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COMPARATIVE STUDIES ON WATER STRESS TOLERANT *MUSA SPECIES* PRODUCTION THROUGH *IN VITRO* PROPAGATION

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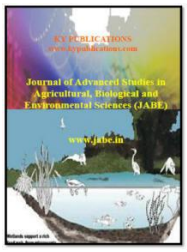

ABSTRACT

Water stress now-a-days enforces a serious threat to banana productivity. Banana plant productivity is greatly affected by environmental stresses such as drought, water and cold. Plants respond and adapt to these stresses to survive under stress condition at the molecular and cellular levels as well as at the physiological and biochemical levels. Therefore, the attempts to develop tolerant lines are of massive worth to increase cop productivity. Physiological responses to soil water deficit are the feature that is most likely to determine the response of the crop to irrigation. In banana, water deficit usually affects growth and lowers the yield. To overcome the major problem, this study has been aimed at the development of stress tolerant banana plants during their early stages of growth to protect the plants from the natural calamities. A stress inducer, Polyethylene glycol (PEG) has been added in the growth media of Grand Naine, a banana variety through Micropropagation method and the results were recorded based on the growth. Observations were positive in the growth which did not show much effect during the growth stages thus developed the tolerant lines.

Key Words: Water Stress Tolerant, *Musa Species*, *In Vitro* Propagation, Polyethylene glycol.

1. INTRODUCTION

Edible bananas do not produce seeds and therefore are clonally propagated using a number of methods. The methods include; tissue culture derived plantlets, suckers and split corms sometimes called bits. For good establishment sources and selection of suckers are very important. A new and most promising planting material consists of *in vitro* plants which are small suckers produced from meristem culture (Swennen, 1990). Planting materials can also be collected from an existing old field, and or a multiplication plot planted only for the production of suckers. Seed propagation is only possible in wild bananas which produce vast seeds from open pollinations. Banana is one of the main plants of horticultural interest, which are multiplied by micropropagation, compared with the conventional planting methods. (Siamak Shirani Bidabadi *et al.,* 2012). Banana (Musa spp.) is the fourth most important food crop in the world as well as in India (Ganapathi *et al.,* 1999).

Plant tissue culture technique has great potential as a means of vegetative propagation of economically important species, especially for those difficult to propagate by conventional methods (seeds or cuttings). Tissue culture is important to establish and/or maintain a virus free stock somatic hybridization induction and selection of mutants and germplasm conservation (Conger, 1980; Vuylsteke, 1989). Healthy

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plants can be grown in the laboratory at any time. *In vitro* culture techniques of banana plants can produce thousands of plants in a relatively shorter time either using somatic embryo or apex explants which require different culture media for shoot multiplication and root differentiation (Cronauer and Krikorian, 1988).

Presently, banana is grown in around 150 countries across the world on an area of 4.84 million ha producing 95.6 million tones. Asia, Africa and Latin America are the major banana producing continents. Among the major producers, India alone accounts for 27.43 % (26.2 million tonnes) followed by Philippines, producing 9.01 million tonnes and China, Brazil and Ecuador, with production ranging from 7.19 to 8.21 million tonnes. Production in India, China and Indonesia has witnessed giant leaps in the last eight years. In the last five decades, the per cent contribution of the African continent has witnessed marginal reduction from 12 % to 9 % while the decline has been prominent in Latin American countries, from 40 % to 30 %. This is mainly due to the Asian countries achieving substantial increase from 38 to 60 % (Eglal M. Said *et al.,* 2015).

Drought is a major environmental factor that determines the growth, productivity and distribution of plants. It is the most serious and worldwide yield reducing stress in agriculture. The increase in drought stress threatens the global agriculture production and food availability. It has been estimated that two thirds of the yield potential of major crops are routinely lost due to drought stress.

Utilization of tissue culture techniques for quantifying stress tolerance of various crops has been increasing rapidly. Tissue culturing systems are useful for the evaluation of tolerance to environmental stresses because stress conditions can be easily controlled *in vitro.* Drought stress-induction is one of the most popular approaches takes use of high molecular weight osmotic agents, such as Polyethylene Glycol (PEG). These agents have no detrimental or toxic effects on the plant; however, they inhibit the plant's growth by lowering the water potential of the culture medium in a way similar to soil drying, so that cultured explants are unable to take up water (Krishna Surendar *et al.,* 2013).

