

Original Research Article

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Effect of Plasma and Nanochitosan Treatment on Growth Indices of Wheat (*Triticum aestivum* L.) Seeds

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ABSTRACT

Keywords

Nanochitosan treatment, Plasma treatment, Seed coat etching, Target action

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A water culture experiment was conducted with eight treatments; these were absolute control, plasma seed treatment with 2, 4, 6, 8 and 15 minutes, nanochitosan (50 ppm) and nanochitosan (50 ppm) integration with NPK mixture at G B Pant University of Agriculture & Technology, Pantnagar during *rabi* season year 2017-18. While pot experiments were conducted with twelve treatments; which were control with full dose of fertilizer, nanochitosan (50 ppm) treatment with full dose of fertilizer, plasma treatment with full dose of fertilizer, control with 3/4th dose of fertilizer, nanochitosan (50 ppm) treatment with 3/4th dose of fertilizer, plasma treatment with 3/4th dose of fertilizer, control with 1/2 dose of fertilizer, nanochitosan (50 ppm) treatment with 1/2 dose of fertilizer, plasma treatment with 1/2 dose of fertilizer, control with no fertilizer, nanochitosan (50 ppm) treatment with no fertilizer and plasma treatment with no fertilizer. Maximum root length, shoot length, root and shoot dry weight were obtained in seed treated with 6 minute plasma treatment seeds under *in vitro* condition, whereas pot culture morphological indices were reported higher with full dose of fertilizer along with nanochitosan (50 ppm) treatment.

Introduction

Optimum use of mineral nutrients (fertilizers) by crops is essential for sustainable agricultural production. With increasing world demands for food and energy, this is set to become an ever increasing priority. Fertilizers

are costly to produce and apply, both financially and environmentally. There is an absolute requirement to maximize efficiency (Hawkesford, 2012). This can be achieved by use of upcoming new technologies such as plasma and nanotechnology. Plasma denominates as a state with a high, unstable energy level that has more or less equal

number of positive and negative charged particle or reactive species, created when gas become ionized. When plasma comes in contact with seed coat layer, the surfaces get modified and change their important properties, such as the surface etching, wettability of surface, rate of imbibition etc., which helps in accelerating the physiological processes of plants. In addition, together with surface etching effect results in increased permeability towards seed coat, surface modification of the seed coat, stimulated seed germination and growth of seedling.

Nanotechnology has proved its worth in agriculture. It is a powerful technology which can have ability of creating massive changes in food and agriculture system. Nanofertilizers release the nutrients as per the requirement of crop, preventing from premature interaction with soil as well as water environment and the microorganisms; the released nutrients directly assemble into the plant system effectively. These unique characteristics can improve the nutrient use efficiency of the crop (DeRosa *et al.*, 2010). Chitosan nanoparticles have good association and loading efficiency values of a model substance which is showing its ability as a nano carrier for nutrient delivery systems (Aiping *et al.*, 2006) and also act as a slow release fertilizers that is only a bit soluble in water or slowly broken down by microbial action (Prasad *et al.*, 2009). Thus, the rate of nutrient liberation from slow release fertilizers is related to their water solubility, microbiological degradation and chemical hydrolysis and NO_3^- -N leaching decreased by applying slow release fertilizers coated with nano-materials. A plasma seed treatment bestows to seed germination enhancement and raises the plant productivity. Nanochitosan get easily applied to seed's surface and enter the nano pores of cell wall and cell membrane of the seed cause increase in harvest index, crop index and mobilization index of yield factors, as compared with factor treated with normal

non-fertilized and normal fertilized (Aziz *et al.*, 2016) seeds.

Materials and methods

Plasma seed treatment

The wheat seeds have been treated in a capacitively coupled plasma reactor consists of Stainless Steel (SS) chamber with rotary pump for creating necessary sub-atmospheric vacuum (Fig. 1). A Petri dish with wheat seeds was put on the grounded lower electrode (size 120 mm) made of SS for plasma treatment experiments. The plasma treatment of wheat seeds have done using air plasma formed using radio frequency supply (13.56 MHz) with treatment time 2, 4, 6, 8 and 15 minutes with fixed 50 watts coupled power and 0.7 mbar pressure. These plasma treatments were done at Institute for Plasma Research, Gandhinagar, Gujarat, India.

