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Validating Protocol and Deciphering the Nanoparticulate Seed Treatment in Enhancing Seed Quality of Soybean, Pigeon Pea and Groundnut

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RG and KU designed the study. Authors KU and BR performed experiments and analysed data. AuthorsRG, KU and BR wrote initial drafts of the manuscript. Authors KU and BR performed the statistical analysis. AuthorsRG, BR and KJS wrote the final version of the manuscript.All authors read and approved the final manuscript.

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ABSTRACT

Silicon nanoparticles have attracted huge interest as a rapidly growing class of materials for many agricultural applications. It provided new insight of the potential growth promoting effects of the nanoparticle (SiO_2) on plant system. Looking in to its importance, a comprehensive study was conducted to standardize the protocol on method of seed treatment with Silicon dioxide (SiO_2) nanoparticle for enhancing seed quality in soybean, groundnut and pigeon pea. Among the

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treatment combinations, soybean, groundnut and pigeon pea seeds treated with SiO_2 nanoparticle in powder form along with polymer found to be superior for all the tested seed quality parameters *viz.,* germination (95.00, 95.33 and 94.67%), seedling length (39.60, 35.14, 25.56cm), mean seedling dry weight (45.53, 28.33 and 50.47mg/seedling), seedling vigour index-I (3763, 3348 and 2421), seedling vigour index-II (4332, 2700 and 4780) for soybean, pigeon pea and groundnut, respectively. However, compared to untreated control, these seed quality attributes were statistically on par with CMC+NP treatment. These findings suggested that seed treatment with SiO_2 nanoparticle in powder form along with polymer coating significantly enhanced seed quality of soybean, groundnut, and pigeon pea.

Keywords: Silicon dioxide; nanoparticle; soybean; groundnut; pigeon pea; Seed quality.

1. INTRODUCTION

The nanoparticles have been of great scientific value since they came to bridge the gap between bulk materials and atomic / molecular structures, the SiO_2 nanoparticles are most intriguing due to the high surface area by volume ratio, the surface of the nano particles are so important and should be controlled because a change in the size of the surface can generate a change in the physical and chemical properties of the nanoparticles [1-4]. But what factors influence the characteristics of these nanoparticles? What can we do so that their attributes change? When particles reach a size of 1-100nm, their properties change at the electrical, chemical, and physical levels, indicating that the properties are directly related to size, and that changing their size and shape allows for control of properties such as temperature, redox potential, colour, conductivity, chemical stability, electrical qualities, optics, and so on [5-7]. Extensive studies have shown that the size, morphology, stability and properties specifically of the nano silicon dioxide particles are greatly influenced by the experimental conditions of their synthesis, the kinetics of the reaction, the interaction of the ions with the reducing agents and the absorption processes of the stabilizing agent used [7] so that the specific control regarding its shape, size, distribution of the desired nano particle falls on the synthesis method that is selected.

Better seed germination and early seedling establishment are important for increasing production and productivity of soybean, groundnut, and pigeon pea. As a result of producing a deep root system before the top layers of soil dry up, harden, or become exposed to extreme temperatures, rapid and uniform seedling emergence promotes effective establishment. Yet, due to diminished vigour during dry storage especially when kept incorrectly seeds frequently show sluggish and uneven germination. As opposed to conventional approaches, recent uses of certain nanomaterials can speed up plant germination and production and provide excellent plant protection with minimal environmental impact [8,9]. A lot of metabolic and physiological plant processes depend on silicon. When silicon fertilisers are applied to soil that lacks silicon, plants grow more vigorously, become more resistant to disease, cold, and heavy metals like manganese, iron, aluminium, and copper, and as a result, their photosynthesis is enhanced [10]. By encouraging potassium absorption while inhibiting sodium absorption, silicon fertilization raises the potassium/sodium selection ratio, aids in the accumulation of potassium, nitrogen, and sulphur in plants, and enhances plant nutrition. In addition to the impact of Si on plant protection, various other beneficial effects of Si have been reported, such as amelioration of the adverse effects of biotic and abiotic stress in plants [10]. SiNPs have physiological features that permit them to penetrate plants, transport into the plant leaves and manipulate plant metabolic actions [11].

