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Exploration on impact of GA_3 encapsulated silica nanoparticles ($nSiO_2$) on seed germination and seedling vigour of ten months aged maize (Zea mays L.) varieties seeds.

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ABSTRACT

In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Nanoparticles can serve as 'magic bullets', containing plant growth regulators, herbicides, chemicals or genes, which target plant parts to release the content. The use of silica nanoparticles in the growth of plants and for the breaking dormancy, improve seed germination and increasing the vigour of maize seed. The present study was aimed evaluating that effect of GA_3 encapsulated silicon nanoparticles (SiO_2) comparative efficiency of invigoration technology on seed germination and early seedling growth of maize varieties. The ten month aged seeds of six maize varieties, along with seven invigoration treatments *i.e.* control, nanosilica 8g/l, GA_3 100 ppm, 100 ppm GA_3 encapsulated nanosilica, 125 ppm GA_3 encapsulated nanosilica, 150 ppm GA_3 encapsulated nanosilica and 100 ppm PEG encapsulated nanosilica. D-765 maize variety had significantly maximum value for seed quality parameters including standard germination percentage (77%), seedling length, seedling dry weight, seedling vigour index- II, speed of germination, index of synchrony germination, Relative growth index, mean germination time, germination value, germinable energy, mean daily germination and peak value. The nano-invigorated ten month aged seeds of maize variety D-765 invigorated with 150 ppm GA_3 encapsulated nanosilica showed highest value for standard germination percentage (88%) and other seedling quality parameters. It was concluded that GA_3 encapsulated nanosilica ($nSiO_2$) has produced better seed germination potential and seedling growth, as they are able to penetrate seed coat and delivery of GA_3 in seed embryo.

INTRODUCTION

Maize is one amongst the foremost vital cereal crops of the planet and contributes to food security in most of the developing countries. In India, after rice and wheat, maize is third most crucial nascent crop. In India, maize impart about 9 % in the national food basket and engaged over 100 million man-days at the farm and downstream agricultural and industrial sectors, apart from that it also contributes Rs. 100 billion to a greater extent in the agricultural GDP [5].

Quality seed is an indispensable trait for crop production and food security, peculiarly during the increasing incertitude due to climate change. Innovative crop production collectively the science of agriculture additionally affirms that whereas not quality seed we have a tendency to cannot have a successful crop production. Crop improvement and the deliverance of fine quality seeds and planting materials of selected variety to farmer is vital for ascertain better crop production and meeting growing environmental challenges. Gibberellic acid is most important growth regulator for breaking of seed dormancy and seed germination. Seed germination is a physico-chemical process during seed germination Gibberellic acid was increase secretion of α-amylase enzyme and other important hormone which was helpful for germination and seedling growth.

Nanoparticles are known to show unexampled variations in their properties that unit size dependent. They move with close substrate/cells and concerned by cells via endocytosis or perhaps may move within through intact cell membranes and infiltrate internal tissues. Functionalization of nanoparticles with chemicals and organic/inorganic corpuscle could lead to development of merchandise that may be used to clear many trouble related to seed germination, smart targeted and long term delivery of agrochemicals like fertilizers/pesticides/growth regulators and in fabrication of sensors for on-field speedy catching of contaminants. Green-synthesis and microbic deduction of nanomaterials for their agricultural use may be very important as they are naturally encapsulated with mother protein, therefore, more stable and safer to biological system. Nanosilica is a crucial metal compound that covers all major fields of science and technology together with industrial, physical science and medicine applications [10]. It has gained bigger aid as a result of its extremely reactive surface-to-volume magnitude relation property. The introduction of nanoparticles into plants might need vital impact and so, it will be used for agricultural applications for higher growth and yield. An earlier study showed that the addition of nanosilica in soil increased growth of maize (*Zea mays* L.)[26].

Materials and method

The 10 months aged (28 October 2016-25September 2017) seeds of 6 maize varieties (Amar, PSM-3, D-765, Tarun, Sweta and Navin) were treated with the SiO_2 nano-particles (10-20 nanometer size) 8 g/liter, GA_3 100 ppm concentration, different concentration (100 ppm, 125 ppm and 150 ppm) of GA_3 encapsulated silica nanoparticles and 100 ppm Poly Ethylene Glycol (PEG) encapsulated nanosilica solution. The six maize varieties seed were soaked in different prepared solution for 12 hours at 25 ^{0}C. After 12 hr of soaking the solution was drained out from the beaker and presoaked seeds dried at room temperature till the original weight. The dried seeds were used for germination as per ISTA procedure in seed testing laboratory at G.B. Pant University of Agriculture and Technology, Pantnagar. The experimental data were analyzed statistically as per the method described for Factorial Completely Randomized Design [8].

Seeds were considered to have germinated when the radicle emerged 2 mm in length. Germination was counted in 24 hrs intervals and continued until no further germination occurred. At 7thday after germination count, ten seedlings from each sample were taken to record data on seedling length of individual seedling manually with help of scale. The dry weight was recorded after oven drying of ten seedling. Seedling vigour index II were measured at 7thday of germination test. For calculating speed of germination, seeds were checked daily after they began to germinate at approximately the same time each day. This procedure was continued until all seed that were capable of producing a normal seedling had germinated.

Standard germination (%)

Final count was taken on 7th day for maize seed after the seed kept for germination **(ISTA, 2007)**. Only normal seedlings were counted on the 7th day to record the data.

$$\text{Standard Germination \%} = \frac{\text{Number of normal seedlings germinated}}{\text{Total number of seeds}} \times 100$$

Seedling length (cm)

Ten seedlings in each replication were randomly selected for measurement of root and shoot length on the day of final count and the mean length was expressed in centimeter.

Seedling dry weight (g)

Seedling dry weight was recorded in the seed germination test on 7th day. The ten normal seedlings were dried in the oven at 80ºC ±2 for 24 hrs. The dried seedling was weighed on an electronic balance and expressed in gram.

Seedling vigour index- II

The seedling vigour index II was calculated as per the following formula **(Abdul Baki and Anderson, 1973)**:

Seedling vigour index -II =Standard germination (%) × Seedling dry weight (g)

Relative growth index (RGI)

Relative growth index of seedlings was calculated according to the formula given by **Brown and Mayer (1986)** as under:

$$RGI = \frac{\text{No. of seed germinated at first count } (4^{th}\text{ Days})}{\text{No. of seed germinated at final count } (7^{th}\text{ Days})} \times 100$$

Germination index (GI)

Germination index (GI) was calculated as described in the **Association of Official Seed Analysts (1983)** using the following formula:

$$GI = \frac{\text{No. of germinated seeds}}{\text{first count } (4^{th}\text{ days})} + \dots\dots\dots + \frac{\text{No. of germinated seeds}}{\text{final count } (7^{th}\text{ days})}$$

Mean germination time (MGT)

Mean germination time (MGT) was calculated to the equation of **Ellis and Roberts (1981)** as under:

$$MGT = \frac{\Sigma Dn}{\Sigma n}$$

Where,

n is the number of seeds which were germinated on day D, and D is the number of days counted from the beginning of germination.

