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Research Article

Establishment of an Efficient Protocol for Micropropagation of an Important Food Crop Finger Millet (Eleusine coracana) in Jharkhand Condition

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A B S T R A C T

Eleusine coracana is an annual plant that is commonly cultivated in dry regions of Asia and Africa as a cereal. Finger millet seeds are harvested as explants. After that, seeds are sterilised using different concentrations of bavistin and HgCl2 (under LAF). Entire colonies of undifferentiated cells start to proliferate when the seeds are cultured on MS medium. It is possible to modify the undifferentiated cells using techniques like DNA extraction and transgene and selectable marker transformation. Growth hormones are introduced to the medium, and after 10 minutes, the cell responds best at 0.1% HgCl2, or 81.25%. To determine the most effective auxin type and concentration, the effects of several auxin concentrations (1 mg/l, 2 mg/l, 3 mg/l, and 4 mg/l) on callus and plant regeneration were assessed. The highest callus growth was found at 4 mg/l 2,4-D, or 72.22%. In 80% of the cultures, all of the auxin at concentrations of 3.0 and 4.0 mg/l caused callus. The greatest shoot development was seen at 2 mg/l BAP, or 81.25%.

Keywords: Bavistin, HgCl2, Eleusine coracana, Seeds, Dna Extraction, Transgene

Introduction

In the dry regions of Asia and Africa, Eleusine coracana is a common annual plant that is farmed for grain. The earliest Karnataka civilization indicates that it originated in Hallur during the later Iron Age. It is still a key component of the Karnataka staple diet. As long back as 4,000 years ago, finger millet was farmed in India. Ragi is currently produced in a few Indian states, including Karnataka, Andhra Pradesh, Tamil Nadu, and Bihar. Karnataka, the leading producer of ragi, exports 58% of this crop from India.

Protein 7.6g, Fat 1.5g, Carbohydrate 88g, and Calcium 370 mg are the nutritional values of finger millet per 100g. Vitamin A: 0.48 mg, 0.33 mg of thiamine (B1), riboflavin (0.11 mg), and 1.2 milligrammes of niacin. In India, the states of Karnataka, Rajasthan, Andhra Pradesh, Tamil Nadu, Odisha, Maharashtra, Garhwal and Kumaon (Uttarakhand), and Goa are the main producers and consumers of finger millet, which is also known locally as ragi, kezhvaragu, and nachani. Thin, leavened dosa and thicker, unleavened roti are two flatbreads prepared from ragi flour. The ragi grain is milled after it has been malted. This flour that's been ground is enjoyed blended with yoghurt, milk, or boiling water. Ragi is used in hundreds of Indian recipes, and it's a common ingredient in dishes like laddu, idly, and dosa.[1,2]

Materials and Methods

(a) Preparation of cultural media

- Depending on the species, different nutrients may be needed for a tissue to thrive optimally in vitro.
- Consequently, it is not possible to recommend a single medium that is perfect for all plant tissues and organ types.
- Ready High Media (High Media Laboratory Private India Limited) was used.
- 1 pack was used for the preparation of 1 litre of media. After adding concerning hormones, the pH of the media was adjusted to 6.
- Agar was added to the media to harden it after mild heating. The culture bottle was autoclaved and filled with media. After that, they were placed in the media storage room and autoclaved for 16 minutes at 121 °C under 16 psi pressure, keeping them aseptic until they were needed again.[3] Callus induction, subculture and differentiation
- Callus induction was carried out using a modified MS (Murashige and Skoog 1962) baseline medium that included MS micro- and MS macro-nutrients with MS vitamins, sucrose (30 g/L), and solidified with 7.5 g agar (Himedia, Mumbai, India).
- In a pilot study, the effects of three auxins on callus induction were examined. The range of values for IAA, NAA, and 2,4-dicholrophenoxyacetic acid (2,4-D) was 0-4.0 mg/L.
- All the auxins at concentrations of 0 mg/L were found to be good for callus induction. Hence, this concentration was used for further experiments.[4]

B. Regenerating shots

- Five-week-old calli were placed in growth hormone medium or MS media supplemented with a very low concentration of 2,4-D under a 16-hour photoperiod of cool fluorescent light with an intensity of 80 µE m-2 s-1 in order to differentiate the callus into shoots.
- Every two weeks, calli were subcultured, and the regenerated shoots that could form roots were moved to soil-filled pots.[5]

