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Influence of Nanoparticles on Plant Growth, Seed quality, Yield and Genetic changes in Maize (*Zea mays* L.)

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ABSTRACT: The study illustrates the potential usage and beneficial effects of nanochemicals through seed priming. The experiment was conducted on maize cv.MAH 14-138 F_1 hybrid pot culture under polyhouse condition with few selected nanochemicals *viz.*, silicon dioxide, titanium dioxide and iron oxide with two levels (500 and 1000 mg/kg of seeds). The results of the study revealed that polymer coating of SiO_2NPs @500 mg per kg of seeds recorded significantly higher plant height (336.27cm), number of leaves (15), stem girth (4.77cm) as compared to untreated control (251.07cm, 13 and 3.09cm, respectively) at 90th day of sowing. Increased cob length (15.95 cm), cob diameter (6.81cm), cob seed weight (105.41 g), cob pith weight (63.90 g), total cob weight (170.64 g), number of seeds per cob (456) and 100 seed weight (26.63 g) recorded in T_4 (dry dressing with TiO_2 500 mg per kg of seeds) over control (9.87cm, 14.50cm, 45.97g, 21.70g, 71.0g, 300 and 16.14g, respectively). The resultant seeds were harvested at physiological maturity and evaluated for initial seed quality parameters. Seeds dry dressed in TiO_2 500 mg/kg recorded maximum germination (95%), shoot length (19.61cm), root length (18.52cm), seedling length (38.13cm), dry weight of seedling (60.79mg/seedling), seedling vigour index-I (3791), seedling vigour index-II (5937), lower electrical conductivity of seed leachate (12.07µS/cm) and higher total dehydrogenase activity (0.547). The findings of the present study clearly suggest that both silicon dioxide and titanium dioxide nanochemicals @500 mg/kg of seeds with or without polymer mediated coating significantly improve maize seed production. Thus, nanoparticles have the potential to improve the growth and production of crops through various mechanisms.

Keywords: Maize hybrid, nanochemicals, seed dry dressing, seed polymer coating, pot culture.

INTRODUCTION

Cereals are the most important food grain crops, which are generally grown in various agro climatic regions of the country. India is the second largest producer of rice, wheat, maize, and other cereals, which accounts 54% of total agricultural production; even these can be handled, transported and stored with minimum spoilage. Among the cereal crops, maize (*Zea mays* L.) contributes as the third major principle cereal and India ranks seventh worldwide in maize production. The crop is widely grown in a wide range of soils under temperature varying from 21 to 35°C. Major growing states of maize are Karnataka, Maharashtra, Tamil Nadu, Telangana, and Madhya Pradesh.

Nanopriming uses nanoparticles which have particle size of below 100 nm, by means of priming methods; several biotic and abiotic stress tolerance techniques have been developed so far. However, several researchers demonstrated that nano priming could improve seed germination and plantability of wide range of agricultural and horticultural crops with their enhanced vigour potential. Besides, nanopriming has got the ability to break the seed dormancy, but the reason is obscure. Meanwhile, usage of nanoparticles was also observed with toxicological effects on crops due to their unsuitability and over dosage. Nevertheless, nano-encapsulated pesticides and fertilizers are an alternative method of improving crop performance. Nano based inputs in agricultural production can reduce excess chemical fertilizer usage, thereby controlling environmental pollution, tolerance towards different type of stresses in plants. Besides, seed nanopriming also utilized for seed protection as many nanoparticles have antimicrobial activities and protect crops from pests and diseases.

