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Evaluation of GA_3 loaded silica nanoparticles ($nSiO_2$) effects on germinability of ten month aged maize (*Zea mays* L.) varieties seeds

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Abstract
The present study was aimed to evaluate the Effects of silica nanoparticles and GA_3 loaded silica nanoparticles (SiO_2) effects on comparative efficiency of invigoration techniques on seed germinability of maize varieties. The present investigation was carried out at G.B.P.U.A.T., Pantnagar, Uttarakhand under laboratory condition. The ten month aged seeds of six maize varieties *viz.* Amar, PSM-3, D-765, Tarun, Sweta and Navin along with seven invigoration treatments *i.e.* control, nanosilica 8g/l, GA_3 100 ppm, 100 ppm GA_3 loaded nanosilica, 125 ppm GA_3 loaded nanosilica, 150 ppm GA_3 loaded nanosilica and 100 ppm PEG loaded nanosilica. Results also depicted that D-765 maize variety had significantly maximum value for seed quality parameters including standard germination percentage, germination value, germinative energy, mean daily germination and peak value after 10 month of ageing. The results of experiment showed that ten month aged seeds invigorated with T_5 recorded higher value for all the seed quality parameters and increase 23% germination as compared to control. Ten month aged seeds of D-765 maize variety reported highest value for seed quality parameters including standard germination percentage, germination value, germinative energy, mean daily germination and peak value. The results showed that the variety D-765 was minimum deteriorated after the ten month of ageing. Therefore, it is very clear that delivery of GA_3 through nanosilica ($nSiO_2$) has a significant impact on the seed germination potential.

Keywords: seed invigoration, nanosilica, germination, GA_3, *Zea mays*

Introduction
Crop improvement and the delivery of high quality seeds and planting materials of selected variety to growers is necessary for ensuring improved crop production and meeting growing environmental challenges. Maize is one of the most important cereal crops of the world and contributes to food security in most of the developing countries. In India, maize is emerging as third most important crop after rice and wheat. Maize in India, contributes nearly 9% in the national food basket and more than Rs. 100 billion to the agricultural GDP at current prices apart from that also generating employment over 100 million man-days at the farm and downstream agricultural and industrial sectors (Ministry of Agriculture & Farmers Welfare, 2017) [3].
The development of seed invigoration treatments really started with seed priming, as first described by Heydecker *et al.* (1973) [8]. Seed priming or osmo conditioning is one of the physiological methods that improves seed performance and provides faster and synchronized germination. It is an easy, low cost and low risk technique and recently being used to overcome the salinity problem in agricultural lands. It entails the partial germination of seed by soaking in either water or in a solution of salts for a specified period of time and then re-drying them just before the radicle emerges (Neto and Tabosa, 2000) [15]. Seed priming stimulates many of the metabolic processes involved in the early phases of germination, and it has been noted that seedlings from primed seeds emerge faster, grow more vigorously, and perform better in adverse conditions (Cramer and Quarrie, 2002) [5]. Plants generally require silica to control biotic and abiotic stress (Ma, 2004) [13].The presence of silicon reduces toxic metal elevation and increases water-use efficiency and photosynthesis rate in plants. Furthermore, silicon also acts as a bioprotectant against fungal attack (Datnoff *et al*., 1997) [2]. Nanoparticles show promise in different fields of agricultural biotechnology (Majumder *et al.*, 2007) [14]. However, application of silica nanoparticles in agriculture is still considered a novel approach as they are more reactive than their bulk counterparts. Silica nanoparticles (SNPs)

Would be required in smaller quantity to improve crop protection. Nanosilica is an important metal oxide that covers all major fields of science and technology including industrial, electronics and biomedical applications (Dinda *et al*., 2012) [7]. It has gained greater attention because of its highly reactive surface-to-volume ratio property. The introduction of nanoparticles into plants might have significant impact and thus, it can be used for agricultural applications for better growth and yield. An earlier study showed that the addition of nanosilica in soil enhanced growth of maize (*Zea mays* L.) (Yuvakkumar *et al*., 2011) [11].