Germination value

Germination value was calculated according the following formula:

Germination value = Peak value x Mean daily germination

Germinative energy

Germinative energy was calculated according to following formula:

$$\text{Germinative energy} = \frac{\text{Germination at peak period}}{\text{Total number of seeds taken}}$$

Mean daily germination

Mean daily germination was calculated according to following formula:

$$\text{Mean daily germination} = \frac{\text{Standard Germination (\%)}}{\text{Total number of days}}$$

Peak value of germination

Peak value was calculated according to following formula:

$$\text{Peak Value} = \frac{\text{Standard Germination (\%)}}{\text{Number of days to reach maximum germination}}$$

Instruments:

The zeta potential and size of the mesoporous nanosilica and urea with gibberellic acid encapsulated silica pellet were confirmed by dynamic light scattering and laser Doppler principles, respectively using Microtrac Nanotrac Wave II (Microtrac Instruments) with a scattering angle of 180°. The absorption spectra were recorded in the range of 254 nm by UV-Vis spectrophotometer UV-2600 (SHIMADZU Technologies). All samples were diluted 5-fold in distilled water before the treatment given for the data recoding. The Fourier-transform infrared spectroscopy (FT-IR) study was done using a Nicolet iS50FT-IR spectrometer (Thermo Scientific, USA) and the spectra was measured between 4000 and 400 cm−1 in Attenuated Total Reflectance (ATR) for nanosilica characterization and 4000 and 720 cm-1 in Omni-Cell/Traditional Liquid Transmission Cell holder (ZnSe) for liquid sample characterization.

Synthesis of mesoporous nano-silica pellets

Firstly, 800 mg mesoporous nano-silica was weighed by the electronic balance and it is rinsed by adding 100ml methanol and stirred by Coring stirrer (Model PC-420D) for 30 minutes at 320 rpm. The solution was centrifuged by Remi centrifuge (model no. C-24BL) for 5 minutes at 5000 rpm. The supernatant was removed with the help of micropipette from the tube and the process is repeated thrice and at last precipitate materials (pellet) was used to treat the seeds with and without gibberellic acid loading.

Encapsulation of GA_3 on the mesoporous nano-silica

The gibberellic acid was stabilized with urea and non-ionic adjuvant to overcome the problem of denaturation. The collected precipitated materials (pellet) was added in the 100 ml methanol solution with the varying concentration of gibberellic acid (stabilized with urea and non-ionic adjuvant) such as 100 ppm, 125 ppm and 150 ppm. The different concentration of a mesoporous nano-silica with gibberellic acid solution in the beaker was kept on a stirrer for 24 hours at 150 rpm. After, 24 hours the solution is centrifuged for 5 minutes at 5000 rpm. Then, supernatant was removed from the centrifuge tube and pellet was collected for further seed invigoration treatment. The prepared pellet (gibberellic acid loaded mesoporous nano-silica) was used to prepare final solution in distilled water (100 ml) for the conditioning of maize seeds prior to tetrazolium test.

Preconditioning of seed and viability Test

The seeds of different maize varieties were preconditioning overnight (12 hrs.) in the gibberellic acid encapsulated, nonencapsulated nanosilica and gibberellic acid solution and dried under shade to maintain the proper moisture content. Further, treated seeds were used for the germination test and seed viability test. The three concentrations of tetrazolium chloride salt aqueous solution (0.1%, 0.2% and 0.5%) was prepared. The seed viability test using tetrazolium was conducted. The procedure laid down by International Seed Testing Association (ISTA) was followed.

After the preconditioning of seed with nanosilica and gibberellic acid for a prescribed period (12 hours) each seed was bisected longitudinally through the center of the embryo so that each half had a part of the plumule and the radicle. One half of each seed was then placed in four petri dish and poured 2,3,5-triphenyl tetrazolium chloride solution (0.1%, 0.2% and 0.5%) over the cut seeds until they were completely immersed. The petri dish was kept at 25 ^{0}C temperature for 30 minutes to proper staining of embryo and other essential structure of seeds. The sample (100 seeds) is satisfactorily stained when tissues develop interpretable staining characteristics and the analyst can 'sense' embryo condition. After proper staining the seed was removed from the tetrazolium solution, rinsed 2-3 times in water and then evaluated according to the staining pattern. During the course of observation on seed viability, seeds were kept in little water to prevent drying. For the clearing of solution 2-3 drops of lactophenol was added. The distinct staining of the vital parts of the embryo was evidence of ability to produce a normal seedling [8]. The viability percentage tested by staining of 2,3,5-Triphenyl Tetrazolium Chloride indicated the percentage of normal viable seeds.

Results and discussion

Characterization

Particle Size Analyzer (PSA)

By particle size analyzer (PSA) technique, one may record data on the hydrodynamic diameter and the aggregation state of nanosilica prepared in methanol solution. In addition, the hydrodynamic diameter of nanosilica refers to the particle size with electrostatic potential radius around it. The size distribution of nanosilica and urea with Gibberellic acid encapsulated nanosilica pellet were measured by PSA and is shown in Fig. 1a and b, respectively. The volume (42.4 %) of nanosilica in Fig. 1a is 273.7 nm and some volume (57.6 %) is 985 nm in diameter. In Fig. 1b, most of volume (85.2 %) of nanosilica is 4340 nm and some volume (14.8 %) of urea with GA_3 encapsulated nanosilica is 347 nm which increased to 3355 nm and 74 nm in the case of Fig. 1a, respectively[18].

