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Kinetin coated Metal Nanoparticle for in-vitro Regeneration of Recalcitrant Green Gram (*Vigna radiata L.)*

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Abstract—*Kinetin is a type of Cytokinin that promotes cell division, initiation of Inter-fascicular Cambium, Dormancy of seeds, Promotion of Chloroplast Development. kinetin coated silver nanoparticle efficacy is much more than the normal kinetin. It is present in nano-form thus it is very easily penetrable in the plant cell .Metal acts as catalyst here that is why the regeneration from recalcitrant green gram is very smooth. The experiment was conducted on Mung bean(Vigna radiate L.) variety sonali at university of Calcutta Department of Agronomy. The experiment was executed by the following treatments.**T1** (0.25 PPM nanofabricated kinetin) **T2**(0.25 PPM nanofabricated kinetin and 0.5 mg/lNAA and 1 mg/l BAP) **T3**(0.50 PPM nanofabricated kinetin) **T4** (0.50 PPM nanofabricated kinetin and 0.5 mg/l NAA and 1 mg/l BAP) **T5** (0.5mg/l kinetin and 0.5 mg/l NAA and 1 mg/l BAP). along with MS media and with 2% sucrose and 0.1 % Agar. They are incubated in 16 hours photoperiod in 25^{0}C .In Green gram Organogenic Calli developed with in 4-6 weeks. In case of **T4(** 0.50 PPM nanofabricated kinetin and 0.5 mg/l NAA and 1 mg/l BAP) the callus formed in 2-3 weeks followed by **T2** (0.25 PPM nanofabricated kinetin and 0.5 mg/l NAA and 1 mg/l BAP) **T3** (0.50 PPM nanofabricated kinetin) **T5**(0.5mg/l kinetin and 0.5 mg/l NAA and 1 mg/l BAP) **.**After all of the experiments it is analysed that after transferring the rooted plantlets to the soil and the success full grown plants with productivity in best dose **T4** (0.50 PPM nanofabricated kinetin and 0.5 mg/l NAA and 1 mg/l BAP) is nearly 85 %..*

Keywords*: Mung bean, Kinetin, Metal Nano particle invented by Dr. Nilanjan Deb has 11 International patents. Recalcitrant, Regenaration, MS Media, PPM, BAP, NAA.*

1. INTRODUCTION

Mung Bean is one of the most important protein rich pulse for human consumption. It improves the soil fertility by association with nitrogen fixing bacteria and accumulation of organic matter. Convention method for cultivation of mung bean face the problem with the low productivity of this crop and the production has been stagnant over the years because of its susceptibility to various biotic and abiotic stresses and cultivation as an orphan crop (Sahoo and Jaiwal 2008).It is a self pollinating crop .So it has a bottleneck to make up this problem by plant breeding (Jaiwal and Gulati 1995).But there is an opportunity to develop the characteristics by regeneration of whole plant through tissue culture. Usually legumes are highly recalcitrant and genotype specific for in vitro regeneration (Somers et al. 2003) and progress in transgenic development in legumes, specifically in green gram, has been very slow owing to their recalcitrant nature in culture (Dita et al. 2006; Eapen 2008. But, in most of these studies, the regeneration potential remained very low except few reports using cotyledonary node explants (Amutha et al. 2006; Vijayan et al. 2006; Yadav et al. 2010a, b; Sagare and Mohanty 2015) .Nano scale structure of kinetin can easily penetrate to the cell and enhance the total growth parameters of the recalcitrant plant. How ever the growth regulator coated matal nanoparticle significantly increased other nutrient uptake absorption and assimilation for very fast callusing, root and shoot growth from callus and regeneration. all modified characters can be developed before planting the rooted tissue cultured planlets to the field.

2. MATERIALS AND METHOD

Seed material of the variety sonali collected from Seed Certification office of Govt. of West Bengal. Uniform and good quality seeds of sonali were washed with distilled water two–three times and surface sterilized by rinsing with 70 % ethanol for 1 min followed by 0.1 % (w/v) aqueous $HgCl_2$ solution for 8–10 min. Then the seeds were thoroughly washed with sterilized distilled water for 4–5 times to remove any residual disinfectant and kept for germination (Fig-1)Shoot tip explants were excised from 3 day-old aseptically germinated seedlings of sonali genotype. The seed coat was removed and cut the seedling approximately 2 mm below the nodal region followed by a horizontal division along the middle of the seed leading to production of shoot tip explants with embryoaxis comprising proximal half of the two cotyledons attached at the cotyledonary node. These explants were inoculated in vertically upright position in shoot bud induction media sonali supplemented with different concentrations of BAP,NAA and nano –kinetin as different treatments **T1** (0.25 PPM nanofabricated kinetin) **T2**(0.25 PPM nanofabricated kinetin and 0.5 mg/lNAA and 1 mg/l