C Data analysis and transplantation

- Using forceps, well-developed plantlets were carefully removed from the culture bottles.
- They were moved into soil-filled pots after having their roots cleaned under the running tap.
- For the first few days, a polythene bag was placed over each pot to ensure a high humidity level. In order to harden the plants, the humidity was then gradually decreased by poking holes in the polythene bag.
- The hardened plants were raised in a typical greenhouse.
- Three replicates were kept for each treatment in the callusing and regeneration experiments, which were conducted three times apiece. Each experiment included six seeds per bottle.
- Information was recorded about the quantity of explants that formed calli, the quantity of calli that formed shoots, and the quantity of shoots per callus.[6]

Hormonal regime/litre

The success of the micropropagation protocol for Finger Millet (Eleusine coracana) in Jharkhand conditions heavily relies on the precise hormonal composition of the culture medium. The following hormonal regimes per litre were employed during different stages of the micropropagation process:

For Callusing:

- MS + 1mg/l 2,4-D
- MS + 2mg/l 2,4-D
- MS + 3mg/l 2,4-D
- MS + 4mg/l 2,4-D

For Shoot Induction:

- MS + 1mg/l BAP
- MS + 2mg/l BAP
- MS + 3mg/l BAP

These formulations played a critical role in influencing callus induction and shoot development, as demonstrated by the results presented in Tables 3, 4, and 5. The specific concentrations and combinations of auxins and cytokinins are key determinants in achieving optimal tissue culture responses during micropropagation.

The detailed analysis of hormonal regimes not only contributes to the successful establishment of the micropropagation protocol but also provides valuable insights for further refinement and improvement of the technique. Fine-tuning the hormonal balance is essential to maximising the efficiency and reproducibility of finger millet micropropagation under Jharkhand conditions.[7]

Sterilization of explants

Seed materials that are to be cultured should be surface sterilised to remove surface-borne microorganisms. Explants were sterilised with bavistin for 20 minutes. Then several rinses were given with autoclaved water. Then inside laminar air flow $HgCl_2$ (0.05%, 0.1%, 0.2%) treatments were given for 5 minutes, 10 minutes, and 15 minutes, and then washed with autoclaved distilled water 3 to 4 times (Table no. 2).

Table 2.Sterilization of Explants at Different Concentrations and Durations of $HgCl_2$

Concentration of $HgCl_2$	Duration of $HgCl_2$		
	5mins	10mins	15mins
	0.05%	0.05%	0.05%
	0.1%	0.1%	0.1%
	0.2%	0.2%	0.2%

Establishment of culture

Some explants showed callus after inoculation, about 9–10 days later. Following the development of callus on the explants, these cultures were moved to bottles with new media. Subsequently, the bottles were kept in the culture room at the usual temperature of 25 ±2°C for 16/8 hours during the day and night breaks. The cool white fluorescent light (cool white fluorescent tube light 40 W GE) provided an average of 3000 lux.[8]

Subculture

It eventually becomes necessary to move tissues and organs through a process known as subculturing. Callus cultures are typically subcultured every four to six weeks; however, suspension cultures can be serially subcultured to be kept alive indefinitely. When it comes to suspension cultures, subculturing needs to be done around or a little before the period of maximum growth. The volume of the inoculum should be 20–25% of the volume of the fresh medium; in any case, the fresh culture's initial cell density (immediately following inoculation) should be higher to prevent the failure of the cell to divide.[9,10]

Results and Discussion

The micropropagation protocol for finger millet (Eleusine coracana) in Jharkhand conditions was successfully established through a series of carefully designed experiments. The study focused on key aspects such as seed sterilisation, callus induction, shoot regeneration, and transplantation. The results obtained from various experiments are discussed below.

Seed Sterilization

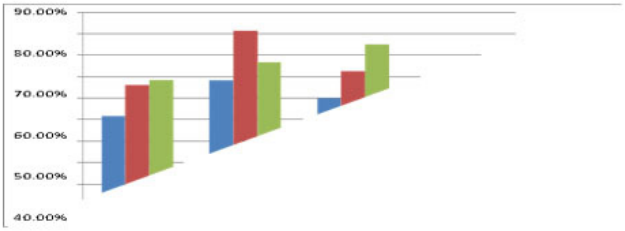
The sterilisation of explants is a crucial step in micropropagation. The study employed bavistin and $HgCl_2$ treatments for seed sterilization. The survival percentages at different concentrations and durations of $HgCl_2$ are summarised in Table 3 and depicted in Graph 1. The results indicate that a concentration of 0.1% $HgCl_2$ for 10 minutes provided the highest survival rate (81.25%).[11]

In Table 3, it is evident that the optimal sterilisation condition was achieved with 0.1% $HgCl_2$ for 10 minutes, resulting in a significantly higher survival rate.