Nano particles can easily penetrate the plasma membrane, also induces or enlarge the pores which enhance the uptake of essential micronutrients compared to the conventional bulk particles (Navarro *et* *al.*, 2008). Nano-silica has beneficial effects by establishing plant species with their enhanced growth, varied stress resistances (Mushtaq *et al*., 2017; Choudhary and Budania 2022) and defense mechanism against fungi by enhancing phenolic accumulation and enzymes like peroxidase, polyphenol oxidases, etc., acts as a modulator of host resistance to specific pathogens (Cherif *et al.,* 1994). Nano titanium dioxide significantly improved the germination of low vigour seeds of spinach (Zheng *et al.,* 2005) and in wheat (Feizzi *et al.,* 2012) when compared to application of bulk titanium particles. These encounters the water deficit conditions supported by several stress related genes and influenced the growth characteristics in wheat (Tumbura *et al.,* 2017; Jaberzadeh *et al.,* 2013). Productivity of different crops has been hindered by stresses like water logging, drought and toxicity of heavy metals, etc. Several researchers found that treatment with nano silica, nano titanium etc., facilitated the germination and productivity of maize crop under saline conditions (Zhang *et al.,* 2007; Ghodrat and Rousta 2012; Abdel Latef and Tran 2016). TiO_2 nano particles have many insightful impacts on plant morphological, physiological and biochemical features (Mishra *et al.,* 2014). Waqas *et al.* (2022) proved that seeds treated with zinc oxide nanoparticles increased the seed and straw yield under water deficit environment and it also reduced MDA content in rice plants under water stress conditions. Zinc improved the SOD activities (Kasim, 2007) and helped in alleviating ROS induced damages and which ultimately improved the nutrient profiles of cereal crops (Powell, 2000). It also documented increased plant biomass in the nano treated seeds (Khan *et al.,* 2021; Rizwan *et al.,* 2019; Mahakham *et al.,* 2016). Iron content plays major role in cell metabolism and it involved in photosynthesis, respiration, enzyme activity and other physio process (Bakhtiari *et al.,* 2015). Nano iron treated with durum wheat (Preco variety) seeds exhibited maximum plant height in lower concentrations (Ali *et al.,* 2022) since the iron nutrient drives the higher plant growth, chlorophyll production and redox process in plant development (Briat *et al.,* 2007). Therefore, with these evidences, a pot culture experiment was taken up at the Seed Technology Research Unit in GKVK, Bangalore during *Kharif* 2022 to study the real impact of nanochemicals (which proved to enhance seed quality in our previous laboratory work) on crop growth and its performance.

MATERIAL AND METHODS

Source of seeds: The freshly harvested seeds maize F_1 hybrid MAH 14-138 were procured from Breeder Seed Production Unit, National Seed Project (crops), University of Agricultural Sciences, GKVK, Bengaluru and manually cleaned and dried to obtain uniform and safer moisture content (< 9%).

Dry dressing nano treatment: Seeds were treated with selected nano chemicals *viz.*, Silicon dioxide (SiO_2), Titanium dioxide (TiO_2) and Iron oxide (FeO) each at the concentration of 500 and 1000 mg per kg of seeds (Fig. 1). 2 to 3 ml of 2% CMC as binding agent was utilized for uniform coating of nanochemicals over the seed. The treated seeds were subjected to air drying in room temperature for few hours to obtain equilibrium moisture level in seed mass and then were evaluated for various seed quality parameters.

Polymer mediated nano coating: 3 ml of water-soluble synthetic polymer (pink colour) was mixed with nano chemicals each @ 500 and 1000 mg per kg of seeds and uniformly coated with polymer (Fig. 2). Then, the seeds were kept for shade drying to achieve moisture equilibrium and were evaluated for several quality attributes.

Pot culture experiment: Treated seeds with three replications were planted in high density plastic pots of 14" diameter filled with pot mixture of red soil, sand, compost and the crop was managed with required NPK nutrients in the form of complex fertilizers uniformly for all the treatments, regular watering, weeding and other cultural operations until crop maturation (Fig. 3). Observations were recorded on seedling emergence, plant growth and yield parameters. Further, the seed quality attributes were also recorded on resultant crop seeds as per the procedure prescribed by ISTA (2021). The statistical analysis of experimental data was carried out by using completely randomized design method.