Materials and Method

Laboratory experiments were conducted to know the effect of invigoration with different concentration of GA_3 loaded nanosilica (SiO_2) on ten month aged seeds of maize varieties. The interaction effects due to all different combinations among different maize varieties and GA_3 loaded nanosilica treatments were also studied. The laboratory experiments were conducted at G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India by following the procedure outlined by International Seed Testing Association (ISTA, 2007) [9]. The 10 month old seeds of 6 maize varieties (Amar, PSM-3, D-765, Tarun, Sweta and Navin) were treated with the SiO_2 nano-particles 8 g/liter, GA_3 100 ppm concentration, different concentration (100 ppm, 125 ppm and 150 ppm) of GA_3 loaded silica nanoparticles and 100 ppm Poly Ethylene Glycol (PEG) loaded nanosilica solution. The six maize varieties seed were soaked in different prepared solution for 12 hours at 25 ^{0}C. 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 shoot and root length of individual seedling manually with help of scale. Fresh weight of ten seedlings was measured at the same time and then dry weight was recorded after oven drying. 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) [9]. Only normal seedlings were counted on the 7th day to record the data.

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

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 valueof germination

Peak value was calculated according to following formula:

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

Results and Discussion

The standard germination percentage was recorded on 7th days of germination test only normal seedling was counted. The data presented in Table 1regarding 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. Among the different nano-invigoration treatments, 150 ppm GA_3 loaded nanosilica treatment had highest standard germination percentage (85%). The highest 23% increase of standard germination was recorded in nanosilica with 150 ppm GA_3 loaded treatment as compared to untreated control. Maize variety D-765 invigorated with 150 ppm GA_3 loaded nanosilica was showed highest standard germination percentage (88%) and lowest standard germination percentage was found in untreated Sweta variety (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. Alam *et al.* (2015) [2] which show edthatiron nanoparticles concentration helps to increase germination and growth response of wheat seedlings. Adhikari *et al.* (2013) [1] observed good germination of seeds in the presence of $nSiO_2$ and Mo nanoparticles in rice. Karunakaran *et al.* (2012) [11] observed that nanosilica promoted seed germination percentage (100%) in maize than conventional Si sources, Khodakovskaya *et al.* (2009) [12] reported that carbon nanotubes increased germination and growth of tomato due to the ability of carbon nanotubes to penetrate into the seed coat and stimulate waterabsorption. Rad *et al.* (2014) [16] concluded that silica nanoparticles concentrations could significantly increase the seed germination in comparison to control of maize seed similar results was reported by Roohizadeh *et al.* (2015) [17] in *Viciaafaba* L., Siddiqui and Al-Whaibi (2014) [18] in tomato.

Germination value

Germination value is the mean daily germination multiplied by peak value. Finding towards the germination value shown in Table 1indicated that there was significant variation obtained among maize varieties and nano-invigoration treatments. The maize variety D-765 had maximum germination value 239.07 followed by Navin variety (227.68), whereas lower germination value was recorded for Sweta variety (193.25). Data revealed that among the treatments maximum germination value was observed for 150 ppm concentration GA_3 loaded nanosilica invigoration treatment (335.15) and minimum mean value for germination value was observed for untreated control (135.09). The maximum increase of germination value was recorded for nano-invigoration with 150 ppm concentration GA_3 loaded nanosilica. An overall mean value observed that the interactions between treatment and variety had non-significant effect on germination value. This agreed result of Azimi *et al.* (2014) [4] 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 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). Among the treatments 150 ppm concentration of GA_3 loaded nanosilica invigoration treatment was reported maximum germinative energy (0.66) followed by 150 ppm concentration of GA_3 loaded 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. 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) [20]. The result agreed with Janmohammadi and Sabaghnia (2015) 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.

Mean daily germination (seeds/day)

The mean daily germination is the average daily germination calculated as germination percentage divided by number of days to reach the highest germination. The statistical analysis of the data presented in Table 2 depicted that highly significant difference was observed among the maize varieties and nano-invigoration treatments. Among the maize varieties maximum mean daily germination was recorded for D-765 (11.01 seed per day) whereas lowest mean daily germination was recorded for Sweta (9.84 seeds per day).The result of the comparison data revealed that mean daily germination among all treatments varied from 8.76 seeds per day to 12.07 seeds per day. The maximum mean daily germination was reported for 150 ppm concentration GA_3 loaded nanosilica invigoration treatment (12.07 seeds per day). However, minimum mean daily germination was found in untreated control (8.76 seeds per day). A close examination of data revealed that interaction between maize varieties and nano-invigoration treatment had non-significant effect on mean daily germination. The above findings are in agreement with the results of Azimi *et al.* (2016) which showed thatnSiO_2 nanoparticles had positive effect on mean daily germination of tall wheatgrass (*Agropyron elongatum* L.). Janmohammadi and Sabaghnia (2015) reported similar results on nanosilica priming treatments and have significant effect on mean daily germination (MDG) of sunflower (*Helianthus annus*).