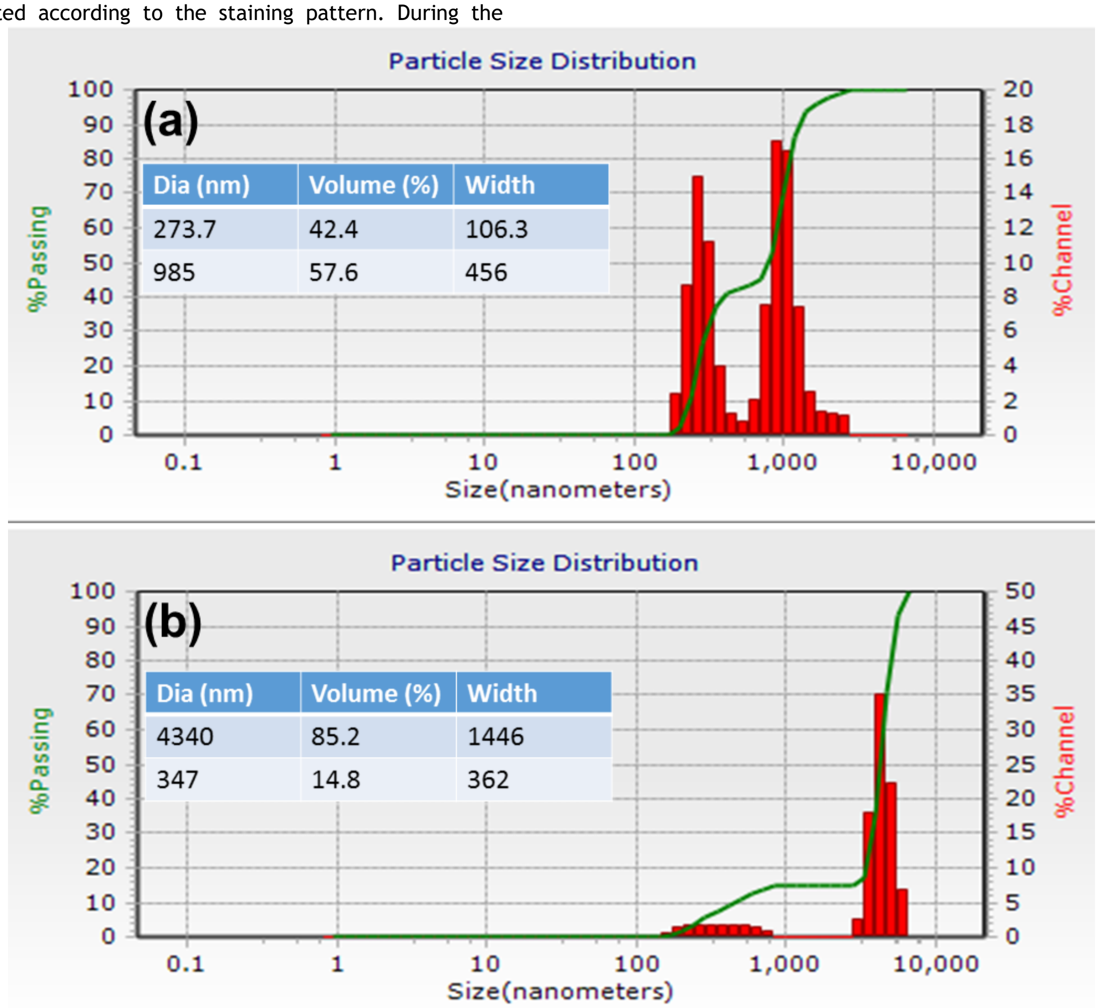

Fig.1 PSA spectra analysis (**a**) mesoporous nanosilica (**b**) Urea with GA_3 encapsulated mesoporous nanosilica.

UV-Visible Spectrophotometer Analysis

In the last few decades, nanosilica and synthesis of nanosilica encapsulated with gibberellic acid has been an active research area in agriculture field because of their vital optical merits, which is strongly dependent on shape, size and nano-composition, and can be analyzed with help of the optical instruments like UV-visible spectroscopy analysis. Even though, the absorption spectra were carried out using UV-visible spectrophotometer to check the stability of urea with GA_3 encapsulated nanosilica at different concentrations like 100, 125, and 150 ppm (in Fig. 2). Fig. 2 shows the maximum absorption peak of urea with Gibberellic acid encapsulated nanosilica pellet at different concentration without adjuvant (black line) and minimum absorption peak of urea-gibberellic acid encapsulated nanosilica pellet at different concentration with adjuvant (red line)[18].

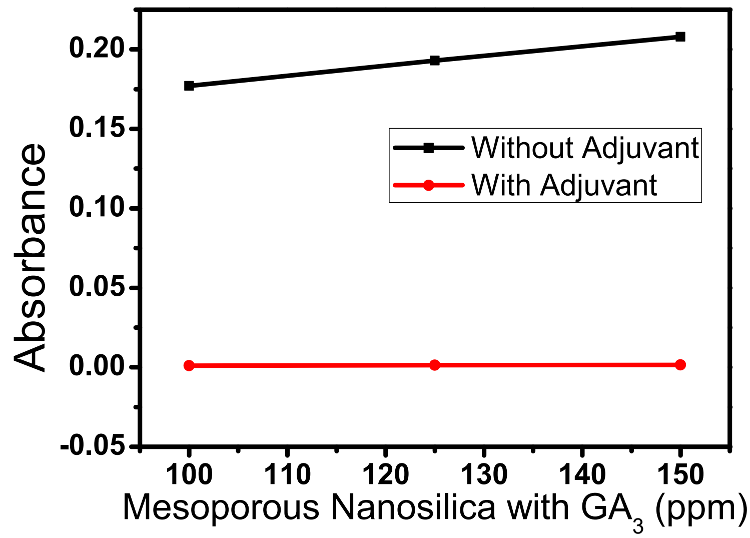

Fig.2 UV-Vis spectra analysis of urea with GA3 loaded nanosilica pellet without adjuvant (black line) and with adjuvant (red line).

FT-IR spectrometer

Fig.3a presents the FT-IR results of nanosilica. The FT-IR spectra of nanosilica sample shows large bands of O-Si-O stretching at 1,079.81 and 793.67 cm^{-1}. The bands at 3365.07 and 1634.28 cm^{-1} correspond to the O-H stretching and bending vibrations as shown in Fig.3a. Furthermore, the FT-IR spectrum of urea (black line), urea with gibberellic acid (red line) and urea with gibberellic acid encapsulated nanosilica (blue line) is shown in Fig. 3b. In Fig.3b, the C=O stretching frequency shows at 1681 cm^{-1}. The N-H stretching and vibrational frequencies show at 3433 cm^{-1} and 1606 cm^{-1}, respectively. In addition, the C-N stretching frequency shows at 1455 cm^{-1}. Compared with urea, the conjugate of urea- gibberellic acid does not appear a peak in red line at 1606 and 1681 cm^{-1}, which could be ascribed to the amide bond like the CO-NH stretch. Furthermore, another characteristic peak of gibberellic acid at 1752 cm^{-1} corresponded to the stretch of carboxyl group expressively lost in the FT-IR spectrum of urea- gibberellic acid (20). The FT-IR results showed that the carboxylic groups of gibberellic acid reacted with the NH_2 of urea. Furthermore, O-H stretching and bending vibrations peaks (blue line) do not appear due to reaction of urea with gibberellic acid as shown in Fig. 3b.

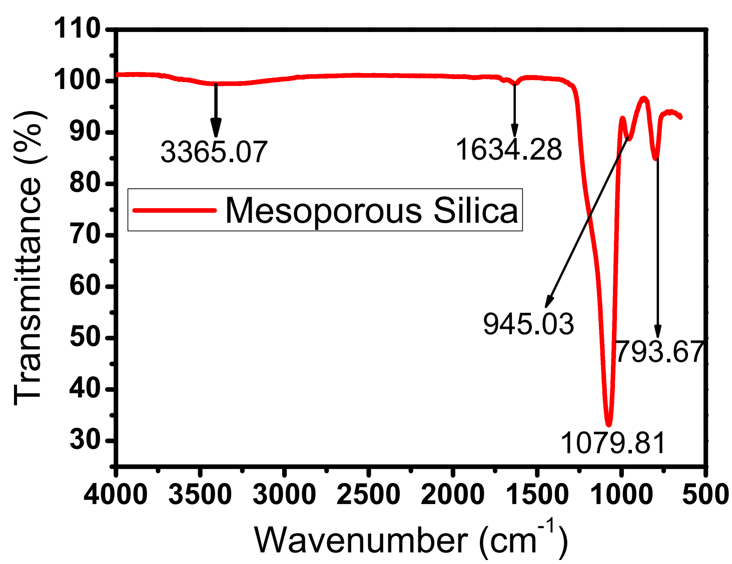

Fig.3a FT-IR transmission spectra of nanosilica.