Molecular analysis of genetic changes: Seven days old fresh leaves were taken for DNA isolation by CTAB method (Doyle and Doyle, 1990) with slight modifications at the Molecular Lab of Seed Technology Research, AICRP on NSP (Crops), University of Agricultural Sciences, GKVK, Bangalore. Identified SSR markers (Phi 053, Bnlg 1520 and bnlg 198) of maize hybrid were used for PCR analysis (Humera, 2017). The DNA profiles were examined along with the control and compared for any changes in genomic level of nanoparticle treated seeds, The DNA profile data are used to interpret the results based on the presence or absence of corresponding bands at the known base pair.

RESULTS AND DISCUSSION

The studies on the pot culture experiment under polyhouse conditions revealed that seeds treated with selected nano chemicals showed early emergence when compared to the untreated control. The plant height was significantly higher in T_9 (polymer coated with SiO_2 @ 500 mg per kg of seeds) in all plant growth stages *viz.*, 30, 60 and 90 days after sowing (117.13, 328.65 and 336.27 cm, respectively) compared to control (94.53, 243.07 and 251.07 cm, respectively). The number of leaves were maximum in T_9 (12, 14 and 15) at 30, 60 and 90 days, respectively after sowing when compared to control (11, 12 and 13). The increased stem girth was also found in T_9 (4.43, 4.60 and 4.77 cm) at 30, 60 and 90 days after sowing and lowest in control (2.97, 3.17 and 3.09 cm) respectively (Table 1). Similarly, research findings of Siddiqui *et al.* (2014) revealed increased germination due to nano-silicon seed treatment in wheat. Shah *et al.* (2021) also reported increased germination due to nano titanium in maize under salinity stress. Nano ferric oxide improved the several plant growth traits of durum wheat like plant height, root length, number of leaves, inter nodal length, 1000 grain weight, relative water contents according to Ali *et al.* (2022).

Further, various cob quality parameters from maize hybrid MAH 14-138 plants grown under pot culture were assessed after physiological maturity stage and presented in Table 2. Among all the treatments, increased cob length (cm), cob diameter (cm), cob seed weight (g), cob pith weight (g), total cob weight (g), number of seeds per cob, 100 seed weight (g) were noticed in T_4-dry dressed with TiO_2 NPs @ 500 mg (15.95 cm, 6.81 cm, 105.41 g, 63.90 g, 170.64 g, 456 and 26.63 g, respectively), however they were statistically on par with T_9-polymer coated with SiO_2 500 mg (15.50 cm, 5.74 cm, 89.97 g, 57.33 g, 145.97 g, 402 and 24.51 g, respectively) when compared to T_1-untreated control (9.87 cm, 4.17 cm, 45.97 g, 21.70 g, 71.0 g, 300 and 16.14 g, respectively). According to Farooq *et al.* (2013); Shah *et al.* (2019) nanoparticles priming accelerate the metabolic activities during germination stage and thereby improves the seedling quality of maize under saline condition.

The resultant crop seeds were evaluated for seed quality attributes to know the influence of nanochemicals treatment in maize MAH 14-138 hybrid and presented in Table-3. The results revealed increased germination (%), shoot length (cm), root length (cm), seedling length (cm), dry weight of seedling (mg), seedling vigour index-I, seedling vigour index-II, total dehydrogenase activity and decreased electrical conductivity (µS/cm) of seed leachates with T_4 seeds dry dressed with TiO_2 NPs 500 mg/kg of seeds (95%, 19.61 cm, 18.52 cm, 38.13 cm, 60.79 mg, 3791, 5937, 0.547, 12.07 µS/cm, respectively) compared to T_1-untreated control (70%, 15.77cm, 17.00cm, 32.77cm, 43.30mg, 2316, 3056, 0.430, 9.29 µS/cm, respectively). These research findings are in agreement with Suriyaprabha *et al*. (2012) wherein nano-silicon resulted in increased germination due to absorption and utilization of silica by seeds and had significant increase in fresh weight and dry weight of seedlings. Further, Haghighi *et al.* (2014) also noticed that TiO_2 NPs recorded 100 per cent germination in onion and radish and acted as good priming agent for horticultural crops. Besides, root length increases upon nano iron treatment with lower concentrations in both *Capsicum annuum* and *Arachis hypogea* (Yuan *et al.*, 2018; Rui *et al.*, 2016).

