nanoparticles by *Allium cepa* root tip assay at high exposure concentrations. PLoS ONE 9(2):e87789

Palmqvist NGM, Bejai S, Meijer J, Seisenbaeva GA, Kessler VG (2015) Nano titania aided clustering and adhesion of beneficial bacteria to plant roots to enhance crop growth and stress management. Sci Rep 5:10146

Patlolla AK, Berry A, May L, Tchounwou PB (2012) Genotoxicity of silver nanoparticles in *Vicia faba*: a pilot study on the environmental monitoring of nanoparticles. Int J Environ Res Public Health 9(5):1649–1662

Rafique R, Arshad M, Khokhar MF, Qazi IA, Hamza A, Virk N (2015) Growth response of wheat to titania nanoparticles application. NUST J Eng Sci 7(1):42–46

Raliya R, Nair R, Chavalmane S, Wang WN, Biswas P (2015) Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (*Solanum lycopersicum* L.) plant. Metallomics 7(12):1584–1594

Rastogi A, Zivcak M, Sytar O, Kalaji HM, He X, Mbarki S, Brestic M (2017) Impact of metal and metal oxide nanoparticles on plant: a critical review. Front Chem 5:78

Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL (2011) Interaction of nanoparticles with edible plants and their possible implications in the food chain. J Agric Food Chem 59(8):3485–3498

Singh M, Upadhyaya HD, Bisht IS (eds.) (2013). Genetic and genomic resources of grain legume improvement. Elsevier, Amsterdam.

Song U, Shin M, Lee G, Roh J, Kim Y, Lee EJ (2013) Functional analysis of TiO_2 nanoparticle toxicity in three plant species. Biol Trace Elem Res 155(1):93–103

Sun TY, Conroy G, Donner E, Hungerbühler K, Lombi E, Nowack B (2015) Probabilistic modelling of engineered nanomaterial emissions to the environment: a spatio-temporal approach. Environ Sci: Nano 2(4):340–351

Sytar O, Kumari P, Yadav S, Brestic M, Rastogi A (2019) Phytohormone priming: regulator for heavy metal stress in plants. J Plant Growth Regul 38(2):739–752

Tiwari DC, Pukhrambam D, Dwivedi SK et al (2017) PPy/TiO_2(np)Polymer nanocomposite material for microwave absorption. J. Mater Sci. Mater Electron. https://doi.org/10.1007/s10854-017-8076-y

Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH (2009) Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Can Res 69(22):8784–8789

Verma RC (2004) Radiation, and EMS Induced translocation and inversion heterozygotes in *Vicia faba* L. J. Cytol. Genet. 5(1):45–52

Vevers WF, Jha AN (2008) Genotoxic and cytotoxic potential of titanium dioxide (TiO_2) nanoparticles on fish cells in vitro. Ecotoxicology 17(5):410–420

Yang L, Watts DJ (2005) Particle surface characteristics may play an important role in phytotoxicity of alumina nanoparticles. Toxicol Lett 158(2):122–132

Yang F, Liu C, Gao F, Su M, Wu X, Zheng L, Yang P (2007) The improvement of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. Biol Trace Elem Res 119(1):77–88

Zheng L, Hong F, Lu S, Liu C (2005) Effect of nano-TiO_2 on strength of naturally aged seeds and growth of spinach. Biol Trace Elem Res 104(1):83–91