This study involves the production of water stress tolerant plant of various *Musa species* in the *in vitro* stage involving various trials that is responsible for the improvement of stress tolerant plant. PEG of high molecular weight is a non-penetrating inert osmoticum lowering the water potential of nutrient solutions without being taken up or being phytotoxic. PEG as a stress inducer, trialed in the micropropagation stages and the difference in the plant growth parameters were observed. This activity helps in the production of water stress tolerant plants.

2. MATERIALS AND METHODS

2.1 Source Collection

The plant source material was a mature *Musa accuminata*, acquired from Genewin Biotech, R and D, Hosur and maintained in the Mother plant Pedigree at Genewin Biotech.

Fig: 1. Showing Mother Plants (matured *Musa accuminata)*

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2.2 Preparation of MS Medium

Murashinge and Skoog medium (MS- medium) is a plant growth medium used in the laboratories for cultivation of plant cell culture (Murashige and Skoog, 1962). Media were supplemented with 30g/L sucrose and 6g/L agar and the pH was maintained at 5.8 before autoclaving.

2.3 Explant Initiation

The new sucker explants processes from the mother plant corm were soaked in antifungal and antibacterial solution, carbendazim (0.1%) and streptocycline (0.1%) for 15 minutes. The sterilization was followed by cleaning of leaves very carefully using 70% ethanol with sterilized cotton. Finally, explants were treated using detergent, Polysorbate 20 for 20 minutes. The explants were washed with sterile water three times to ensure the complete removal of foam. The explants then treated with 0.1% Mercuric chloride for 10 min and inoculated in the initiation media for its growth.

Initiation stage gives rise to regeneration of new shoots from the selected explants in 15 days. The growth was frequently monitored every week and recorded. The surface sterilized explants were inoculated in following MS basal media treatments + Sucrose 3% with various growth regulator concentrations.

Silver nitrate : 1 to 5 mg/L
6 (BenzylAminoPurine) : 1 to 5 mg/L
IndoleAcetic Acid : 0.1 to 0.5 mg/L

The explants were placed in the prepared media; each treatment consisted of 3 replicates and the mean parameters were calculated. The inoculated jars were incubated.

2.4 Multiplication of Emerged Shoots

The shoots arised from the explant was further transferred to multiplication stage for rapid propagation. The emerged shoots were properly trimmed and inoculated in the media consisted of basal MS media + 3% Sucrose with the following.

MS + Silver nitrate : 1 - 5mg/L
6-(BAP) : 1 to 5 mg/L
PEG + 6BAP – Water stress tolerant : 0.5 to 3% + 5 mg/L

The above trial media were prepared and the trimmed shoots were inoculated and incubated. 4 Subculturing was done every 3-4 weeks in order to increase the multiplication ratio.

2.5 Rooting Stage

The shoots raised from the explant were further transferred to Rooting stage for the emergence of roots. Root inducing media were prepared and compared the withstanding of banana plant's growth using a water stress tolerant in the rooting media as well. The plants were inoculated in the media consisted of basal MS media + 3% Sucrose with the following.

IBA + Kinetin+ PEG : 1mg/L + 1 mg/L+ 2.5%
IBA + Kinetin : 1 mg/L + 1 mg/L

The above trial media were prepared and the trimmed shoots were inoculated and incubated. Rooting efficiency was tabulated.

Culture Conditions

The inoculated plants were subjected to light intensity for 10-12 h in the growth room for rapid multiplication. Photoperiod provided by cool white fluorescent lamps of 1500-3000 lux, temperature of about 25 ± 2 °C and humidity of 35 - 40%.

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4. RESULTS AND DISCUSSION

4.1 Mortality Rate

The inoculated explants were monitored every week and Mortality rate was found out.