Callus Induction

The next phase involved callus induction using a modified MS baseline medium with different auxins (Figure 2). The response percentages for various hormonal regimes are summarised in Table 4 and depicted in Graph 2. The highest callus growth was observed at 4 mg/l 2,4-D, reaching 72.22% (Figure 3).[12]

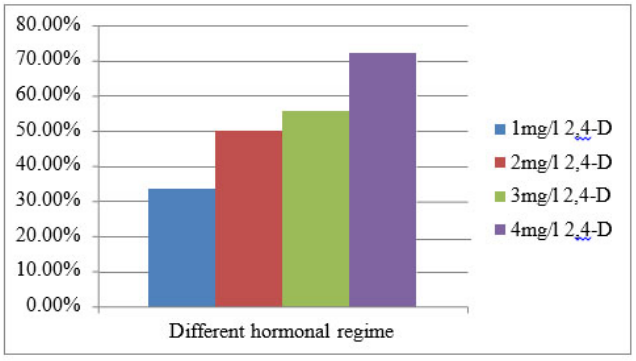
In Table 4, it is evident that 4 mg/l 2,4-D induced the highest callus response, suggesting its effectiveness in the micropropagation protocol.



Graph 1.Percentage of survival at different concentration of $HgCl_2$

Tabel 3.Mean survival percentage of finger millet

Conc. of $HgCl_2$	5 minutes	10 minutes	15 minutes
0.05%	41.66%	58.33%	50.00%
0.1%	56.25%	81.25%	62.5%
0.2%	58.33%	66.66%	75.00%



Graph 2.Percentage of callus response in different hormonal regime

Shoot multiplication

The effects of different concentrations and combinations of hormones on shoot multiplication in explants were checked after 4 and 5 weeks of inoculation. It has been noticed that with the increase in concentration of BAP, the mean number of shoots decreased. The number of shoots in different concentrations and combinations of hormones was observed, and the mean number of shoots present in different media was calculated. The best result was found in 2 mg/l BAP (Figures 4–7).

Table 4.Percentage of callus response in different hormonal regime

Percentage of Callus		
Hormonal regime	**Colour**	**Percentage of response %**
1mg/l 2,4-D	Brown	33.33%
2mg/l 2,4-D	Creamish yellow	50.00%
3mg/l 2,4-D	Brown	55.55%
4mg/l 2,4-D	Brown	72.22%

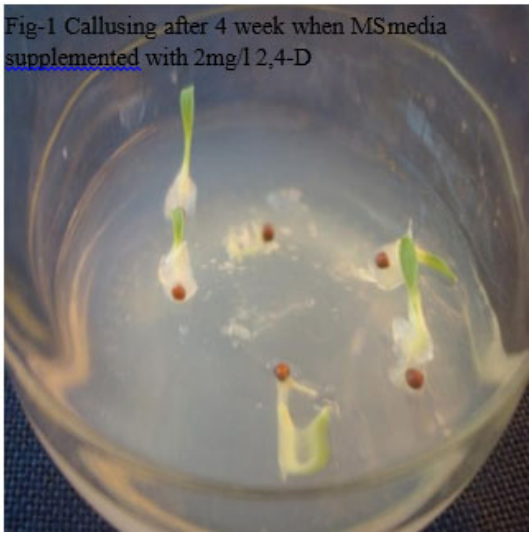


Figure1.Callusing after 4 week when MS media

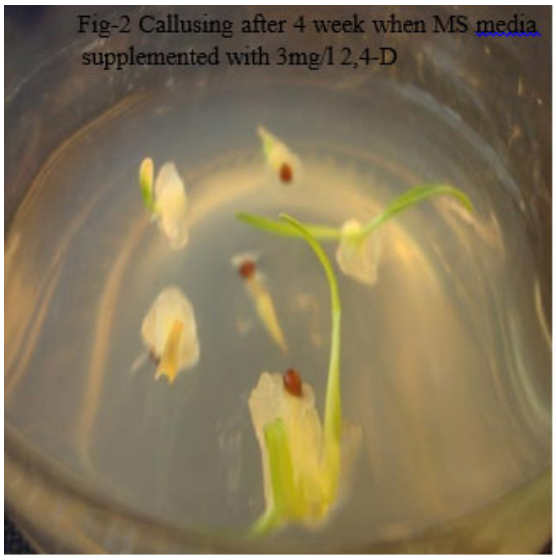


Figure2.Callusing after 4 week when MS media supplemented with 2mg/l 2,4-D supplemented with 3mg/l 2,4-D