Three SSR primers like Phi 053, Bnlg 1520 and bnlg 198 were used for the study the genetic changes, if any due to seed treatment with nano-chemicals. All the primers showed single, uniformly amplified banding pattern for all the treatments along with the untreated control. Based on the presence or absence of band and its size, we can infer that no genetic variations observed in genomic level due to selected nano-chemicals (Fig. 4). As the nano-chemicals treated seeds produced identical monomorphic banding pattern it is suggested the genetic stability. These findings were also supported by Lopez-Moreno *et al.* (2010) where ZnO @ 2000mg/L showed no changes in RAPD profile in soybean but ZnO NPs @4000 mg/L showed a new band due to change in priming sites leading new annealing events and deletions and homologous recombination which indicates genotoxicity.

Table 1: Influence of nanochemicals on plant height (cm), number of leaves and stem girth (cm) grown under polyhouse conditions in maize hybrid cv. MAH 14-138.

Treatments	Plant height (cm)			Number of leaves			Stem girth (cm)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	94.53	243.07	251.07	11	12	13	2.97	3.17	3.09
T_2	108.10	290.83	293.40	12	14	14	3.21	3.57	3.72
T_3	111.67	277.18	278.81	12	13	13	3.63	4.01	4.01
T_4	113.81	327.25	330.70	12	15	15	3.70	3.88	4.02
T_5	110.07	274.03	283.39	12	13	13	3.61	4.18	4.40
T_6	110.83	279.88	290.93	11	13	13	3.22	4.00	3.71
T_7	109.70	308.93	319.41	11	14	14	3.28	3.34	3.40
T_8	111.97	268.75	279.87	12	13	14	3.67	3.81	3.87
T_9	117.13	328.65	336.27	12	14	15	4.43	4.60	4.77
T_{10}	104.08	307.13	329.78	11	13	14	3.04	3.33	3.23
T_{11}	105.40	309.89	327.90	12	13	14	3.29	3.34	3.37
T_{12}	104.69	306.78	320.92	11	14	14	3.53	3.70	3.98
T_{13}	105.90	317.41	319.87	12	14	14	3.61	3.83	3.97
T_{14}	100.17	303.30	316.98	11	13	13	3.27	3.47	3.55
T_{15}	101.47	256.87	280.57	11	14	14	3.00	3.22	3.41
Mean	107.30	293.33	303.99	11	13	14	3.43	3.70	3.77
S.Em ±	2.75	8.29	8.75	0.24	0.32	0.26	0.17	0.14	0.09
CD(0.05P)	7.92	23.90	25.22	0.70	0.93	0.74	0.48	0.39	0.27
CV (%)	4.44	4.90	4.98	3.66	4.11	3.21	8.36	6.41	4.29

*mg/kg of seed

T_1 - Control; T_2-Dry dressing of SiO_2 NP @ 500mg; T_3 - Dry dressing of SiO_2 NP @ 1000mg; T_4 - Dry dressing of TiO_2 NP @ 500mg; T_5 - Dry dressing of TiO_2 NP @ 1000mg; T_6 - Dry dressing of FeO NP @ 500mg; T_7 - Dry dressing of FeO NP @ 1000mg; T_8 - Polymer only; T_9 - Polymer coating with SiO_2 NP @ 500mg; T_{10} - Polymer coating with SiO_2 NP @ 1000mg; T_{11} - Polymer coating with TiO_2 NP @ 500mg; T_{12} - Polymer coating with TiO_2 NP @ 1000mg; T_{13} - Polymer coating with FeO NP @ 500mg; T_{14} - Polymer coating with FeO NP @ 1000mg; T_{15} - Spinatorum @ 0.8 %

Table 2: Influence of nanochemicals on cob characters of maize hybrid cv. MAH 14-138.