The Mortality rate was calculated by,

$$\% \text{ Mortality } = \frac{\text{Explants contaminated}}{\text{Total no of Explants}} \text{x100}$$

Table:1. Mortality Rate of Inoculated Explants

Treatment	No. of Explants Taken	No. of Explants Dead	Mortality Rate (%)	Mean Rate of Shoot Regeneration (%)
0.1% $HgCl_2$ – 8 min	10	3	30	17
0.1% $HgCl_2$ – 9 min		6	60	26
0.1% $HgCl_2$ – 10 min		3	30	60
0.1% $HgCl_2$ – 11 min		2	20	78
0.1% $HgCl_2$ – 12 min		0	0	89

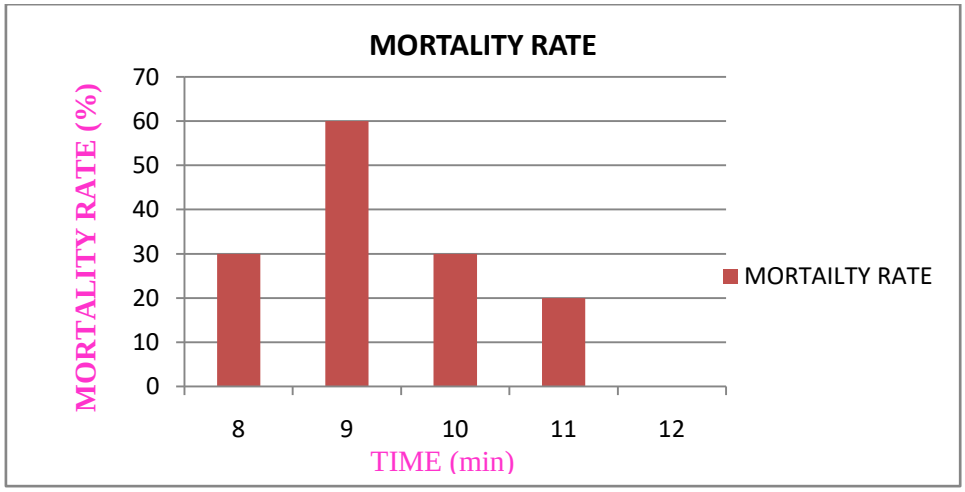

Fig: 2. Graph showing the Mortality Rate V_S Time

4.2 Initiation

Table: 2. Initiation of shoot length with Silver Nitrate (SN)

S. No.	SN (mg/L)	Mean shoot length (cm)	Response (%)	Contamination (%)
1	1	1.1 ± 0.43	20	80
2	2	1.05 ± 0.56	15.6	84.4
3	3	1.19 ± 0.63	15	85
4	4	1.18 ± 0.7	16	84
5	5	1.18 ± 0.7	11	89

SN- Silver nitrate

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Table: 3. Initiation of shoot length with 6 (BAP)

S. No	6 (BAP) (mg/L)	Mean shoot length (cm)	Response (%)	Contamination (%)
1	1	2.2 ± 0.98	40	60
2	2	2.77 ± 1.05	55	45
3	3	3.01 ± 1.32	62	38
4	4	3.45 ± 1.44	70	30
5	**5**	**3.91 ± 1.65**	**90**	**10**

BAP- Benzylaminopurine

Fig: 3. Initiation Responses

Table: 4. Initiation of shoot length with supplementation of Indole Acetic Acid (IAA)

S. No	IAA (mg/L)	Mean shoot length (cm)	Response (%)	Contamination (%)
1	0.1	1.271 ± 0.43	25	75
2	0.2	1.25 ± 0.56	22	78
3	0.3	1.29 ± 0.63	25	75
4	0.4	1.28 ± 0.7	16	84
5	0.5	1.38 ± 0.7	10	90

IAA –Indole Acetic Acid

During Initiation, it was observed that the G9 suckers responded in all the growth regulators stimulating the shoot growth, but the least contamination was recorded in the media containing 6BAP as growth stimulator that responded well with highest shoot length and the minimum contamination at concentration 5 mg/L (**Table: 3)**.

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4.3 Multiplication

Table: 5. Multiplication of shoot length with supplementation of 6 Benzyl amino purines (BAP) + PEG (Polyethyleneglycol)

Trial Media	Mean Shoot Length (Cm)	Mean Number of Shoots (Cm)	Multiplication Ratio			
6 BAP (mg/L) + PEG			1st Sub-Culture	2nd Sub-Culture	3rd Sub-Culture	4th Sub-Culture
5 + 0.5 %	0.70	1	1	1.1	1.21	1.33
5 + 1 %	0.98	3	0.9	1.1	1.23	1.58
5 + 1.5 %	0.96	3	0.95	1.21	1.41	1.67
5 + 2%	1.71	6	1.21	1.51	1.8	2.22
5 + 2.5 %	1.49	3	1.08	1.31	1.49	1.66
5 +3%	1.41	1	1	1.1	1.28	1.31