Surabhi et al.[12] reported that SiO_2 nanoparticle significantly increased pigeon pea germination, field emergence, seedling length, seedling vigour index and lowered electrical conductivity. Similarly, studies discovered that tomato seedlings treated with SiO_2 NPs had improved seed germination [8]. Alsaeedi et al. [13] revealed that exogenous application of nanosilica improves germination and growth of cucumber by maintaining K+/Na+ ratio under elevated salt stress conditions.

Furthermore, researchers have exposed that seed priming techniques may enhance seed germination speed via inducing several biochemical changes in the seed system. These changes are required to start the germination process such as imbibitions and enzyme activation, dormancy breaking, leaching of inhibitors, hydrolysis or breakdown of food reserves and their mobilization, etc. Therefore, the present research was designed to validate the protocol for the seed treatment with Silicon dioxide nanoparticle (SiO_2) for enhancing seed quality in soybean, pigeon pea and groundnut.

2. MATERIALS AND METHODS

2.1 Seed Source

Seeds of soybean cv. JS-335, groundnut cv.GKVK-5 and redgram cv. BRG-5 varieties were obtained from the National Seed Project, University of Agricultural Sciences, Bengaluru and shade dried to safer and uniform moisture level (< 9%).

2.2 Characterization of Nanoparticles

In order to confirm the particle size of SiO_2 (obtained from M/S Sigma-Aldrich, Bangalore) whether it is in the range of nano or not. It was subjected to different characterization techniques for determination of the size, morphology, absorbance, its surface nature and chemical composition through dynamic light scattering theory (Zeta sizer) for particle size analysis, UV-Visible Spectrophotometer analysis, Fourier Transform Infrared Spectroscope (FT-IR) analysis, X- Ray Diffraction (XRD) analysis, Scanning Electron Microscope (SEM) analysis, Chemical composition/ elemental analysis, before using it for further studies. The characterization of the selected chemical has been done at the Nanotechnology Research Center, College of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka.

2.3 Methods of Treatments

The seeds were subjected to different modes of treatment with Silicon dioxide (SiO_2) at 500ppm concentration to standardize the method of seed treatment for each crop.

2.3.1 Dry treatment

Seeds were treated with Silicon dioxide nanoparticle at 500ppm by using binding agent 2% Carboxy Methyl Cellulose (CMC) of laboratory reagent grade along with charcoal at the ratio of 1:3 as filler material. Firstly the seeds were coated with binding agent (CMC), after that powder form of nanoparticle and charcoal is added and mixed thoroughly for uniform coating and then shade dried for few hours for adequate adhering of chemical.

2.3.2 Wet treatment

The SiO_2 nanoparticle solution is constituted by dissolving 500mg of nanopowder in one litre distilled water using the Ultra Sonicator (60 watts for 20 minutes) for proper mixing, uniform dispersion and de-agglomeration of the nanoparticle. Then seeds were soaked with the chemical solution for 2 to 6 hours (2h for soybean and Redgram & 6h for groundnut) at room temperature (28 ± 2ºC). The treated seeds are then dried under shade (<9%) for moisture equilibration and uniform absorption of nanoparticles.

Soaking duration for all the crops has been standardized by immersing 2g of seeds in 2ml of water for different durations (1:1 ratio). The duration at which the seeds attained maximum imbibition (uptake of water) without seed coat damage has been considered for further studies. From the study, it is noted that maximum imbibition at 2h for soybean, redgram and 4h for groundnut seeds was obtained without any imbibition injury (Fig. 1).

2.3.3 Spray method

About 20g of seeds were placed in petri plates and 0.5ml of nano formulations was sprayed on to the seeds and stirred with glass rod continuously for two minutes to obtain uniform distribution and then shade dried for few hours.