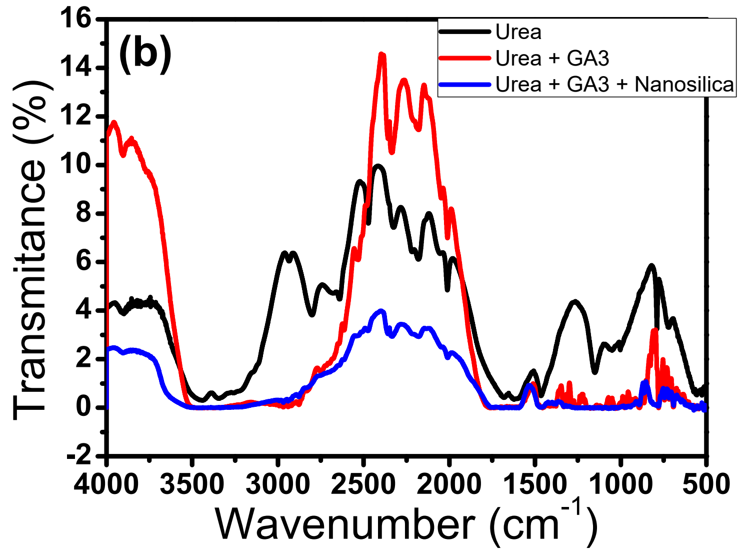

Fig.3b FT-IR transmission spectra of urea (black line), urea with gibberellic acid (red line), and urea with gibberellic acid encapsulated nanosilica (blue line).

Germination at first count

The germination of normal seedling was counted on fourth days from the start of germination test. Data presented in the **Table 1**at first count had a statistically significant effect among different genotypes under different GA_3 encapsulated silica nanoparticles invigoration treatments and interaction also. Data exhibited that among the varieties D-765 had maximum germination percent (52%) at first count stage. Perusal of data depicted that maximum germination percentage (65%) was recorded for seed invigorated with 150 ppm GA_3 encapsulated nanosilica at first count stage and minimum (32%) seed germination at first count stage was observed in untreated control. The interaction also revealed that highly significant difference from each treatment combination. The result of the comparison data revealed that highest germinative seed (70%) was recorded in D-765 variety seed invigorated with 150 ppm GA_3 encapsulated nanosilica (V_3T_5) and lowest (30%) germinative seed was found in untreated Sweta variety (V_5T_0).

Standard Germination percentage

The standard germination percentage was recorded on 7th days of germination test and only normal seedling was counted. The data presented in **Table 1**regarding germination per cent in maize reveal that significant differences were founded among different treatments, varieties and their interaction. Among the varieties the D-765 variety was observed highest (77%) standard germination percentage. The mean values of germination per cent among treatments varied from 62% to 85% per cent and among varieties it is varies from 69% to 77 %. Among the nano-invigoration treatments, 150 ppm GA_3 encapsulated nanosilica treatment had highest standard germination percentage (85%). The highest 23 % increase of standard germination was recorded in nanosilica with 150 ppm GA_3 encapsulated treatment as compared to untreated control. Maize variety D-765 invigorated with 150 ppm GA_3 encapsulated nanosilica was showed highest standard germination percentage (88%) and lowest standard germination percentage was found in V_5 T_0 (58%). It was observed that in all the treatments, there was an increase in germination percentage at final count due to increase water use efficiency by nano-invigoration treatment. Iron nanoparticles concentration helps to increase germination and growth response of wheat seedlings [4]. In rice good seed germination was observed in the presence of $nSiO_2$ and Mo nanoparticles [3]. Nanosilica promoted seed germination percentage (100%) in maize than conventional Si sources [16], Carbon nanotubes increased germination and growth of tomato due to the ability of carbon nanotubes to penetrate into the seed coat and stimulate water absorption [17]. Silica nanoparticles concentrations could significantly increase the seed germination in comparison to control of maize seed [12] similar results were reported by [21] in *Viciaa faba* L., and in tomato [22].

Seedling length (cm)

The ten seedling of each replication was randomly selected and their length is measured with the help of scale and expressed in centimeters. The results showed that highest seedling length was reported for D-765 (37.39 cm), whereas lowest seedling length was observed for Sweta (31.82 cm). The results indicated that D-765 variety was observed for highest seedling growth (**Table 1 and fig. 1, 2, 3**). The 150 ppm concentration GA_3 encapsulating nanosilica invigoration treatment (39.81cm) showed highest value of seedling length among the all invigoration treatments. However minimum value of seedling length was found in untreated control (29.98 cm). The 150 ppm GA_3 encapsulated nanosilica nano-invigoration treatments had 9.83 cm more seedling length in comparison to untreated control. Among interactions the maximum value in term of seedling length (43.47 cm) was observed for maize variety D-765 invigorated with 150 ppm GA_3 encapsulated nanosilica (V_3T_5). The untreated Sweta variety had lowest seedling length (27.93 cm). Similar findings was observed that silica nanoparticles increased root length, shoot length and also increase seedling length as compared to the control in tall wheatgrass [6]. Seedling length of barley and maize was enlarged with the concentration increase of both Fe/$nSiO_2$ nano particles [11]. Seed priming with silicon dioxide nanoparticles improve germination and also increase root length, shoot length, root and shoot ratio and seedling length of deteriorated soybean seed [19].

Seedling dry weight (g)

Results showed that among the maize varieties highest seedling dry weight was measured for D-765 variety (1.567 g), while the lowest seedling dry weight was found in Sweta variety (1.166 g). It was observed that increased GA_3 encapsulating concentration on nanosilica increases the seedling dry weight. Among nano-invigoration treatments, seedling dry weight varied from 1.017 g to 1.664 g. Results indicated a maximum dry weight was recorded in 150 ppm GA_3 encapsulated nanosilica invigoration treatment (1.664 g) and minimum value in term of seedling dry weight was observed for untreated control (1.017 g). Among the interaction maize variety D-765 invigorated with 150 ppm GA_3 encapsulated nanosilica (V_3T_5) showed maximum seedling dry weight (1.873 g). Seeds invigoration treatment increase imbibition rate and also increase relative water content as well as the increase enzymatic activity which increases respiration rate and increases the dry matter of seedlings. The application of $nSiO_2$ germination (%), root length and fresh weight of tomato seedlings were increased under NaCl stress [29]. The application of N-Si increased the plant water use efficiency, rate of photosynthesis and mesophyll conductance of tomato plants under saline stress [14]. N-Si had significant effects on seed germination, seedling growth and yield, as well as the metabolism activity in tomato plant [26].