PEG - Polyethylene glycol; BAP- Benzylaminopurine

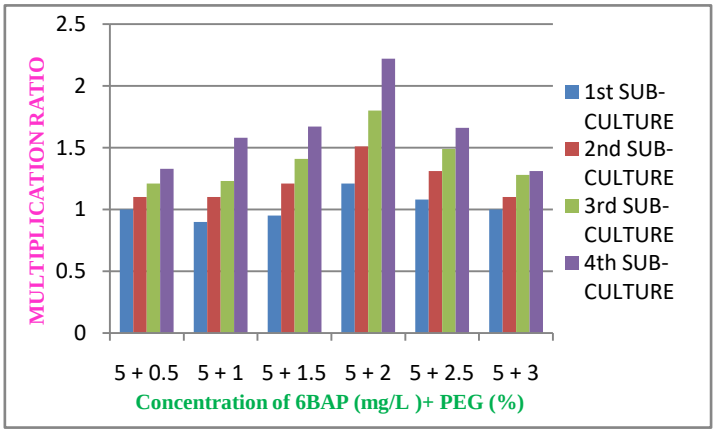

Fig: 4. Graph showing the multiplication efficiency with PEG

Fig: 5. showing the multiplication efficiency with PEG-Water Stress Tolerant

Table: 6. Multiplication Efficiency with 6BAP

Trial Media	Mean Shoot Length (Cm)	Mean Number of Shoots (cm)	Multiplication Ratio			
6 BAP (mg/L)			1st SUB-CULTURE	2nd SUB-CULTURE	3rd SUB-CULTURE	4th SUB-CULTURE
1	0.65	1	0.95	1.03	1.11	1.27
2	0.86	2	0.82	1.02	1.15	1.37
3	0.91	3	0.67	1.04	1.27	1.49
4	1.43	5	1.03	1.24	1.56	1.87
5	1.51	2	0.96	1.11	1.31	1.51

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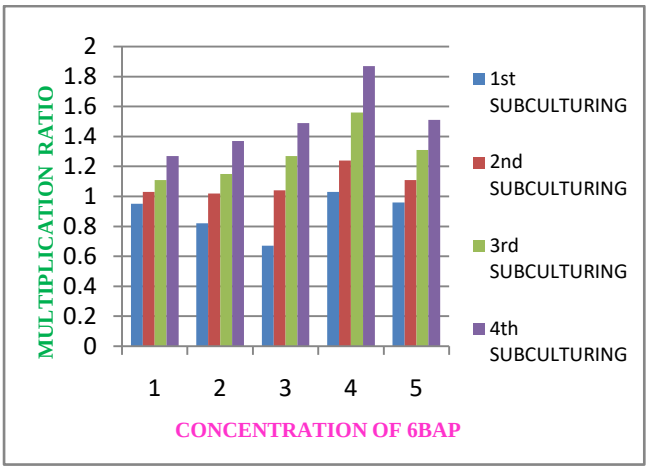

Fig: 6. Graph showing the multiplication Efficiency with 6BAP

Fig: 7. showing the multiplication efficiency with 6BAP

Table: 7. Multiplication Efficiency with Silver Nitrate

Trial Media	Mean Shoot Length (Cm)	Mean Number of Shoots (Cm)	Multiplication Ratio			
Silver Nitrate (mg/L)			1st Sub-Culture	2nd Sub-Culture	3rd Sub-Culture	4th Sub-Culture
1	0.55	1	0.65	0.91	1.03	1.12
2	0.46	2	0.73	0.97	1.07	1.14
3	0.51	3	0.67	0.88	1.09	1.18
4	1.23	3	1.06	1.16	1.28	1.32
5	1.31	2	1.03	1.18	1.32	1.43