2.3.4 Seed coating

Seeds were subjected to polymer coating at 3ml/kg seed along with 500mg of SiO_2/kg in powder form using small drum seed treater and then dried under shade. The best form of polymer coating was standardized by coating the seeds both in solution and powder form.

After the different modes of treatments, all the treated seeds were tested by Paper Strip Method (PSM), a special method adapted to record germination and seedling growth without disturbing seedlings. The seed germination along with other parameters was recorded to determine the better mode of seed treatment.

2.4 Assessment of Seed Quality in the ISTA Member Lab

Treated seeds with three replications were used to determine various quality aspects like seed germination (%) as per ISTA [14], epicotyl length (cm), hypocotyl length (cm), total shoot length

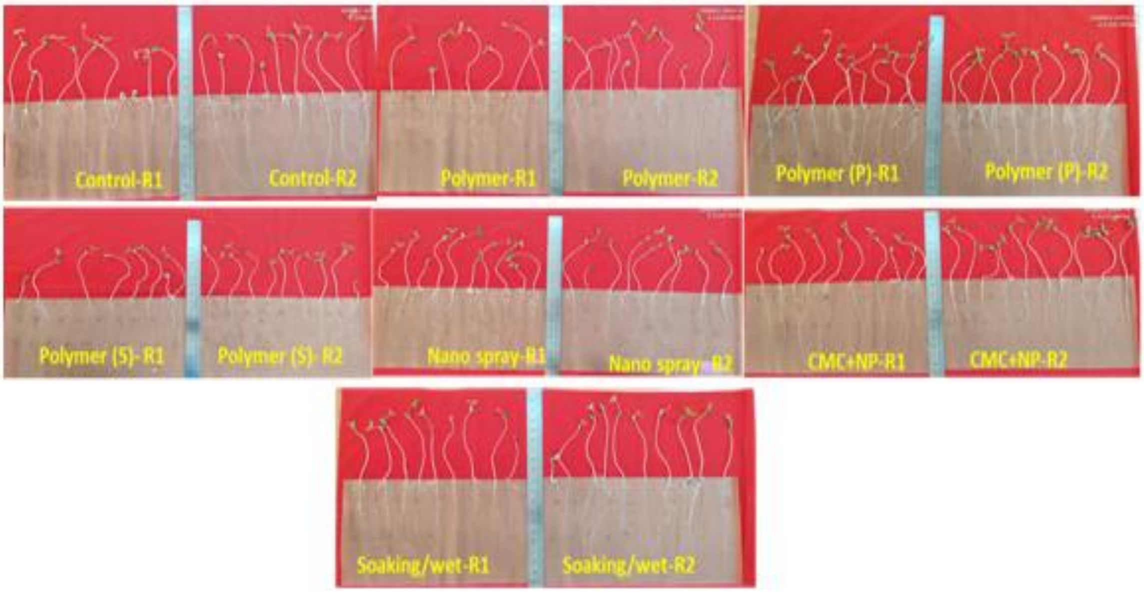

Fig. 1. Influence of seed treatment with SiO_2 nanoparticle on germination of soybean seeds in paper strip method

(cm), root length (cm), seedling length (cm), seedling dry weight (mg/seedling), seedling vigour index-I and seedling vigour index-II [15]. The mean data obtained were statistically analyzed by using suitable ANOVA and the results were presented as mean ± standard deviation (SD) and by comparing each experimental value with its corresponding control. The critical differences were calculated at five per cent level of probability, wherever 'F' test was significant.

3. EXPERIMENTAL RESULTS

3.1 Final Germination Percentage (FGP)

Nano seed treatment improved the germination of seeds. Treating SiO_2 nanoparticle with soybean, pigeon pea and groundnut along with polymer coating significantly increased germination (95.00, 95.33 and 94.67%, respectively). On the other hand, it was similar with carboxy methyl cellulose + SiO_2 nanoparticle treatment when compared to untreated control (Tables 1, 2 & 3; Figs. 2, 3 & 4).