Seedling vigour index-II

The estimation of seedling vigour index from the seedling dry weight was more efficient than the seedling vigour index estimated from seedling length. Among the maize varieties highest seedling vigour index-II was recorded for D-765 (122.68), whereas lower seedling vigour index-II was noticed in Sweta (82.02). Results indicated that maximum seedling vigour index-II was observed for 150 ppm GA_3 encapsulated nanosilica invigoration treatment (140.92) and minimum seedling vigour index-II was observed in untreated control (62.93). Results revealed that maize variety D-765 invigorated with 150ppm GA_3 encapsulated nanosilica (V_3T_5) had maximum seedling index-II (164.80) and minimum mean value of seedling vigour index-II was observed in V_5T_0 (48.17). The maize variety with higher seedling vigour index-II showed the highest level of activity. The level of enzymatic activity was increased by the nano-invigoration treatment and also by interaction of maize varieties and nano-invigoration treatments. Similar findings was also reported on maximum seedling vigor index II (11.3) was found in the multi-walled carbon nanotubes (MWCNTs) 100 $\mu g.ml^{-1}$ concentration in castor [11] and in sunflower [20].

Speed of germination

The maize variety which produces the highest number of germinated seeds at the preliminary count will produce fastest growing seedlings and the fastest stand establishment. The data presented in **Table 3** depicted that there was significant difference for Speed of germination was found among maize varieties and nano-invigoration treatment. Among the maize varieties maximum speed of germination was observed in D-765 variety (22.54) and minimum speed of germination was observed for Sweta variety (20.61). The result of the comparison data revealed that Speed of germination for all treatment was positively influences and increase from 16.23 to 26.87. Perusal of data depict that overall mean value for maximum Speed of germination was observed for 150 ppm GA_3 encapsulated nanosilica invigoration treatment (26.87) followed by 125 ppm GA_3 encapsulated nanosilica invigoration treatment (25.25) and minimum Speed of germination was observed in untreated control (16.23). Perusal of data revealed that on an average mean value of speed of germination among interaction between maize varieties and nano-invigoration treatment showed non-significant variation.

4.3.13 Index of synchrony germination

The index of synchrony germination indicates frequency of germination and the low value is indicator of more synchronize germination. The statistical analysis of the data presented in **Table 3** depicted that significant difference for index of synchrony germination among maize varieties, nano-invigoration treatment and interactions. The maize variety PSM-3 had maximum value of synchrony germination (0.397), followed by Tarun (0.375) and lowest value of index of synchrony germination was recorded in D-765 variety (0.368) which showed more synchronized germination. The result of the comparison data revealed that mean index of synchrony germination was range from 0.365 to 0.398 among the nano-invigoration treatments. It was observed that maximum value of index of synchrony germination was observed for 8 g/l concentration nanosilica invigoration treatment (0.398) followed by 150 ppm concentration GA_3 encapsulated nanosilica invigoration treatment (0.378) and minimum value for index of synchrony germination was observed in untreated control (0.365). Perusal of data depicted that maximum value of index of synchrony germination was observed for V_2T_1 interaction (0.469) followed by V_3T_1 (0.413) and minimum value of index of synchrony germination was found V_3T_6 interaction (0.333).

4.3.14 Relative growth index (RGI)

The relative growth index is indicator of plant strategy with respect to seedling vigour under favorable condition. The statistical analysis of the data in **Table 4.3.1** depicted that there was significant variation present among the maize varieties, nano-invigoration treatment and their interactions for trait relative growth index. Among the maize varieties highest relative growth index was perceived in Amar variety (68.96) followed by Sweta variety (66.62) whereas minimum relative growth index was reported in Tarun variety (65.69). Perusal of data revealed that an average mean value of relative growth index (RGI) increases from 51.57 to 77.32 among the nano invigoration treatments. Perusal of data depicted that overall mean value for maximum relative growth index was observed for 150 ppm GA_3 encapsulated nanosilica invigoration treatment (77.32) followed by 125 ppm concentration GA_3 encapsulated nanosilica invigoration treatment (76.27). However, minimum value of relative growth index was observed for untreated control (51.57). Results indicated that all treatment increased relative growth index as compare to untreated control. An overall mean value indicated that the maximum value in term of relative growth index (84.44) was observed for V_1T_5 followed by V_3T_5 (79.92) and minimum value for relative growth index was observed for V_6T_0 (49.78). Results showed that relative growth index (RGI) was significantly increase by nano-invigoration treatment, significantly differ with variety to variety and also showed significant variation with variety and treatment interaction. The relative growth index was important vigour quality of seed calculated with help of germination percent at first count and germination percent at final count.

4.3.16 Mean germination time (days)

Mean time to germination (MGT) is a measure of the rate and time spread of germination. The mean germination time not only measured rate of germination of seeds, but lag period for each seed between the start of imbibition and germination also measured. Finding towards the mean germination time shown in **Table 4** significantly reflected among maize varieties, nano-invigoration treatments and their interactions. The highest value of mean germination time among the varieties was recorded for PSM-3 (5.11 days) and lowest mean germination time (4.87 days) was reported for Amar and D-765 varieties. The mean germination time was decrease with increase GA_3 encapsulating concentration on nanosilica from 4.91 days to 4.05 days. Maximum mean germination time was recorded for untreated control (5.91 days), whereas minimum mean germination time was observed in 150 ppm concentration GA_3 encapsulated nanosilica invigoration treatment (4.05 days) followed by 125 ppm concentration GA_3 encapsulated nanosilica invigoration treatment (4.27 days). It was observed that nano-invigoration treatment with 150 ppm concentration GA_3 encapsulated nanosilica was maximum decrease in mean germination time (1.86 days) among all nano-invigoration treatment. All nano-invigoration treatments significantly decreased mean germination time as compared to untreated control.

Maize varieties and nano-invigoration treatments interactions significantly decreased mean germination time from 6.49 days to 3.96 days. The results depicted that maximum mean germination time was recorded for V_2T_0 (6.49 days), while minimum mean germination time was observed for V_2T_5 (3.96 days). MGT is an accurate measure of the time taken for a lot to germinate and lower mean germination time (MGT) represents a faster germination speed. It focuses instead on the day when most germination events have been occurring. This agreed with results of **Azimi *et al.* (2014)** who revealed that significant increase in seed germination, MGT, germination rate, seedling weight and vigor index was found by prechilling and $nSiO_2$ nanoparticles treatment. **Disfani *et al.* (2016)** stated that synthesized $Fe/nSiO_2$ nanoparticles with different iron content had significant effect on mean germination time (MGT) with the lowest of 0.58 day for barley and 0.79 day for maize; at 15 and 5 mg kg-1 nano $Fe/nSiO_2$, respectively. **Siddiqui and Al-Whaibi (2014)** demonstrated that treatments, 8 g L1 of nSiO2 improved germination percentage, mean germination time, seed germination index, seedling vigour index, seedling fresh weight and dry weight. **Janmohammadi and Sabaghnia (2015)** reported that mean germination time (MGT) achene priming in 0.2, 0.4 and 0.8 mM nano-silicon solutions could appreciably reduce the MGT as compared to intact achenes of sunflower (*Helianthus annus*).