Scanning electron microscopy analysis

The surface morphology of plasma treated and untreated wheat seeds are investigated by Scanning Electron Microscopy (SEM) analysis. The treated and untreated wheat seeds surfaces were analyzed using High Vacuum Mode (HVM) operation of SEM in detail without damaging seed coat surface during analysis.

Nano chitosan seed treatment

Nanochitosan purchased from nanoshell, Wilmington (USA), that have $\text{C}_6\text{H}_{11}\text{NO}_4$ (molecular formula), > 99 % purity, 1.4 specific gravity, cost price 1000 Rs/gram, white appearance and 50ppm of it used for experiment purpose. For preparing 50 ppm nanochitosan solution weighed the nanochitosan at 0.050 gram or 50 mg and blended in 1 liter distilled water with the help of magnetic stirrer for 30 minutes, for equal

distribution of it into the distilled water. The seeds of wheat were immersed in a sodium hypochloride solution for 2 minutes for surface sterilization of seeds then they were soaked in nanochitosan 50 ppm solution for 3 hours. Treated seeds were shade dry over muslin cloth for 24 hours. This process was performed one day before the sowing of wheat seeds.

Water culture and plasma seed treatments

Water culture experiments of 15 days were conducted in moorashige and skoog media for plasma treated wheat seeds. The experiments were laid out in completely randomized design (CRD) having three replications of plasma treated wheat of HD-2967 variety. Morphological indices for water culture experiment viz., germination percentage, root length (cm), shoot length (cm), fresh weight (g) and dry weight (g) were recorded during experiments. All the data were subjected to one way analysis of variance (ANOVA), using the STPR statistical package. For comparison of means, the Duncan's multiple range tests ($p < 0.05$) were used in this study.

Pot experiment

The pot experiment was conducted during the *Rabi* season, year 2017 at the Norman E. Borlaug crop research centre of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The pots were filled with soil taken from the N.E. Borlaug crop research centre where field experiment was conducted and pot experiment also done in open field condition. The soil of experimental site was silty clay loam in nature with neutral pH of 7.4 and EC of 0.419 dS/m medium in organic carbon (0.72%), low in available nitrogen (216.25 Kg/ha), but available phosphorus (12.14 Kg/ha) and potassium was in medium range (139.08 Kg/ha). The experiment was laid out in factorial CRD

design with three replications. The treatments were included four different doses of fertilizer in main plot and seed treatments in subplot. The treatments comprises four doses of recommended dose of fertilizers (150:60:40 NPK Kg/ha) viz; Full dose of fertilizer (N1), 3/4 dose of fertilizer (N2), 1/2 dose of fertilizer (N3) and without use of fertilizer (N4) in main plot and Control / without seed treatment (S1), Plasma treated seeds (S2) and Chitosan nanoparticles (S3) in subplot. The observations viz., emergence count (%), plant height (cm), green seeker, tillers/pot were recorded during experiments. Data was analyzed using the STPR statistical package.

Results and Discussion

SEM surface analysis of wheat seed coat

HVM-SEM was used to characterize the modification of surface morphology of wheat seed coat and results were showed in Figure 2. Typical mesh structures were clearly observed on wheat seed coat for untreated seed (Fig. 2 (A)), whereas such mesh structures were gradually soften with air RF plasma treatment and for 6 min treated shown in Figure 2 (B). While further increase in plasma treatment time such structures were destroyed and also cracks (marked with arrows) were formed on seed coat confirmed the degradation of seed coat (Fig. 2 (C)). Such surface modification may improve the water uptake and hence germination of seeds in case of 6 min treatment.

Water culture and plasma seed treatment

Maximum root length and shoot length were reported with 6 minutes plasma treatment, which was significantly higher than control, 2, 4, 8 and 15 minutes of plasma treatment (Fig. 3). Their high surface energy helped them to stimulate early root and shoot development.

