International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 03-01-2022, Accepted: 18-01-2022, Published: 03-02-2022
Volume 7, Issue 2, 2022, Page No. 123-126

Assessing the growth of *Eleusine coracana* seeds in artificial medium under *In-vitro* conditions

Neha Pal, Sharada Prajna*, Ramesh B S, Jayarama Reddy
Department of Botany, St. Joseph's Post Graduate and Research Centre, Bengaluru, Karnataka, India

Abstract
An efficient reproducible plant regeneration of seed derived culture has been developed, a complete regeneration system has been developed using mature seeds as explants. The seeds of ragi were germinated and were successfully initiated in Murashige and Skoog's media. Mesocotyl and leaf base tissue derived which gave shoot buds. The callus induction was seen on media with hormones mainly the MS media was supplemented with 9gm/lt agar along with hormones like auxin (IAA), cytokinin (BAP) both with 0.005 gm. /lt, sucrose 30gm/lt was added as the sole carbon source. Callus was successfully initiated in Vacin and Went media. MS Media showed seed germination and later gave out root and shoot, attributing that, MS media promoted direct organogenesis, whereas Vacin and Went media promoted indirect organogenesis by callus formation.

Keywords: *Eleusine coracana, In-vitro*, micropropagation, plant regeneration, callus, direct organogenesis, indirect organogenesis

Introduction
Plant tissue culture is an 'art' or technique of culturing plant cells, tissues and organs on synthetic nutritive media under aseptic and controlled conditions of light, temperature, and humidity. The principle of Plant tissue culture is totipotency (Shahzad *et al*, 2017) [11]. Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones (Bhatia, 2015) [2]. In 1902, Haberlandt being the pioneer of Plant Tissue Culture, established it by culturing *Tradescantia* plant species' tissue. Since then, PTC has evolved into one of the most important and frequently used biotechnology techniques, from basic experiments to agricultural applications and production of plant-derived metabolites of important commercial value (Yemets *et al*., 2013; Abebaw *et al*., 2021) [14, 1].

About the Mother Plant- *Eleusine coracana* (Finger Millet)
Finger millet, belonging to the family Poaceae, commonly known as ragi or madua in India, rapoko in South Africa and dagusa in Ethiopia (Obilana&Manyasa, 2002) [9], is the staple food for millions of people in tropical dry land regions. Finger millet has a relatively higher content of minerals and micronutrients density compared to other millets, superior to that of rice and is on par with that of wheat. Ragi is vulnerable to abiotic stresses like drought and salinity stress and biotic stresses like fungal blast (Pandey *et al*., 2017) [10]. *Eleusine coracana (L.)* has been recognised with a potential of being a 'super cereal' by the United States National Academies being one of the most nutritious among all major cereals (Kumar *et al*., 2016) [5]. Millets are grown in poor soils with limited inputs. They are a major source of income for resource-poor farmers of the tropical areas ("Farmers Turn to Millets as a Climate-Smart Crop," 2018) [3]. Ragi has been in talks for a possible application as an alternative grass source for bioethanol production in countries with temperate climate of Eastern Europe (Yemets *et al*., 2013) [14].
In this paper, *Eleusine coracana* seeds were used as an explant and checked for their growth in a controlled artificial environment. The exact conditions required to initiate and sustain plant cells in culture, or to regenerate intact plants from cultured cells, are different for each plant species. Each variety of a species will often have a particular set of cultural requirements (Molnár *et al*., 2011) [6]. *Murashige and Skoog's media* was used for the experiment (Murashige & Skoog, 1962) [7].

Materials and Methods
Callus induction
MS media was prepared with a desired amount of hormonal and vitamin supplements.
The MS media was supplemented with Plant Tissue Culture-Grade Agar for solidification, along with hormones like Auxin (IAA) and Cytokinin (BAP), and sucrose (3%) was added as the sole carbon source with pH – 6 being maintained.
The Vacin and Went media was supplemented with Plant Tissue Culture-Grade Agar for solidification, along with Auxin (NAA) and sucrose (2%) was added as the sole carbon source with pH – 6-6.5 being maintained.

The media was later poured into glass culture bottles and autoclaved at 121°C for 15 minutes. The media was solidified and stored in sterile conditions for 2 days before inoculation.

Plant material and Media-
The seeds of Eleusine coracana were bought from a farmer in Mandya for the purpose of this research (Figure 1).

Fig 1: Various stages of *Eleusine coracana* – A) Ragi Crop; B) Inflorescence showing flowers; C) Inflorescence showing seeds; D) Harvested Ragi seeds (Fig Courtesy- Agro Vista Farming)

Surface Sterilization
Outside LAF- Seeds were washed with tap water for 10 mins and then with 10% Teepol solution for 15 minutes and washed in double distilled water for 3 rounds, making sure there is no Teepol residue left.
Inside LAF- The double distilled water bottle with the seeds was carried into the LAF, where the seeds transferred into 0.1% mercuric chloride solution and rinsed continuously for 5 minutes (Take care not to touch the solution with hand, as it is very toxic). The seeds were again washed in double distilled water for 2 rounds to get rid of mercuric chloride traces and transferred into 6% hydrogen peroxide solution and washed for 30 seconds as a part of the final sterilization step. The seeds were immediately transferred into double distilled water and ready for inoculation (Gadakh Santosh Ashok, 2017) [4].