4.3.18 Germination value

Germination value is the mean daily germination multiplied by peak value. Finding towards the germination value shown in **Table 5** indicated that there was significant variation obtained among maize varieties and nano-invigoration treatments. The maize variety D-765 had maximum germination value 239.05 followed by Navin variety (227.68), whereas lower germination value was recorded for Sweta variety (193.25). The means towards germination value indicated that germination value increased with increase in GA_3 loading concentration on nanosilica and ranged from 135.09 to 335.15. Data revealed that maximum germination value was observed for 150 ppm GA_3 encapsulated nanosilica invigoration treatment (335.15) followed by 125 ppm GA_3 encapsulated nanosilica (306.60) and minimum mean value for germination value was observed for untreated control (135.09). It was observed that all treatment was showed increased germination value as compared to untreated control and had highly significant effect on germination value. The maximum increase of germination value was recorded for nano-invigoration with 150 ppm GA_3 encapsulated nanosilica. An overall mean value observed that the interactions between treatment and variety had non-significant effect on germination value. The maximum germination value was observed for V_3T_5 (336.40) and V_1T_5 (333.62) minimum germination value was noticed for V_5T_0 (120.21). Germination Value was integrated measure of seed quality in a single figure and expression of total germination at the end of the test period with an expression of germination energy or speed of germination. This agreed result of **Azimi *et al*. (2014)** who revealed that nano-sized $nSiO_2$ concentrations significantly increases MGT, GV, MDG, PV and vigor index of tall wheatgrass seedling.

Germinative energy

Germinative energy is the speed with which the seeds germinate, sometimes expressed as a percentage of the seeds germinated within the first week of analysis with respect to overall germination. Perusal of **Table 5** data revealed that there was highly significant difference was recorded among maize varieties and nano-invigoration treatments. Among the maize varieties highest germinative energy was recorded for D-765 variety (0.52) and lower value of germinative energy was observed for Sweta variety (0.47). It was found that among the nano-invigoration treatments germinative energy varied from 0.32 to 0.66. The 150 ppm concentration of GA_3 encapsulated nanosilica invigoration treatment was reported maximum germinative energy (0.66) followed by 125 ppm GA_3 encapsulated nanosilica invigoration treatment (0.61) and minimum germinative energy was observed for untreated control (0.32). The result of the comparison data revealed that mean for germinative energy all treatment had highly significant increment from each treatment and also compare to untreated control.

Perusal of data revealed that interaction between maize varieties and nano-invigoration treatment showed non-significant difference for germinative energy. The maximum value of germinative energy was recorded for V_3T_5 (0.69). Results revealed that germinative energy increased with different invigoration treatment by affecting the physiological process ***i.e.,*** synthesis of GA_3, secretion of α-amylase which is essential for metabolic process and transpiration for rapid ATP production (**Zheng *et al*., 2005**). The result agreed with **Janmohammadi and Sabaghnia (2015)** who reported that germinative energy was increase of sunflower achene rehydrated at lower concentration of nanosilica 0.2, 0.4 and 0.6 mM as compare to control.

Peak value of germination

The peak value represents the maximum cumulative germination percentage divided by the number of days to reach this germination percentage. The index of peak value was an expression of speed and totality of germination, and their interaction. The statistical analysis of the data presented in **Table 6** depicted that there was highly significant difference among the maize varieties and nano-invigoration treatments on peak value of germination. Among the maize varieties highest peak value of germination was recorded for D-765 (21.29) followed by Navin (20.86) whereas minimum peak value of germination was recorded for Sweta (19.12). The result of the comparison data revealed that peak value of germination among the nano-invigoration treatment varied from 15.39 to 27.77. Maximum peak value of germination was recorded for 150 ppm GA_3 encapsulated nanosilica invigoration treatment (27.77) followed by 125 ppm GA_3 encapsulated nanosilica invigoration treatment (26.67). However, lowest peak value of germination was reported in untreated control (15.39). The nano-invigoration treatment with 150 ppm GA_3 encapsulated nanosilica was found superior among the all nano-invigoration treatment and showed highest increase of peak value of germination (12.38) as compared to control. Interaction between maize varieties showed non-significant difference for peak value of germination. The highest peak value of germination was found in V_6T_5 interaction (28.89) and lowest peak value of germination was recorded for V_5T_0 (14.50). **Azimi *et al*. (2016)** also reported that seed treated with 40 and 60 mgL^{-1} nanosilica showed highest germination value (GI), mean daily germination (MDG) and peak value (PV) of tall wheatgrass (*Agropyron elongatum* L.).

Fig 1: Effect of nano-invigoration treatments on seedling growth of ten month aged seeds of maize.

Fig 2: Effect of nano-invigoration treatments on seedling growth of ten month aged seeds of maize.

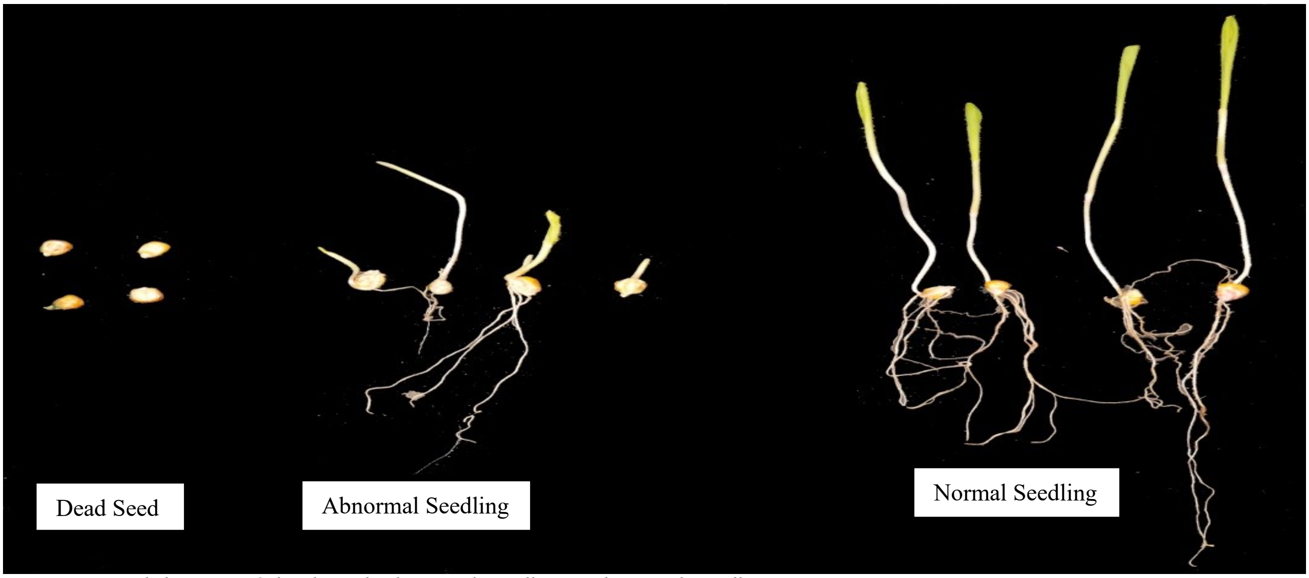

Fig 3: Pictorial diagram of dead seed, abnormal seedling and normal seedling.