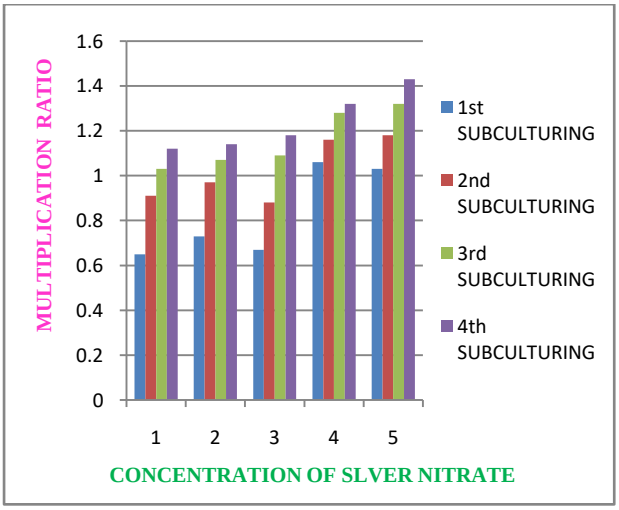

Fig: 8. Graph showing the multiplication efficiency with Silver Nitrate

Fig: 9. showing the multiplication efficiency with Silver Nitrate

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Multiplication efficiency shown highest multiplication ratio with 6BAP and PEG combination (**Table: 5**) than that of silver nitrate and 6BAP alone. Multiplication ratio of 2.22 at the end of 4thsub culturing which showed constant increases in the ratio from the 1stsubculturing.

According to RukundoPlacide *et al.,* 2012**,** sorbitol is a neutral osmotic inducer to study the drought tolerance of banana varieties and showed that the concentration of 0.2 M sorbitol is a suitable concentration to screen for drought tolerant banana varieties under *in vitro* condition. Moreover, the growth parameters were proved to be relevant in identifying drought tolerant banana varieties under *in vitro* condition. The present study reveals that 6 BAP (mg/L) + PEG (5+2%) combination gives highest multiplication ratio at the end of 4th sub culturing. PEG is a water stress inducer and the above mentioned combination is suitable concentration to screen for water stress tolerant banana varieties under *in vitro* condition.

In Ikram-ul-Haq *et al.,*(2011) studies, maximum shoot multiplication was observed on MS2b medium supplemented with BA, while, multiplication rate was decreased significantly in all the stressed cultures. These stresses not only reduced the multiplication shoots among the cultures, but also the shoot height, shoot fresh weight as well as the shoot dry weight were also decreased ($p < 0.05$). Including growth rate of the cultures, photosynthetic pigments were also affected. Chlorophyll *a* was decrease non-significantly, while chlorophyll *b* and total chlorophyll contents were also decreased but significantly. Total carotenoids were increased significantly in all the stressed cultures. A significant increase in the epidermal cells and decrease in aerenchymatous cells ($p < 0.05$) was observed, while anon-significant change in the size of mesophyll cells occurred.

Research studies by Krishna Surendar *et al.,* 2013 suggested that the capability of banana germplasm to rehydrate after the stress period is of importance to drought recovery. Recovery of a banana germplasm from drought is related to its ability to retain green leaf area during that period. Plants with good leaf retention can supply more assimilates to the developing fingers during subsequent recovery. These in turn result in production of a large number of fingers and more number of hands per bunches. Leaf wilting coincided with midday leaf water potentials of approximately -2.0 MPa, beyond which, some leaves become yellow and abscised. Upon re watering, leaves appeared normal (non wilted) and leaf water potential returned to previous levels (-0.6 MPa) in two to three days later. A slow rate of onset of stress may allow for development of adaptive mechanisms such as osmotic adjustment, decreasing leaf area, abscission of leaves, leaf folding, rolling or reorientation of leaves and an increased root growth rate.

4.4 Rooting

Table: 8 Rooting Efficiency with PEG

IBA (mg/L) + Kin (mg/L) + PEG (%)	No. of Shooted Plants Taken	No. of Roots	Length of Roots (Cm)	Mean Rooting Response (%)
1+ 1 + 0.5%	20	6	1.5	30
1+ 1 + 1%		8	1.57	40
1+ 1 + 1.5%		10	2.09	50
1+ 1 + 2%		9	1.63	45
1+ 1 + 2.5%		18	2.91	90