Treatments	Cob length (cm)	Cob diameter (cm)	Cob seed weight (g)	Cob pith weight (g)	Total cob weight (g)	Number of seeds/cob	100 seeds weight (g)
T_1	9.87	4.17	45.97	21.70	71.00	300	16.14
T_2	14.41	5.78	76.48	32.58	109.05	333	22.30
T_3	14.87	5.70	78.07	46.02	127.42	392	21.52
T_4	15.95	6.81	105.41	63.90	170.64	456	26.63
T_5	14.33	5.72	65.30	47.34	107.64	309	24.07
T_6	13.37	5.33	56.93	33.67	90.60	278	22.28
T_7	14.67	5.41	62.67	37.08	99.75	340	21.87
T_8	14.58	5.02	71.28	38.17	109.45	354	20.83
T_9	15.50	5.74	89.97	57.33	145.97	401	24.51
T_{10}	14.42	5.62	61.31	36.34	99.32	290	21.20
T_{11}	13.81	5.28	73.35	39.90	109.92	318	21.38
T_{12}	14.35	5.33	75.77	41.67	117.43	282	22.20
T_{13}	14.95	5.30	65.07	42.82	107.89	271	22.27
T_{14}	14.73	5.41	70.57	45.13	112.37	320	20.92
T_{15}	13.80	6.00	71.10	37.74	108.84	337	21.90
Mean	14.24	5.51	71.28	41.43	112.49	332	22.00
S.Em ±	0.82	0.29	3.22	2.36	3.29	18.60	0.62
CD(0.05P)	2.36	0.84	9.28	6.81	9.47	53.59	1.79
CV (%)	9.97	9.13	7.83	9.87	5.06	9.69	4.89

*mg/kg of seed

T_1 - Control; T_2-Dry dressing of SiO_2 NP @ 500mg; T_3 - Dry dressing of SiO_2 NP @ 1000mg; T_4 - Dry dressing of TiO_2 NP @ 500mg; T_5 - Dry dressing of TiO_2 NP @ 1000mg; T_6 - Dry dressing of FeO NP @ 500mg; T_7 - Dry dressing of FeO NP @ 1000mg; T_8 - Polymer only; T_9 - Polymer coating with SiO_2 NP @ 500mg; T_{10} - Polymer coating with SiO_2 NP @ 1000mg; T_{11} - Polymer coating with TiO_2 NP @ 500mg; T_{12} - Polymer coating with TiO_2 NP @ 1000mg; T_{13} - Polymer coating with FeO NP @ 500mg; T_{14} - Polymer coating with FeO NP @ 1000mg; T_{15} - Spinatorum @ 0.8 %

Table 3: Influence of nanochemicals on seed quality of resultant crop of maize hybrid cv. MAH 14-138.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Dry weight of seedling (mg)	Seedling vigour Index-I	Seedling vigour Index-II	EC (µS/cm)	TDH
T_1	70	15.77	17.00	32.77	43.30	2316	3056	9.29	0.430
T_2	72	15.21	17.30	32.51	53.42	2296	3828	8.18	0.548
T_3	84	15.42	16.17	31.58	55.03	2652	4619	9.80	0.394
T_4	95	19.61	18.52	38.13	60.79	3791	5937	12.07	0.547
T_5	95	15.75	16.03	31.78	59.50	3040	5692	9.98	0.620
T_6	85	16.14	16.61	32.75	58.45	2804	5003	10.35	0.459
T_7	93	17.07	15.90	32.97	55.91	3099	5198	10.21	0.497
T_8	95	16.98	15.80	32.78	55.27	3136	5287	10.15	0.554
T_9	96	18.90	16.94	35.51	59.81	3452	5831	10.44	0.517
T_{10}	90	17.18	15.67	32.84	52.60	2975	4768	10.13	0.522
T_{11}	87	17.34	16.70	34.04	51.83	2960	4507	9.59	0.540
T_{12}	83	17.10	16.23	33.33	53.54	2933	4474	10.48	0.513
T_{13}	89	18.17	13.47	31.63	51.95	2835	4659	9.15	0.532
T_{14}	94	15.31	15.27	30.57	50.01	2917	4718	10.05	0.503
T_{15}	91	16.48	16.61	33.09	52.53	3032	4816	9.36	0.515
Mean	88	16.83	16.28	33.09	54.26	2949	4826	9.95	0.51
S.Em ±	1.69	0.48	0.42	0.76	1.54	75.06	141.86	0.36	0.03
CD(0.05P)	4.86	1.39	1.22	2.18	4.43	216.28	408.76	1.03	0.07
CV (%)	3.30	4.98	4.50	3.96	4.91	4.41	5.09	6.25	8.71