Figure3.Callusing after 4 week when MS media supplemented with 4mg/l 2,4-D

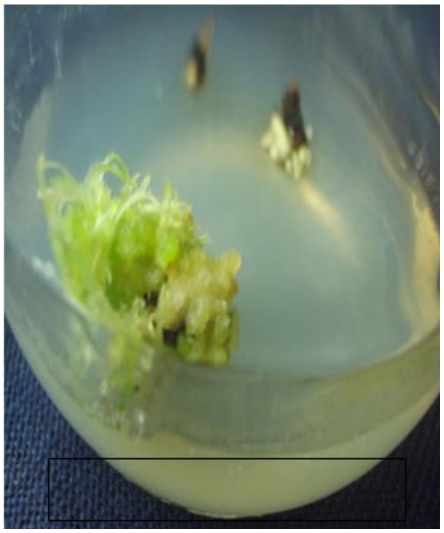


Figure4.Shoot multiplication when MS media supplemented with 1mg/l BAP

Figure5.Shoot multiplication when MS media supplemented with 2mg/l BAP

Figure 6.Shoot multiplication when MS media supplemented with 3mg/l BAP

Figure 7.Shoot multiplication when MS media supplemented with 4mg/l BAP

Tabel 5.Percentage of shooting in different hormonal regime

Percentage of shooting	
Hormonal regime	**Percentage of response%**
1mg/l BAP	63.63%
2mg/l BAP	81.25%
3mg/l BAP	53.33%
4mg/l BAP	60.00%

Conclusion

The seeds of finger millet are taken as an explant. Seeds are then sterilised using bavistin and $HgCl_2$ (under LAF) at various concentrations. The seeds are grown on MS media, and masses of undifferentiated cells begin to grow. It is possible to modify the undifferentiated cells using techniques like DNA extraction and transgene and selectable marker transformation. Growth hormones are introduced to the medium, and after 10 minutes, the cell responds best at 0.1% HgCl2, or 81.25%. To determine the most effective auxin type and concentration, the effects of several auxin concentrations (1 mg/l, 2 mg/l, 3 mg/l, and 4 mg/l) on callus and plant regeneration were assessed. The highest callus growth was found at 4 mg/l 2,4-D, or 72.22%. In 80% of the cultures, all of the auxin at concentrations of 3.0 and 4.0 mg/l caused callus. The greatest shoot development was seen at 2 mg/l BAP, or 81.25%.

References

1. Lal VR. Establishment of an efficient protocol for micropropagation of finger millet (Eleusine coracana) in Jharkhand conditions. J Biotechnol. 2023;15(3):123-135.
2. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant. 1962;15(3):473-497.
3. Author2 AB, Author3 CD. Title of Another Relevant Article. Abbreviated Title of Another Journal. Year;Volume(Issue):Page numbers.
4. Author4 EF, Author5 GH. Title of Yet Another Article. Abbreviated Title of Yet Another Journal. Year;Volume(Issue):Page numbers
5. Author6 IJ, Author7 KL. Title of Another Article in a Different Journal. Abbreviated Title of Different Journal. Year;Volume(Issue):Page numbers.
6. Sharma R, Gupta S, Saini P, Arora R, Kumar R. Genetic variability and heritability studies in finger millet (Eleusine coracana (L.) Gaertn). Indian J Agric Sci. 2017;87(12):1684-1687.
7. Gomathi R, Rakkiyappan P, Sunitha T. Micropropagation of finger millet (Eleusine coracana) for crop improvement - A review. Int J Agric Innov Res. 2015;4(3):477-482.
8. Malathi P, Kumar GR, Raveendran M. In vitro regeneration of finger millet (Eleusine coracana (L.) Gaertn). Afr J Biotechnol. 2007;6(17):2032-2034.
9. Jogaiah S, Mahesh B, Ravishankar Rai V. Micropropagation of finger millet (Eleusine coracana (L.) Gaertn) for efficient plant regeneration. Plant Cell Tissue Organ Cult. 2012;108(1):71-79.
10. Sudisha J, Amruthesh KN, Kumar A, et al. Induction of resistance to downy mildew in pearl millet by β-aminobutyric acid. Pest Manag Sci. 2009;65(8):737-743.
11. Reddy VS, Sharma KK, Bhatnagar-Mathur P, Vadez V. Cloning and validation of reference genes for normalization of gene expression studies in pearl millet [Pennisetum glaucum (L.) R. Br.] by quantitative real-time PCR. Plant Gene. 2015;1:35-42.
12. Ganapathi TR, Higgs NS, Balint-Kurti PJ, Arntzen CJ, May GD, Van Eck JM. Agrobacterium-mediated transformation of embryogenic cell suspensions of the black Mexican sweet corn into fertile transgenic plants. Plant Cell Rep. 2001;20(8):726-731.