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 2 depicts 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), whereas minimum peak value of germination was recorded for Sweta. Among the treatments maximum peak value of germination was recorded for 150 ppm concentration GA_3 loaded nanosilica invigoration treatment (27.77). However, lowest peak value of germination was reported in untreated control (15.39). The nano-invigoration treatment with 150 ppm concentration GA_3 loaded nanosilica was found superior among the all nano-invigoration treatment and showed highest increase of peak value of germination (12.38). Interaction between maize varieties showed non-significant difference for peak value of germination. 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.).

Conclusion

Nanotechnology is an emerging technology working in all fields of science. The application of nanosilica (nSiO_2) may increase significantly standard germination percentage, germination value, mean daily germination (MDG) and Peak value (PV) of ten month aged seed of maize varieties suggest that SiO_2 nanoparticles may be contributed in the metabolic or physiological activity in maize seeds. Consequently, it has proposed that applying SiO_2 nanoparticles together with GA_3 could use as a new alternative potential for seed dormancy breaking of maize seeds.

Table 1: Effects of GA3 loaded silica nanoparticles on standard germination percent, Germination value and germinative energy of ten month old seeds of maize varieties

Character	Standard germination (%)							Germination value							Germinative energy						
	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	V_1	V_2	V_3	V_4	V_5	V_6	M
T_0 (Control)	62	59	64	62	58	64	62	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)	67	65	73	69	65	71	68	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)	65	64	71	64	61	66	65	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)	73	76	82	73	73	82	76	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)	75	80	85	80	77	83	80	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)	84	84	88	83	82	87	85	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+PEG100ppm)	73	72	78	70	67	74	72	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	71	71	77	72	69	75	73	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. (%)	1.62							9.274							5.930						
	SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)				SEm (±)			C.D. (5%)			
Treatment	0.277			0.779				4.699			13.215				0.007			0.019			
Variety	0.256			0.721				4.351			12.235				0.006			0.018			
Variety × Treatment	0.678			1.908				11.510			32.371				0.017			0.047			

Table 2: Effects of GA3 loaded silica nanoparticles on Mean Daily Germination and Peak value of ten month old 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+PEG100ppm)	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					

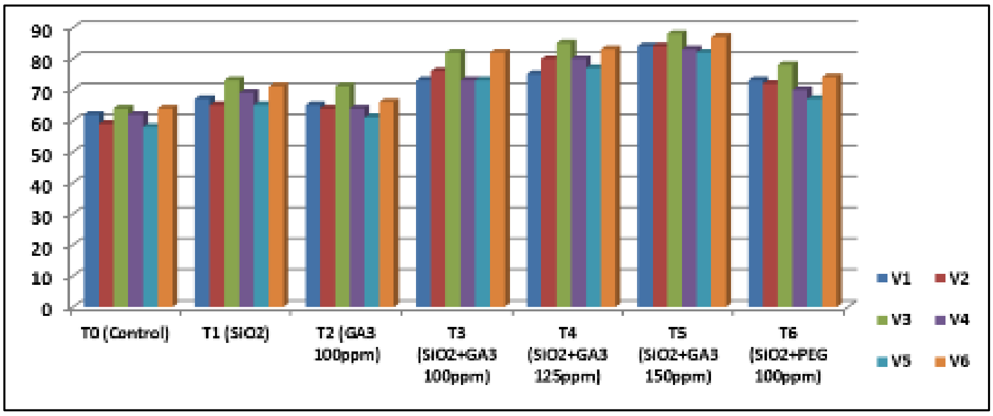

Fig 1: Influence of GA3 loaded nanosilica on Standard germination percent of maize varieties