* mg/kg of seed

T_1 - Control; T_2-Dry dressing of SiO_2 NP @ 500mg; T_3 - Dry dressing of SiO_2 NP @ 1000mg;
T_4 - Dry dressing of TiO_2 NP @ 500mg; T_5 - Dry dressing of TiO_2 NP @ 1000mg;
T_6 - Dry dressing of FeO NP @ 500mg; T_7 - Dry dressing of FeO NP @ 1000mg;
T_8 - Polymer only; T_9 - Polymer coating with SiO_2 NP @ 500mg;
T_{10} - Polymer coating with SiO_2 NP @ 1000mg; T_{11} - Polymer coating with TiO_2 NP @ 500mg;
T_{12} - Polymer coating with TiO_2 NP @ 1000mg; T_{13} - Polymer coating with FeO NP @ 500mg;
T_{14} - Polymer coating with FeO NP @ 1000mg; T_{15} - Spinatorum @ 0.8 %

SiO_2 500mg SiO_2 1000mg TiO_2 500mg TiO_2 1000mg FeO 500mg FeO 1000mg

Fig. 1. Seed treatment through dry dressing with SiO_2, TiO_2 and FeO nanochemicals.

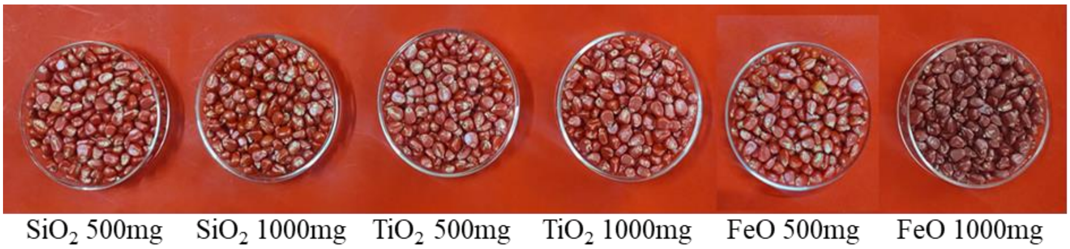


Fig. 2. Seed treatment through polymer mediated coating with SiO_2, TiO_2 and FeO nanochemicals.