3.2 Epicotyl and Hypocotyl Length of Seedling

Similarly, the length of the seedlings was found to be greater in SiO_2 NPs-treated seeds combined with polymer/CMC as compared to control at first count in all the crops. The same pattern of growth was also observed on the subsequent days. Thus, the results of the study revealed that among the tested combinations, polymer + NP treatment and CMC + nanoparticle treatment showed the greatest response on the epicotyl lengths (3.10, 11.40, 2.03, cm and 3.20, 10.10, 2.73 cm) and hypocotyl lengths (16.77, 0.27, 3.23cm and 15.03, 0.27, 3.57cm) for soybean, pigeon pea and groundnut, respectively (Tables 1, 2 & 3; Figs. 2, 3 & 4).

3.3 Mean Seedling Length

Nano seed treatment improved the seedling length of plant system. Treating of soybean, pigeon pea and groundnut seeds with SiO_2 nanoparticle with along with polymer coating significantly increased seedling length (39.60,35.14 and 25.56cm). On the other hand, it was similar to carboxy methyl cellulose + SiO_2 nanoparticle treatment (37.50, 31.00 and 24.47 cm respectively) when compared to control (Tables 1, 2 & 3; Figs. 2, 3 & 4).

3.4 Mean Seedling Dry Weight

Seedling dry weight found to be significantly higher in polymer + nanoparticle treatment (45.53, 28.33 and 50.47 mg/seedling) and carboxy methyl cellulose + SiO_2 nanoparticle treatment (41.57, 26.40 and 48.77 mg/seedling) when compared to any other treatments in soybean, pigeon pea and groundnut, respectively.

Table 1. Influence of seed treatment methods with SiO_2 nanoparticle on seed germination and vigour by PSM method in soybean

Treatments	Germination (%)	Epicotyl length (cm)	Hypocotyl length (cm)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling dry weight (mg/seedling)	SVI-I	SVI-II
Control	90.33	1.97	15.43	17.40	17.03	34.43	36.03	3109	3260
Polymer	85.33	2.10	14.53	16.63	18.73	35.37	40.53	3019	3464
Polymer + NP (P)	95.00	3.10	16.77	19.87	19.73	39.60	45.53	3763	4332
Polymer + NP (S)	85.33	2.10	16.23	18.33	18.23	36.57	39.07	3122	3339
Nano spray	90.33	2.47	14.50	16.97	17.23	34.20	39.07	3090	3534
CMC + NP	95.00	3.20	15.03	18.23	19.27	37.50	41.57	3564	3955
Soaking/wet methods	93.00	2.30	15.73	18.03	17.13	35.17	39.07	3272	3639
Mean	90.62	2.46	15.46	17.92	18.20	36.12	40.12	3277	3646
S.Em±	2.33	0.10	0.25	0.25	0.39	0.60	1.18	113.57	197.46
CD(0.05P)	7.08	0.31	0.76	0.75	1.19	1.81	3.58	344.47	598.92
CV(%)	4.46	7.15	2.81	2.39	3.75	2.86	5.09	6.00	9.38

Note: NP (P)-SiO_2 nanoparticle in powder form, NP(S) - SiO_2 nanoparticle in solution form, No Hard and FUG seeds observed, PSM-Paper strip method, SVI-I: Seedling Vigour index-I; SVI-II: Seedling Vigour index-II

Table 2. Influence of seed treatment methods with SiO_2 nanoparticle on germination and seedling vigour by PSM method in pigeon pea

Treatments	Germination (%)	Epicotyl length (cm)	Hypocotyl length (cm)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling dry weight (mg/seedling)	SVI-I	SVI-II
Control	85.00	8.57	0.23	8.80	19.57	28.37	23.93	2410	2033
Polymer	85.33	8.77	0.27	9.04	20.07	29.11	22.47	2482	1916
Polymer + NP (P)	95.33	11.40	0.27	11.67	23.47	35.14	28.33	3348	2700
Polymer + NP (S)	85.33	8.90	0.27	9.17	16.43	25.60	25.37	2184	2163
Nano spray	80.00	8.60	0.27	8.87	19.10	27.97	24.93	2236	1993
CMC + NP	95.33	10.10	0.27	10.37	20.63	31.00	26.40	2954	2515
Soaking/wet methods	95.33	9.90	0.30	10.20	21.13	31.33	25.90	2986	2468
Mean	88.81	9.46	0.27	9.73	20.06	29.79	25.33	2657	2256
S.Em±	1.58	0.16	0.03	0.17	0.49	0.47	0.59	27.50	39.88
CD(0.05P)	4.80	0.50	0.09	0.52	1.48	1.42	1.79	83.41	120.96
CV(%)	3.09	3.01	20.04	3.05	4.22	2.73	4.03	1.79	3.06