BAP) **T3**(0.50 PPM nanofabricated kinetin) **T4** (0.50 PPM nanofabricated kinetin and 0.5 mg/l NAA and 1 mg/l BAP) **T5** (0.5mg/l kinetin and 0.5 mg/l NAA and 1 mg/l BAP). Total 30 plants were selected for each treatment and these all cultures were multiplied 3 times. Between 12-28 days the plantets developed sequentially from **T4, T2, T3, T5,** and **T1.** Percent shoot induction frequency, mean shoot number and mean shoot length were recorded. In all the media, 0.8 % agar as the solidifying agent and 3 % sucrose as a carbon source were used unless otherwise mentioned. The pH of the media was set to 5.8 before sterilization in autoclave at 121 °C at 15 lbs pressure for 15 min. All these cultures were maintained at 12/12 h light/dark photoperiod under white lamps

In vitro rooting and acclimatization

First the callus (Fig-2) developed and shoot regeneration took place within 7 days (Fig-3)elongated shoots (Fig-4)(2–3 cm) were excised and kept for rooting on ½ MS medium containing BAP, NAA Nano-kinetin with 1.5 % sucrose and cultured for 3 weeks. An observation on frequency of rooting was recorded. (Fig-5). Well rooted plantlets were taken out from the culture bottles carefully and their roots washed thoroughly under running tap water to remove any traces of agar and transferred into polypropylene bags containing autoclaved soil: sand (1:1).(Fig-6). These plantlets were slowly acclimatized for 10–15 days by gradually exposing them to the open environment.(Fig-7). Finally primary hardened plantlets were shifted to field soil without hampering the roots and grown till their maturity. The percentage of survival of plants root length, shoot length, greenness of callus weight, % of callusing was recorded during the growth period during acclimatization of 3 days, 7 days, 15 days after transferring to the media was recorded So approximately after 40 days the tissue cultured best plantlets were transplanted to the field.

Field observation

After transplantation of the rooted plantlets to the field only the half RFD was spread and and finally recalcitrant mungbean harvested with a very high productivity.(Fig-8)

Figure 1: Seeds are prepared for germination and germinated.

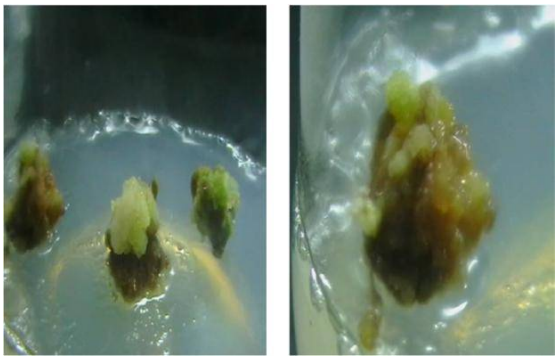

Figure 2.Formation of callus.

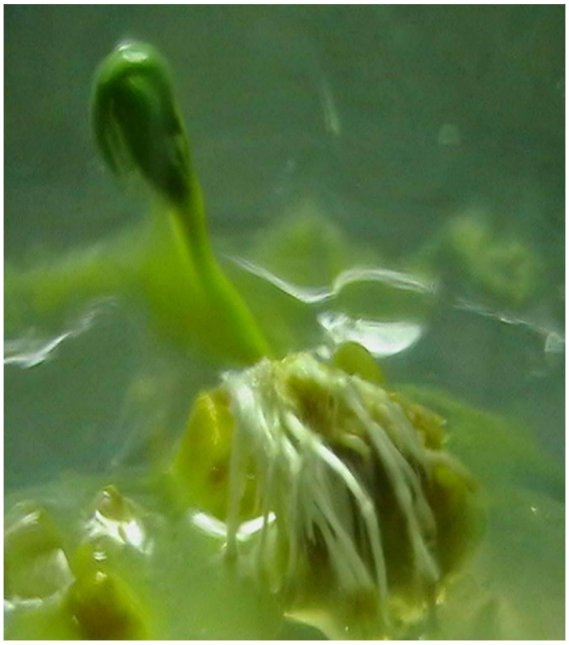

Figure 3. Elongated shoot tip of recalcitrant green gram.

Figure 4: Growing shoot tip.

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Figure 5. Frequency of root recorded for every treatment.

Figure 6. 1:1 soil and sand mixture.

metal and macro kinetin to MS basal medium induced callus .Multiple shoot number increased along with shoot length. Subsequent shoot proliferation and shoot length development depend on the concentration of the growth regulators. Total biomass of callus (Table-1) rate of callus formation(Table-2),root formation (Fig-10), shoot proliferation, root shoot ratio(Table 3) are observed in every 3days, 7 days, 15 days,21 days

Figure 8.Plantlets are exposed in normal environment

Figure 8. Before harvesting from the field.