Callus Induction: For callus induction, seeds were inoculated under fluorescent light in a horizontal laminar air flow chamber with maximum sterility maintained under in-vitro conditions. The seeds were inoculated in 10 MS media bottles and in 3 Vacin and Went media bottles and stored in growth rooms with optimum conditions for callus initiation. (Mohanty *et al*., 1985) [8]. Temperature of 22±2°C and Light intensity of - 2000 LUX for 16 hours photoperiod were maintained.

Results and Discussion
Millets have lagged behind in plant tissue culture due to difficulties in plant regeneration and poor transformation efficiencies (Pande *et al*., 2014) [13]. However in the culture inoculated more than 90% showed successful germination of callus from the explants. Both the cultures were kept under the same condition of temperature, light and humidity for the studies. In MS Media, direct shoot germination was seen, rather than callus formation.. While in MS Media, the explant showed direct organogenesis, in Vacin and Went Media, out of three bottles only one bottle showed positive result with whitish callus formation and later giving out shoots and roots through indirect organogenesis. The success rate was 33%.

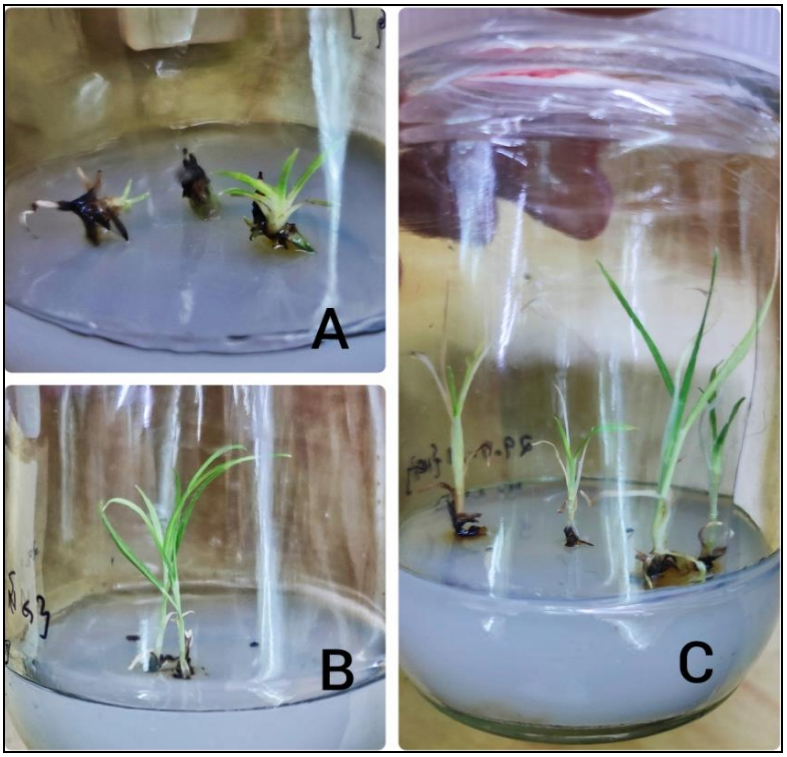

Fig 2: Seed germination of *Eleusine coracana* in MS Media– A) Week 1; B) Week 2; C) Week 3.

Fig 3: Seed germination of *Eleusine coracana* in Vacin& Went Media showing callus formation.

The germination was observed from the first week and a rapid transformation had undergone with the completion of 3 weeks. The germination and regeneration differs for the same explants as the conditions were optimized and modified during media preparation. Similar variations have been reported in *Eleusine* by different investigators, as the response is specific for different cultivators and types. Light and temperature variations can result in alternation of results.

The MS media supplemented with equal amounts of BAP and IAA showed faster shoot growth as it bypassed the callus formation stage (Figure 2). Technically, the equal amounts of auxin and cytokinin supplement should lead to callus formation (Skoog& Miller, 1957) [16], which didn't happen in this case.

The Vacin and Went Media supplemented only with NAA hormone showed callus formation (Figure 3).

Table 3: Explant growth in their respective media.