Note: NP (P)-SiO_2 nanoparticle in powder form, NP(S) - SiO_2 nanoparticle in solution form, No Hard and FUG seeds observed, PSM-Paper strip method, SVI-I: Seedling Vigour index-I; SVI-II: Seedling Vigour index-II

Table 3. Influence of seed treatments method with SiO_2 nanoparticle on seed germination and vigour by PSM method in groundnut

Treatments	Germination (%)	Epicotyl length(cm)	Hypocotyl length (cm)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Seedling dry weight (mg/seedling)	SVI-I	SVI-II
Control	80.67	1.20	2.97	4.17	15.17	19.34	41.73	1560	3368
Polymer	70.00	1.37	2.47	3.84	18.07	21.91	37.90	1533	2654
Polymer + NP (P)	94.67	2.03	3.23	5.26	20.30	25.56	50.47	2421	4780
Polymer + NP (S)	80.67	1.47	2.67	4.14	15.60	19.74	39.87	1592	3217
Nano spray	80.67	1.57	2.83	4.40	18.30	22.70	40.83	1831	3294
CMC + NP	94.67	2.73	3.57	6.30	18.17	24.47	48.77	2317	4619
Soaking/wet methods	95.00	1.97	3.03	5.00	18.17	23.17	49.80	2200	4730
Mean	85.19	1.76	2.97	4.73	17.69	22.41	44.20	1922	3809
S.Em±	1.23	0.05	0.07	0.06	0.46	0.49	1.01	56.38	123.90
CD(0.05P)	3.72	0.15	0.20	0.18	1.40	1.49	3.08	171.02	375.81
CV(%)	2.50	4.80	3.89	2.16	4.51	3.80	3.98	5.08	5.63

Note: NP (P)-SiO_2 nanoparticle in powder form, NP(S) - SiO_2 nanoparticle in solution form, No Hard and FUG seeds observed, PSM-Paper strip method, SVI-I: Seedling Vigour index-I; SVI-II: Seedling Vigour index-II

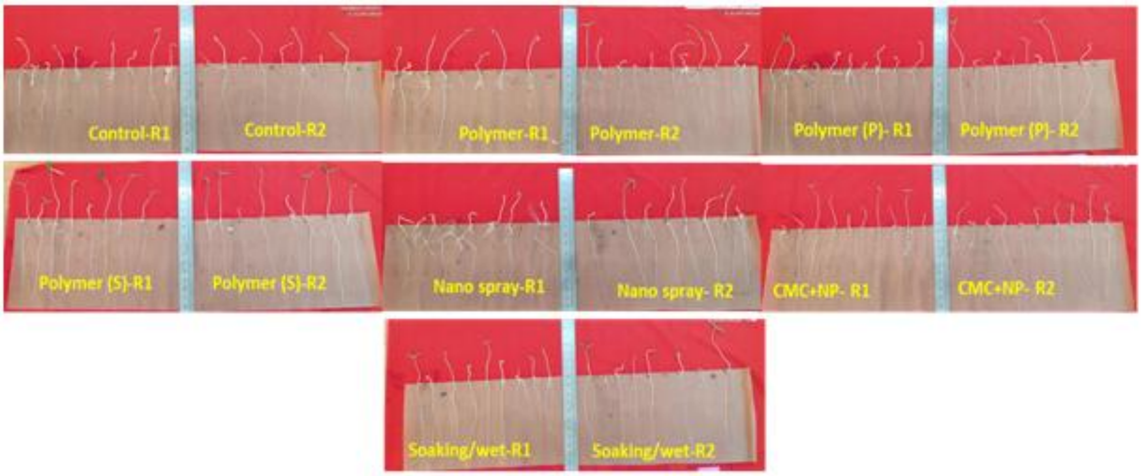

Fig. 2. Influence of seed treatment with SiO_2 nanoparticle on germination of pigeon pea seeds in paper strip method