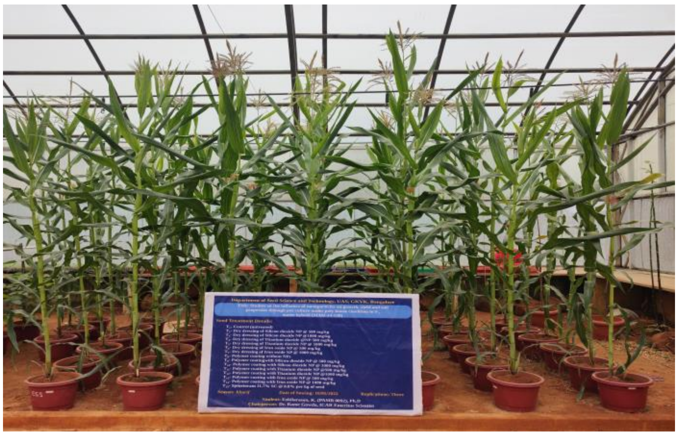
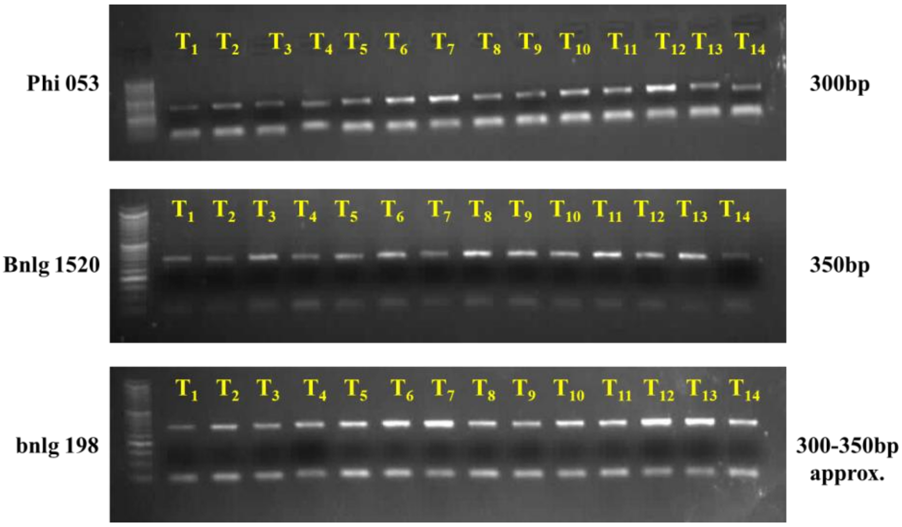


Fig 3. General view of pot culture experiment on nanochemicals in hybrid maize.



T_1 - Control; T_2-Dry dressing of SiO_2 NP @ 500mg; T_3 - Dry dressing of SiO_2 NP @ 1000mg; T_4 - Dry dressing of TiO_2 NP @ 500mg; T_5 - Dry dressing of TiO_2 NP @ 1000mg; T_6 - Dry dressing of FeO NP @ 500mg; T_7 - Dry dressing of FeO NP @ 1000mg; T_8 - Polymer only; T_9 - Polymer coating with SiO_2 NP @ 500mg; T_{10} - Polymer coating with SiO_2 NP @ 1000mg; T_{11} - Polymer coating with TiO_2 NP @ 500mg; T_{12} - Polymer coating with TiO_2 NP @ 1000mg; T_{13} - Polymer coating with FeO NP @ 500mg; T_{14} - Polymer coating with FeO NP @ 1000mg; T_{15} - Spinatorum @ 0.8 %

Fig. 4. SSR markers profiles to reveal genetic changes due to seed treatment with nanochemicals in maize hybrid MAH 14-138.

CONCLUSIONS

The research findings of the study revealed that application of nanochemicals *viz*., silicon dioxide, titanium dioxide, iron oxide significantly improves the crop performance as well as the seed quality attributes of resultant crop of maize. Seed coating with SiO_2 and TiO_2 NPs @ 500 mg/kg of seeds both dry dressing and polymer mediated coating found to be most effective over other nano-chemicals seed treatments for effective and positive influence of crop growth and yield in maize. Nanotechnology is a leading technique in agricultural applications to promote plant germination, quality and improved production, protection from biotic and abiotic stresses. It is important to investigate the influence of metallic and non-metallic nanochemicals on morpho and physiological responses of plants and their mechanisms. Moreover, it is necessary of further studies to know about risks related to the usage of nanochemicals and its dosage because of its potential adverse effects in plants. Besides, it is also better to make comparative study on the green synthesis and commercially available nanoparticles of different kind on seed quality improvement and crop performance to nullify the effects of nanochemicals on soil health and biosafety.

Authors Contributions. Conceived and designed the analysis: Ezhilarasan, K., Rame Gowda; Collected the data: Ezhilarasan, K., Rame Gowda; Contributed data or analysis tools: Ezhilarasan, K., Roopashree, B and Umarani, K; Wrote the paper: Ezhilarasan, K., Roopashree, B and Umarani, K and final edit by Rame Gowda.

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Conflict of Interest. None.

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