Table. 1 Effect of nano-invigoration on germination and seedling length of ten month aged seeds of maize varieties

Character	First count (%)							Standard germination (%)							Seedling length (cm)						
	V_1	V_2	V_3	V_4	V_5	V_6	Mean	V_1	V_2	V_3	V_4	V_5	V_6	Mean	V_1	V_2	V_3	V_4	V_5	V_6	Mean
T_0 (Control)	31	32	33	32	30	32	**32**	62	59	64	62	58	64	**62**	30.08	28.89	32.53	29.84	27.93	30.60	**29.98**
T_1 (SiO_2)	43	44	49	45	44	47	**45**	67	65	73	69	65	71	**68**	32.98	32.49	35.99	33.84	31.40	35.05	**33.63**
T_2 (GA_3 100ppm)	36	35	37	37	35	38	**36**	65	64	71	64	61	66	**65**	32.29	31.49	34.10	31.18	29.73	33.14	**31.99**
T_3 (SiO_2+GA_3 100ppm)	55	58	58	53	52	56	**55**	73	76	82	73	73	82	**76**	35.79	33.70	38.32	35.52	32.54	37.69	**35.59**
T_4 (SiO_2+GA_3 125ppm)	63	59	66	57	57	63	**61**	75	80	85	80	77	83	**80**	37.63	35.75	40.68	37.57	34.59	39.71	**37.66**
T_5 (SiO_2+GA_3 150ppm)	66	64	70	64	61	67	**65**	84	84	88	83	82	87	**85**	39.39	38.10	43.47	39.77	36.35	41.80	**39.81**
T_6 (SiO_2+PEG100ppm)	54	45	49	46	46	48	**48**	73	72	78	70	67	74	**72**	31.20	32.76	36.64	33.96	30.22	35.48	**33.38**
Means	**50**	**48**	**52**	**48**	**46**	**50**	49	**71**	**71**	**77**	**72**	**69**	**75**	73	**34.19**	**33.31**	**37.39**	**34.53**	**31.82**	**36.21**	34.58
C.V. (%)	4.42							1.62							2.149						
	SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)			
Treatment	0.510			1.436				0.277			0.779				0.175			0.492			
Variety	0.473			1.329				0.256			0.721				0.162			0.456			
Variety × Treatment	1.250			3.517				0.678			1.908				0.429			1.206			

Table. 2 Effect of nano-invigoration on seedling dry weight and seedling vigour index-II of ten month aged seeds of maize varieties

Character	Seedling dry weight (g)							Seedling vigour index-II						
	V_1	V_2	V_3	V_4	V_5	V_6	Mean	V_1	V_2	V_3	V_4	V_5	V_6	Mean
T_0 (Control)	0.921	0.868	1.133	1.076	0.830	1.273	**1.017**	57.10	51.25	72.12	67.04	48.17	81.90	**62.93**
T_1 (SiO_2)	1.461	1.293	1.519	1.299	1.044	1.477	**1.349**	97.44	84.45	110.42	89.25	67.60	105.39	**92.42**
T_2 (GA_3 100ppm)	1.156	1.063	1.318	1.231	0.955	1.339	**1.177**	74.78	67.64	93.15	78.76	58.26	87.90	**76.75**
T_3 (SiO_2+GA_3 100ppm)	1.581	1.380	1.724	1.371	1.280	1.583	**1.487**	115.45	104.40	140.84	100.47	93.46	129.82	**114.07**
T_4 (SiO_2+GA_3 125ppm)	1.691	1.487	1.818	1.457	1.359	1.636	**1.575**	126.84	119.44	153.88	116.61	104.19	136.36	**126.22**
T_5 (SiO_2+GA_3 150ppm)	1.730	1.600	1.873	1.575	1.476	1.727	**1.664**	144.83	134.43	164.80	130.72	121.02	149.70	**140.92**
T_6 (SiO_2+PEG100ppm)	1.185	1.236	1.584	1.397	1.216	1.489	**1.351**	86.45	88.62	123.55	98.31	81.47	110.16	**98.09**
Means	**1.389**	**1.275**	**1.567**	**1.344**	**1.166**	**1.503**	**1.374**	**100.41**	**92.89**	**122.68**	**97.31**	**82.02**	**114.46**	**101.63**
C.V. (%)	3.815							4.263						
	SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)			
Treatment	0.012			0.035				1.021			2.872			
Variety	0.011			0.032				0.945			2.659			
Variety × Treatment	0.030			0.085				2.501			7.034			

Table. 1 Effect of nano-invigoration on seed of germination and index of synchrony germination of ten month aged seeds of maize varieties

Character	Speed of germination							Index of synchrony germination						
	V_1	V_2	V_3	V_4	V_5	V_6	M	V_1	V_2	V_3	V_4	V_5	V_6	M
T_0 (Control)	15.32	16.63	16.81	16.27	15.58	16.79	**16.23**	0.350	0.378	0.372	0.364	0.356	0.374	**0.365**
T_1 (SiO_2)	19.34	21.65	21.29	19.75	19.63	20.84	**20.41**	0.365	0.469	0.413	0.384	0.386	0.374	**0.398**
T_2 (GA_3 100ppm)	16.61	17.19	18.57	17.52	16.94	18.19	**17.50**	0.361	0.398	0.349	0.370	0.399	0.370	**0.374**
T_3 (SiO_2+GA_3 100ppm)	22.83	23.89	24.67	22.51	22.30	23.66	**23.31**	0.384	0.375	0.355	0.368	0.361	0.362	**0.367**
T_4 (SiO_2+GA_3 125ppm)	25.56	24.88	26.40	24.67	24.08	25.89	**25.25**	0.391	0.378	0.382	0.379	0.356	0.368	**0.376**
T_5 (SiO_2+GA_3 150ppm)	26.81	26.93	27.55	26.42	25.65	27.84	**26.87**	0.363	0.385	0.375	0.378	0.370	0.396	**0.378**
T_6 (SiO_2+PEG 100ppm)	22.37	21.30	22.50	20.46	20.12	21.15	**21.32**	0.379	0.400	0.333	0.384	0.368	0.369	**0.372**
Mean	**21.26**	**21.78**	**22.54**	**21.08**	**20.61**	**22.05**	**21.56**	**0.370**	**0.397**	**0.368**	**0.375**	**0.371**	**0.373**	**0.376**
C.V. (%)	4.632							5.350						
	SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)			
Treatment	0.235			0.662				0.005			0.013			
Variety	0.218			0.613				0.004			0.012			
Variety × Treatment	0.576			1.621				0.012			0.033			

Table. 1 Effect of nano-invigoration on Relative growth index and Mean germination time of ten month aged seeds of maize varieties