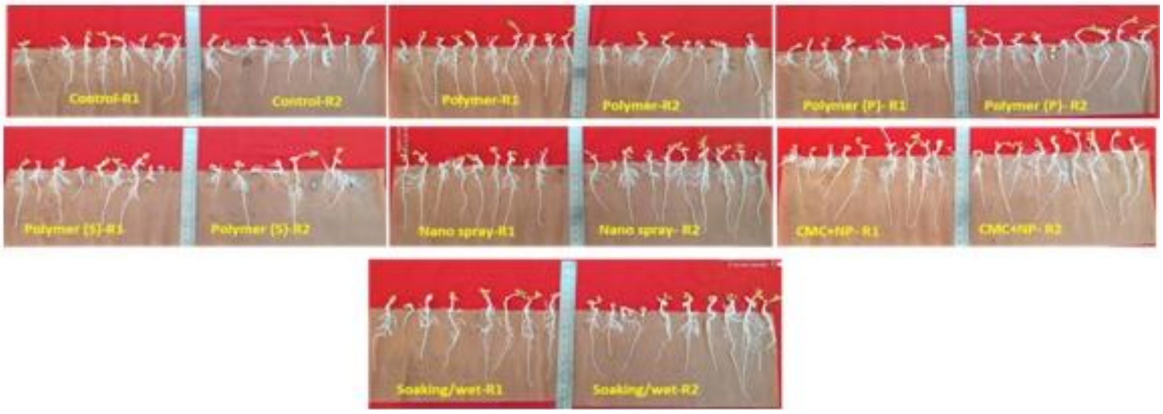

Fig. 3. Influence of seed treatment with SiO_2 nanoparticle on germination of groundnut seeds in paper strip method

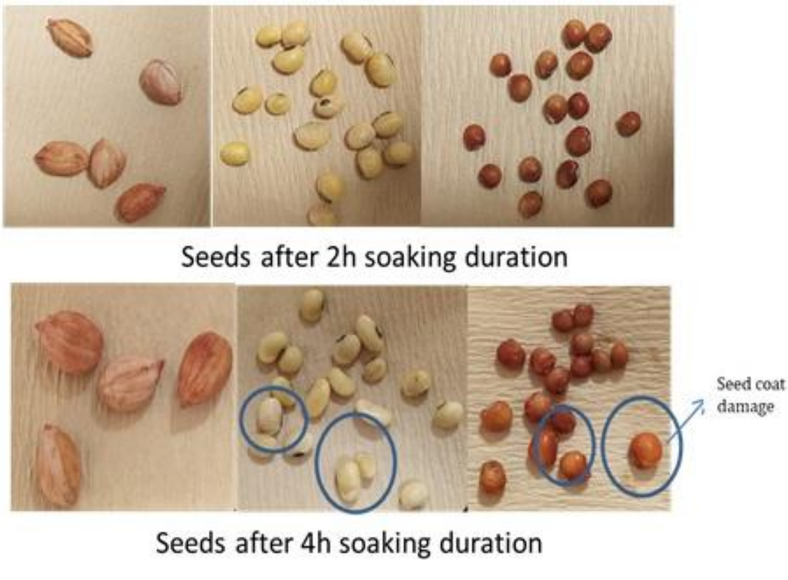

Fig. 4. Seed imbibition studies to optimize the duration of soaking in groundnut, soybean and pigeon pea