Media	Hormones	Growth Week 1	Week 2	Week 3
MS II	BAP And IAA	A little growth was seen.	Shoot formation	Active growth of shoot
Vacin and Went	NAA	No growth seen	Root formation	Root and callus formation seen

This paper attributes that Vacin and Went media promotes callus formation more than the MS media. In the referred papers, the result obtained from the matured seeds for *Eleusine coracana* in MS media with various hormones, mostly 2,4-dichlorophenoxyacetic acid, NAA as auxin, and BA and kinetin as cytokinin were incubated for three weeks and the optimum results were seen during the eighth week and the better results were found to in NAA with maximum callus formation accomplished by shoot formation (Kashyap *et al*., 2018; M *et al*., 2013). But in this paper, the media used were MS media, Vacin and Went. MS media with BAP and IAA showed the maximum success rate in growth with the incubation of one week and shoot growth was seen from the second week itself. Vacin and Went media first showed root formation along with callus, later during the third week, with a lesser success rate compared to MS media.
Therefore, this paper states that– In *Eleusine coracana* seeds, MS media promoted direct organogenesis, whereas Vacin and Went media promoted indirect organogenesis by callus formation.

Acknowledgement
The authors are thankful to St. Joseph's College (Autonomous), Bengaluru for providing us with lab facilities. The guidance received from Dr. Jayarama Reddy and Dr. Martin Paul is also acknowledged and the authors are grateful.

References
1. Abebaw YM, Tobiaw DC, Abate BA, Eshete BK, Seymour SK, Tesfaye K. Plant Tissue Culture Research and Development in Ethiopia: A Case Study on Current Status, Opportunities, and Challenges. *Advances in Agriculture*, 2021, e9979549.
2. Bhatia S. Chapter 2—Plant Tissue Culture. In S. Bhatia, K. Sharma, R. Dahiya, & T. Bera (Eds.), *Modern* Applications of Plant Biotechnology in Pharmaceutical Sciences, 2015, 31-107. Academic Press. https://doi.org/10.1016/B978-0-12-802221-4.00002-9
3. Farmers turn to millets as a climate-smart crop, 2018. The Third Pole.
4. Gadakh Santosh Ashok BAH. Standardization of in-vitro Callus Induction and Regeneration Protocol for Mature Embryo of Proso Millet (Panicum miliaceum L.). International Journal of Current Microbiology and Applied Sciences,2017:6(4):2153-2163.
5. Kumar A, Metwal M, Kaur S, Gupta AK, Puranik S, Singh S *et al*. Nutraceutical Value of Finger Millet [Eleusine coracana (L.) Gaertn.], and Their Improvement Using Omics Approaches. Frontiers in Plant Science,2016:7:934.
6. Molnár Z, Virág E, Ördög V. Natural substances in tissue culture media of higher plants. *Acta BiologicaSzegediensis, 2011, 55.*
7. Murashige T, Skoog F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*,1962:15(3):473-497.
8. Mohanty B, Dutta Gupta S, Ghosh P. Callus initiation and plant regeneration in ragi (Eleusine coracana Gaertn.). Plant Cell, Tissue and Organ Culture,1985:5:147-150.
9. Obilana A, Manyasa E. Millets. *Pseudocereals and Less Common Cereals: Grain Properties and Utilization Potential*, 2002, 177-217.
10. Pandey P, Irulappan V, Bagavathiannan MV, Senthil-Kumar M. Impact of Combined Abiotic and Biotic Stresses on Plant Growth and Avenues for Crop Improvement by Exploiting Physio-morphological Traits. *Frontiers in Plant Science*,2017:8:537.
11. Shahzad A, Sharma S, Parveen S, Saeed T, Shaheen A, Akhtar R *et al*. Historical Perspective and Basic Principles of Plant Tissue Culture. In M. Z. Abdin, U. Kiran, Kamaluddin, & A. Ali (Eds.), *Plant Biotechnology:* Principles and Applications Springer, 2017, 1-36.
12. Sridhar TM, Aswath CR. Review on medicinal plants propagation: A comprehensive study on the role of natural organic extracts in tissue culture medium. American Journal of Plant Sciences,2014:5(20):3073-3088.
13. Pande A, Dosad S, Chawla H, Arora S. In-vitro organogenesis and plant regeneration from seed-derived callus cultures of finger millet (Eleusine coracana). *Brazilian Journal of Botany*,2014:38:19-23.
14. Yemets AI, Bayer GY, Blume YB. An Effective Procedure for In Vitro Culture of Eleusine coracana (L.) and Its Application. *ISRN Botany*, 2013, e853121.
15. Kashyap A, Penak S, Saha A, Singh BR. In vitro Plant Development of Eleusine coracana via Indirect Organogenesis and Somatic Embryogenesis Using Mature Seeds as Explants. Current Science,2018:115:91-98. https://doi.org/10.18520/cs/v115/i1/91-98
16. SKOOG F, MILLER CO. Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symposia of the Society for Experimental Biology,1957:11:118-130.
17. MR, Caesar SA, Duraipandiyan V, Daniel M, Ignacimuthu S. Efficacious somatic embryogenesis and fertile plant recovery from shoot apex explants of onion (Allium cepa. L.). *In Vitro Cellular & Developmental Biology - Plant*, 2013, 49. https://doi.org/10.1007/s11627-013-9510-3.