These findings were concurrent with the findings of Aziz *et al.*, (2016).

Similarly, maximum root and shoot dry weight was obtained with 6 minutes plasma treatment and at par with 4 minutes plasma but found significantly higher over remaining treatments.

This might be possible due to the enhanced the mobilized seed reserves and seed reserve utilization efficiency that was the major factor responsible for early growth and development of seedling, resulted in high root and shoot length of the seedlings. Similar results were also obtained by Ling *et al.*, (2014)

Pot experiment

Full dose of fertilizer along with nanochitosan revealed maximum emergence of seeds, which was statistically at par with 3/4th dose of fertilizer plus plasma for 6 minutes but significantly higher than other treatments (Fig. 4).

Reason behind that nanochitosan increased the rate of gibberallic acid, indole acetic acid (IAA) and reduced mean emergence time. Similar evidences were also obtained by Shao *et al.*, (2005). At 30 DAS, full dose of fertilizer with nanochitosan observed maximum plant height which was at par with 3/4th dose of fertilizer applied to nanochitosan seed treatment. Because nanochitosan might be act as a chelating agent for metals, improve nutrient use efficiency and overall growth of plant.

Similar results were also obtained by Yin *et al.*, (2010). At 60 DAS, Minimum numbers of tillers/pot observed with no dose of fertilizer. Full dose of fertilizer plus nanochitosan @ 50 ppm reported maximum tillers per pot whereas, at par with full dose of fertilizer plus

plasma treatment and 3/4th dose of fertilizer plus nanochitosan but significantly higher over other treatments.

It might be due to plasma improved metabolism of plant that stimulate overall growth and nanochitosan served as a control release formulation that helps to exploit nutrient efficiency which assured the crop requirement at lower doses.

At 30 DAS, Green seeker reading was found maximum with full dose of fertilizer plus nanochitosan @ 50 ppm which was significantly higher than the other treatments. It might be due to vigorous growth and more photosynthesis efficiency of the crop at full dose of fertilizer with nanochitosan treated seeds.

These findings can be supported by the results obtained by Coradini *et al.*, (2010). At 60 DAS, full dose of fertilizer plus nanochitosan obtained maximum green seeker value which was at par with 3/4th dose of fertilizer plus plasma treated seeds for 6 minutes.

Plasma and nanochitosan treated seeds might have excite photosystem I and II, accumulates more photosynthetic pigment, improve relative greenness of the crop. These findings were concurrent with the findings of Zhang *et al.*, (2017).

In efficacy assessment of different plasma treatments, 6 minutes of atmospheric plasma performed better under water culture experiment and in pot culture full dose of fertilizer along with nanochitosan reported maximum growth which was at par with 3/4th dose of fertilizer plus plasma.

Thus, plasma and nanotechnology can become an effective tool for increasing overall production of wheat (Table 1 and 2).

Table.1 Seedling growth indices of wheat as influenced by seed treatment methods

Treatments	Root length (cm)	Shoot length (cm)	Root dry weight (g)	Shoot dry weight (g)
Seed treatments				
Absolute control	5.5	9.5	1.1	2.6
Plasma-2 minutes	8.0	11.4	1.5	3.6
Plasma-4 minutes	11.4	13.6	2.7	3.7
Plasma-6 minutes	19.5	14.7	2.9	2.7
Plasma-8 minutes	18.6	12.3	1.4	2.6
Plasma -15 minutes	12.4	11.4	1.1	2.5
Nano-chitosan	6.5	14.0	2.6	3.4
Nano-chitosan+ NPK	8.3	12.8	2.5	3.0
SEm±	0.41	0.04	0.02	0.03
C.D.(P=0.05)	1.47	0.17	0.10	0.10
C.V. (%)	5.16	4.57	10.47	7.32

Table.2 Interaction effect of fertilizer doses and seed treatments on plant growth indices