3. RESULT AND DISCUSSION

Effect of nano particle mediated hormone specially, kinetin showed a good effect in recalcitrant green gram regeneration explant through tissue culture. In case of the treatment T4 having 0.50 PPM metal nanoparticle mediate kinetin, It is found that the callus regeneration started only in 3 days and the green ness of callus(Fig 9) is distinctly identifiable from other treatments like T5.The callus are yellowish and the rate of regeneration procedure is also slow(T5).compare to other treatments Addition of various concentrations of kinetin coated

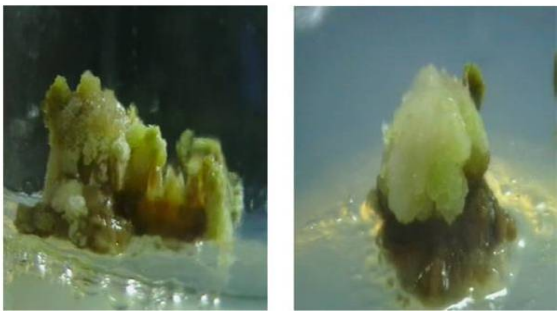

Figure 9.Green callusing from T4, yellowish callusing from T5.

Figure 10: Root formation with nano particle mediated kinetin medium

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Table 1 Total biomass of callus after culturing from 3,7,15,21 days.

Treatment	3 days AC	7 days	15 days	21 days
s	biomass	AC	AC	AC
	(gm)	Biomass	biomass	biomass
		(gm)	(gm)	(gm)
T1	0.20	0.73	1.05	1.25
T2	0.64	0.89	1.66	1.97
T3	0.58	0.76	1.23	2.0
T4	0.67	0.94	1.65	2.22
T5	0.57	0.77	1.25	1.96

The above table is the mean of 30 replication of each treatment. Where it is found that in the case of 0.50 PPM nano particle mediated kinetin (T4)shows the highest weight of biomass in every 3days, 7 days, 15 days, 21 days comparing with other macro kinetin replications(T5)

Table 2: Rate of callusing

Treatments	3 days AC	7 days	15 days	21 days
		AC	AC	AC
T1	1.59%	7.5%	38.4%	69.7%
T2	2.4%	8.4%	45.4%	68.4%
T3	2.2%	7.9%	43.1%	76.4%
T4	4%	9.5%	48.7%	80%
T5	3.4%	9.0%	47.4%	76.4%

The above table is the mean of 30 replication of each treatment. Where it is found that in the case of 0.50 PPM nano particle mediated kinetin (T4)shows the highest percentage of callusing in every 3days(4%), 7 days(9.5%), 15 days(48.7), 21(80%) days comparing with other macro kinetin(0.50 PPM) replications(T5)(76.4% in 21 days).

Table 3: Length of root, Length of shoot, Root Shoot ratio in 3,7,15,21 days

	T1	T2	T3	T4	T5
Root length after 3	0.0	0.1	0.1	0.2	0.2
days AC (cm)					
Shoot length after 3	0.1	0.2	0.3	0.3	0.3
days AC(cm)					
Root lengh :Shoot	0	0.5	0.33	0.66	0.66
length					
Root length after 7	0.2	0.3	0.5	0.9	0.6
days AC(cm)					
Shoot length after 7	0.5	0.7	0.9	1.5	1.0
days AC(cm)					
Root lengh : Shoot	0.4	0.42	0.55	0.6	0.6
length					
Root length after 15	0.7	`1.4	1.3	2.3	1.3
days AC(cm)					
Shoot length after 15	0.9	2.3	2.0	3.2	2.0
days AC(cm)					
Root lengh : Shoot	0.77	0.60	0.65	0.71	0.65
length					
Root length after 21	1.1	2.0	2.10	3.5	2.1
days AC(cm)					
Shoot length after 21	2.0	2.9	3.1	4.6	3.3
days AC(cm)					
Root lengh : Shoot	0.55	0.68	0.67	0.76	0.63
length					

The above table is the mean of 30 replication of each treatment. Where it is found that in the case of 0.50 PPM nano particle mediated kinetin (T4)shows the highest root shoot ratio (0.76) in 21 days where as T4 having 0.50 macro kinetin shows root shoot ratio 0.63 which is quietly lower than the treatment T2 possessing 0.25 PPM kinetin with root shoot ratio of 0.68 in 21 days.

4. CONCLUSION

In this study we have reported maximum induction of shoots with 100 % shoot proliferation frequency. Further, we have found high efficiency with this optimized controlled regeneration protocol. This is the first invented metal nanoparticle mediated hormone activity in recalcitrant green gram of successful regeneration of highest number of multiple shoots and roots and callusing with nearly 80 % frequency. The novel methodology developed in this study are simple and efficient and can be used to develope high efficient high yielding green gram for various traits of interest

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