3.5 Seedling Vigour Index I and II

Mean seedling vigour indices also increased significantly due to nano-particle treatments. In soybean, pigeon pea and groundnut, seedling vigour index I (SVI-I) improved significantly with polymer + nanoparticle treatment (3763, 3348 and 2421) and carboxy methyl cellulose + SiO_2 nanoparticle treatment (3564, 2954 and 2317), when compared to control (3109, 2410, 1560). Even seedling vigour index II (SVI-II) also resulted in significant improvement with polymer + nanoparticle treatment (4332, 2700 and 4780) and carboxy methyl cellulose + SiO_2 nanoparticle treatment (3955, 2515 and 4619), when compared to control (3260, 2033, 3368) in all the three crops, respectively.

4. DISCUSSION

The data depicted in Tables 1 to 3 elucidated that SiNPs treatment along with polymer/ carboxy methyl cellulose succeeded to enhance germination and seedling growth development of soybean, pigeon pea and groundnut seedlings recording higher values for germination and its parameters in contrast to untreated seeds.

Moreover, another possible elucidation for improving germination and vigour via SiNPs might be owing to the ability of SiNPs to initiate some biochemical processes inside the seed system after easily piercing through seed coat because of tiny size of silica nanoparticles [16]. Besides, SiNPs found responsible in removing the free radicals, which inhibit the germination after entering in to the seed. As a result, it activates CAT and SOD enzymes by alerting some oxidation-reduction reactions and thereby produce superoxide ion radical which have an important role in nullifying the free radicals found inside the seed and finally enhance the germination [17]. Moreover, SiNPs can be crucial in releasing the dormancy of seeds by increasing gibberellin secretion and reducing the formation of abscisic acid [18]. To underline these results in the open field, however, additional research is required. This is because SiNPs may undergo some alterations or transformations when put into a complex and open system like the interactions between soil and the environment. Also, considerable biochemical and physiological research is required to clearly define the alterations that could occur within the plant cell as a result of SiNPs treatment. Previous research has revealed at least three mechanisms. The first of these most likely takes place at the level of the control of gene expression. Aquaporins are transmembrane proteins that help biological membranes transport gases and water as well as reactive oxygen species (ROS). "Si" nanoparticles activate the genes that code for these proteins [19]. The second takes place when gibberellins activate the production of gibberellins and hydrolytic enzymes such -amylase, proteases, and lipases [20]. These enzymes facilitate the hydrolysis of organic compounds with larger molecular weights into molecules with lower molecular weights that serve as respiratory substrates for developing cells. Moreover, when the concentration of monosaccharides and other low-molecular-weight compounds rises, the osmotic potential of cells rises, hastening the absorption of seed structures and other storage materials. The link between the amount of oxidative stress and the activity of the antioxidant system in the embryonic cells may vary as a result of the third mode of action of SiNPs [21-26]. Plant cells' antioxidant enzymes (catalase and superoxide dismutase) are activated as a result of stimulation of the production of reactive oxygen species (ROS) such hydrogen peroxide and hydroxyl radicals by SiNPs. These modifications set the proper quantity of ROS in embryonic cells, forming the so-called "Oxidative window," which is required to activate signal pathways that start seed germination and promote improved seedling growth and development.

5. CONCLUSION

This paper discusses the role of powder form of nanosilica (SiNPs) particles to enhance seed germination of soybean, pigeon pea and groundnut and the development of seedlings (vigour) under controlled conditions in paper strip method. The outlined data revealed that seed treatment with SiO_2 nanoparticle along with polymer or carboxy methyl cellulose conspicuously improved seed germination and accelerated the seedling growth and development. Further, all the estimated seed quality parameters like germination and seedling growth of all three crops were significantly superior over untreated control. The application of Silicon dioxide (SiO_2) in nanoform might have penetrated inside the seed due to its nano-size and consequently alerting various biochemical processes such as elimination free radicals by activating CAT and SOD enzymes and thus helps in better promotion of germination and its attributes. Therefore, it is suggested that in future, such amendments (SiNPs) could be advocated in the guidelines of good agricultural practices to improve crop growth and productivity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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