Character	Relative growth index (RGI)							Mean germination time (days)						
	V_1	V_2	V_3	V_4	V_5	V_6	Mean	V_1	V_2	V_3	V_4	V_5	V_6	Mean
T_0 (Control)	50.55	53.64	52.36	51.34	51.74	49.78	**51.57**	5.45	6.49	5.91	5.77	5.97	5.90	**5.91**
T_1 (SiO_2)	64.02	67.35	66.99	65.10	68.56	65.88	**66.32**	5.43	6.09	5.14	5.23	5.14	5.00	**5.34**
T_2 (GA_3 100ppm)	55.21	55.48	52.83	57.33	57.92	58.42	**56.20**	5.40	5.71	5.70	5.72	5.97	5.71	**5.70**
T_3 (SiO_2+GA_3 100ppm)	75.86	76.68	70.61	71.80	70.78	67.92	**72.27**	4.50	4.42	4.39	4.61	4.63	4.43	**4.50**
T_4 (SiO_2+GA_3 125ppm)	84.44	73.83	77.96	71.65	74.36	76.01	**76.37**	4.46	4.16	4.07	4.33	4.34	4.24	**4.27**
T_5 (SiO_2+GA_3 150ppm)	78.57	76.19	79.92	76.70	74.79	77.72	**77.32**	4.16	3.96	3.98	4.07	4.16	4.00	**4.05**
T_6 (SiO_2+PEG100ppm)	74.03	62.81	63.24	65.92	68.17	64.86	**66.51**	4.72	4.97	4.91	4.90	5.10	4.88	**4.91**
Means	**68.96**	**66.57**	**66.27**	**65.69**	**66.62**	**65.80**	**66.65**	**4.87**	**5.11**	**4.87**	**4.95**	**5.04**	**4.88**	**4.95**
C.V. (%)	4.791							5.409						
	SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)			
Treatment	0.753			2.117				0.063			0.178			
Variety	0.697			1.960				0.058			0.164			
Variety × Treatment	1.844			5.185				0.155			0.435			

Table. 1 Effect of nano-invigoration on germination value and germinative energy of ten month aged seeds of maize varieties

Character	Germination value							Germinative energy						
	V_1	V_2	V_3	V_4	V_5	V_6	Mean	V_1	V_2	V_3	V_4	V_5	V_6	Mean
T_0 (Control)	135.90	125.11	144.82	138.81	120.21	145.67	**135.09**	0.31	0.32	0.33	0.32	0.31	0.33	**0.32**
T_1 (SiO_2)	158.77	153.33	189.50	168.50	149.51	181.77	**166.90**	0.43	0.46	0.49	0.45	0.44	0.47	**0.46**
T_2 (GA_3 100ppm)	149.48	144.80	178.54	146.36	132.89	154.14	**151.03**	0.36	0.36	0.37	0.37	0.35	0.38	**0.37**
T_3 (SiO_2+GA_3 100ppm)	211.91	227.48	266.23	192.17	189.45	229.37	**219.44**	0.55	0.57	0.58	0.53	0.52	0.55	**0.55**
T_4 (SiO_2+GA_3 125ppm)	276.30	307.52	341.40	304.89	280.00	329.49	**306.60**	0.64	0.59	0.65	0.59	0.57	0.63	**0.61**
T_5 (SiO_2+GA_3 150ppm)	333.62	334.67	336.40	328.13	320.29	357.78	**335.15**	0.66	0.66	0.69	0.65	0.61	0.69	**0.66**
T_6 (SiO_2+PEG 100ppm)	190.54	203.44	216.44	176.88	160.39	195.57	**190.54**	0.54	0.49	0.50	0.46	0.46	0.48	**0.49**
Mean	**208.07**	**213.76**	**239.05**	**207.96**	**193.25**	**227.68**	**214.96**	**0.50**	**0.49**	**0.52**	**0.48**	**0.47**	**0.50**	**0.49**
C.V. (%)	9.274							5.930						
	SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)			
Treatment	4.699			13.215				0.007			0.019			
Variety	4.351			12.235				0.006			0.018			
Variety × Treatment	11.510			32.371				0.017			0.047			

Table. 1 Effect of nano-invigoration on mean daily germination and peak value of germination of ten month aged seeds of maize varieties

Character	Mean daily germination (seeds/day)							Peak value of germination						
	V_1	V_2	V_3	V_4	V_5	V_6	Mean	V_1	V_2	V_3	V_4	V_5	V_6	Mean
T_0 (Control)	8.76	8.48	9.10	8.90	8.29	9.05	**8.76**	15.50	14.75	15.92	15.58	14.50	16.08	**15.39**
T_1 (SiO_2)	9.52	9.38	10.43	9.81	9.24	10.19	**9.76**	16.67	16.33	18.17	17.17	16.17	17.83	**17.06**
T_2 (GA_3 100ppm)	9.24	9.10	10.10	9.14	8.71	9.38	**9.28**	16.17	15.92	17.67	16.00	15.25	16.42	**16.24**
T_3 (SiO_2+GA_3 100ppm)	10.52	10.76	11.67	10.48	10.38	11.19	**10.83**	20.22	21.06	22.69	18.33	18.25	20.50	**20.18**
T_4 (SiO_2+GA_3 125ppm)	11.05	11.48	12.10	11.43	10.95	11.86	**11.48**	25.00	26.78	28.22	26.67	25.56	27.78	**26.67**
T_5 (SiO_2+GA_3 150ppm)	11.95	11.95	12.57	11.86	11.71	12.38	**12.07**	27.89	28.00	26.86	27.67	27.33	28.89	**27.77**
T_6 (SiO_2+PEG 100ppm)	10.43	10.24	11.10	10.05	9.57	10.57	**10.33**	18.25	19.89	19.50	17.58	16.75	18.50	**18.41**
Mean	**10.21**	**10.20**	**11.01**	**10.24**	**9.84**	**10.66**	**10.36**	**19.96**	**20.39**	**21.29**	**19.86**	**19.12**	**20.86**	**20.24**
C.V. (%)	5.286							6.457						
	SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)			
Treatment	0.129			0.363				0.308			0.866			
Variety	0.119			0.336				0.285			0.802			
Variety × Treatment	0.316			0.889				0.755			2.122			

CONCLUSION

The results of the current study reiterated that controlled imbibition of ten month aged maize kernels in GA_3 encapsulated nanosilica solution followed by dehydration could significantly enhance seed germination. Ten month aged seeds of maize varieties invigorated in GA_3 encapsulated nanosilica solution enhanced germination percentage, seedling shoot length, seedling root length, seedling length, seedling fresh weight, seedling dry weight, seedling vigour index-I, and seedling vigour index-II. The results showed that the maize variety D-765 was less deteriorated after the ten months of ageing. These positive effects of GA_3 encapsulated nanosilica observed in invigorated seeds may suggest that they would exhibit more acceptable agronomic and physiological performance. Seedlings vigour enhancement by the incorporation of GA_3 encapsulated nanosilica in invigoration solution might be due to increased cell division within the apical meristem of seedling. Based on our study, we suggest a potential use of silica nanoparticles to invigorate and accelerate seed germination and produce stronger seedlings in semiarid regions.

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