Treatments	Emergence count (%)				Plant height (cm) at 30 DAS				Tillers/ pot at 60 DAS			
	Seed treatments											
	Control	Plasma	Nano Chitosan	Mean	Control	Plasma	Nano Chitosan	Mean	Control	Plasma	Nano Chitosan	Mean
Full dose of fertilizer	62	74	98	78	15.7	18.3	19.2	17.7	31	33	34	32
¾ dose of fertilizer	58	56	72	62	15.7	17.5	19.1	17.4	29	31	32	30
½ dose of fertilizer	58	94	62	71	14.3	16.3	18.7	16.4	24	25	31	26
No fertilizer	20	38	46	35	13.7	15.5	15.5	14.9	23	24	28	25
Mean	20	66	70		14.9	16.9	18.2		26	28	31	
		SEm±	CD (P=0.05)	C.V. (%)		SEm±	CD (P=0.05)	C.V. (%)		SEm±	CD (P=0.05)	C.V. (%)
Fertilizer		3.54	11.04	4.1		0.03	0.09	1.34		0.37	1.16	3.68
Seed treatments		3.07	9.56	-		0.03	0.08	-		0.32	1.01	-
Interaction		6.14	19.12	-		0.05	0.16	-		0.64	2.01	-

Fig.1(A) Radio frequency plasma treatment system for wheat seed treatment and (B) Closer view of air RF plasma with wheat seed placed in glass Petri dish

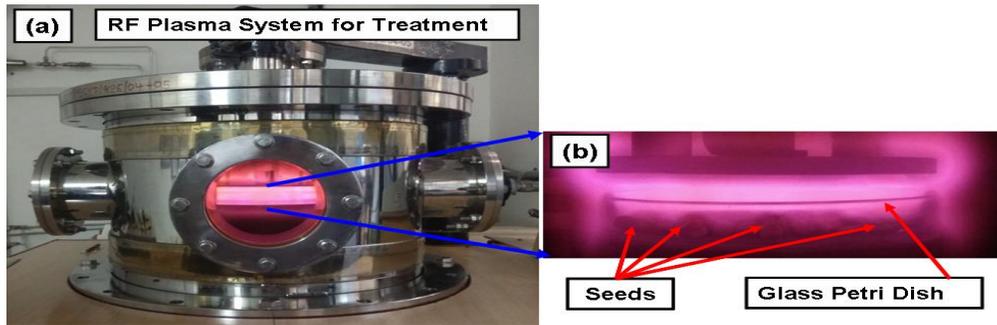


Fig.2 SEM surface images of wheat seed coat taken at 1000X magnification (A) Untreated wheat seed coat (B) 6 min air RF plasma treated wheat seed coat and (C) 15 min air RF plasma treated wheat seed coat with arrows were marked on cracks from due to treatment.

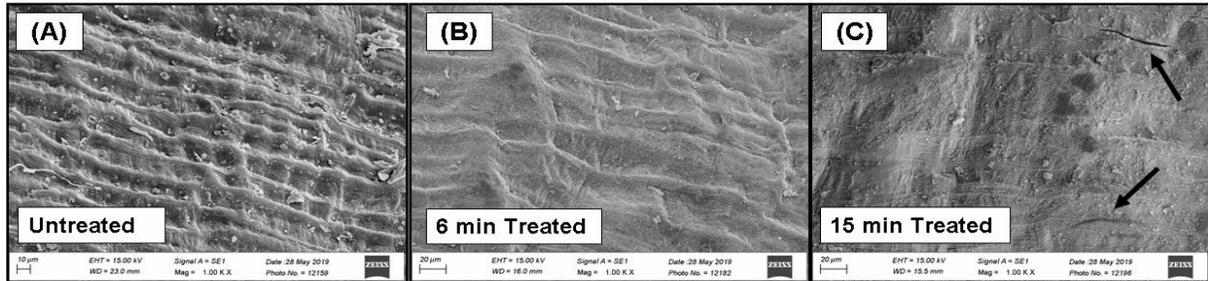


Fig.3 Effect of plasma seed treatments (at control, 6-minute, 8-minute and 15-minute) on seedling growth



Fig.4 Effect of 6- minute plasma and control on emergence and growth



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