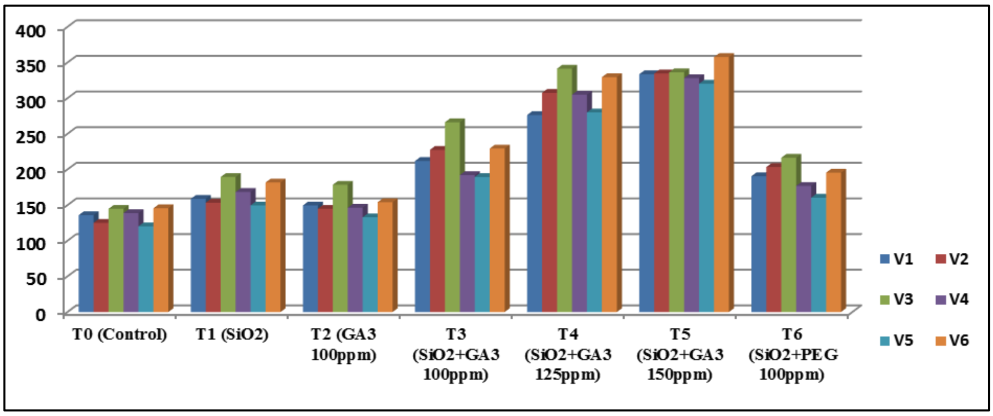

Fig 2: Influence of GA3 loaded nanosilica on Germination value of maize varieties

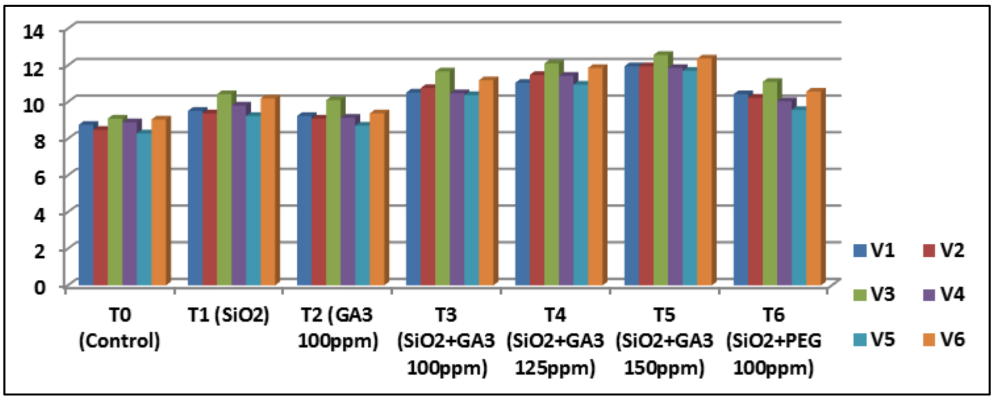

Fig 3: Influence of GA_3 loaded nanosilica on Mean Daily Germination of maize varieties

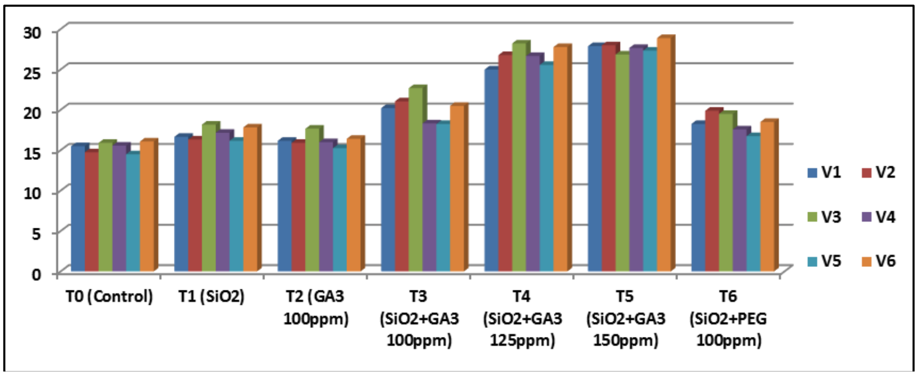

Fig 4: Influence of GA3 loaded nanosilica on Peak value of germination of maize varieties

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