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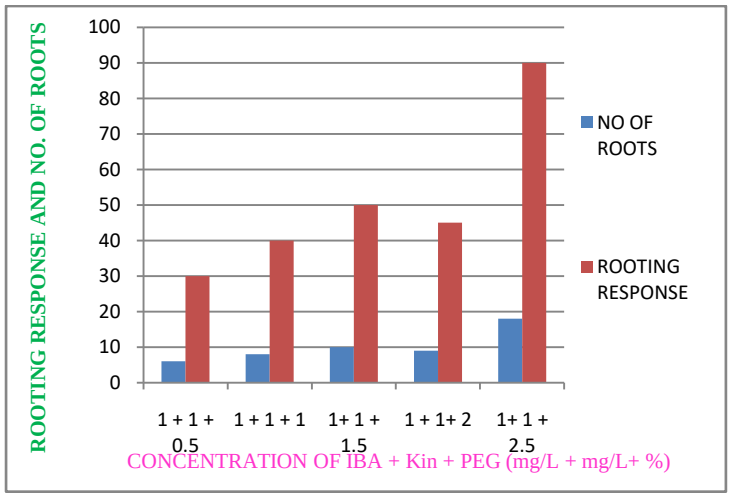

Fig: 10. Graph showing the Rooting efficiency with PEG

Fig: 11. Rooting Response - Trial 1 – IBA + Kin + PEG

Table: 9. Rooting Efficiency without PEG

IBA + Kin (mg/L)	No. of Survived Explants Taken	No. of Roots	Length of Roots (Cm)	Mean Rooting Response (%)
1 + 0.2	20	7	1.53	35
1 + 0.4		8	1.64	39
1 + 0.6		9	1.73	49
1 + 0.8		9	1.84	50
1 + 1		**8**	**2.01**	**61**

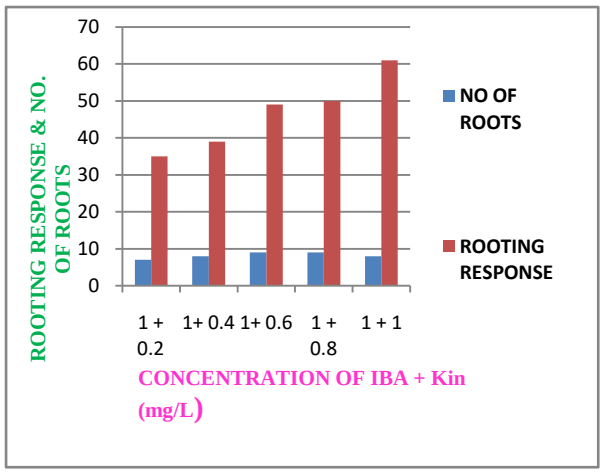

Fig: 12. Graph showing the Rooting efficiency without PEG

Fig: 4.13 Rooting responses – Trial 2 – IBA + Kin

Rooting stage in the banana explants was found highest with the Trial 1**(Table: 8) (Fig: 11)** using PEG as a water stress tolerant which responded about 90% when compared to Trial 2 **(Table: 9) (Fig: 13)** of 61% and the length of the roots was higher in Trial 1 with 2.91 cm whereas in Trial 2, it is 2.01 cm. This stage was very

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successful which the end of tissue culture process is. After a period of 3-4 weeks, the rooted plants were hardened.

In this study, *Musa species*, Grand Naine was selected in order to produce a stress tolerant plant through Micropropagation in which a stress inducer was added as a ingredient in the MS media along with the growth regulators. Multiplication fold rate was found to be high in the stress induced combination with PEG and the multiplication hormone 6BAP which showed the positive response in all the 4 sub-culturing followed by the rooting stage which produced comparatively taller plants and lengthy roots with the response of 90% unlike the trial done without the stress inducer. Banana plants were easily compatible with the stress inducer, PEG and responded well in the culture stage which can be produced as a mass propagation against the natural calamities.

CONCLUSION

This study involves the production of water stress tolerant plant of *Musa species*, Grand Naine (G9) in the *in vitro* stage involving various trials that is responsible for the improvement of stress tolerant plant. PEG of high molecular weight is a non-penetrating inert osmoticum lowering the water potential of nutrient solutions without being taken up or being phytotoxic. PEG as a stress inducer, trialed in the micropropagation stages and the difference in the plant growth parameters were observed. This activity helps in the production of water stress tolerant plants. It was found that the PEG induced in the growth media in the stages such as Multiplication, Rooting were observed to respond equally to non-induced media. Rooting efficiency reached up to 90% unlike in non-induced. PEG as a stress inducer was compatible with the growth regulators during multiplication and rooting which could bring tolerant plants against the natural calamities.

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