



**SRI VENKATESWARA INTERNSHIP PROGRAM
FOR RESEARCH IN ACADEMICS
(SRI-VIPRA)**



SRI-VIPRA

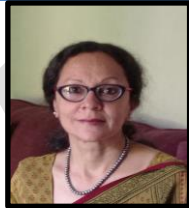

Project Report of 2023: SVP-2317

**“Micropropagation and Biotechnological Aspects in
Improvement in Millets”**


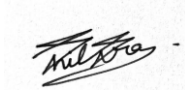

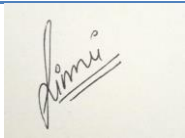

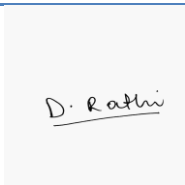
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SRIVIPRA PROJECT 2023

Title : Micropropagation and Biotechnological Aspects in Improvement in Millets

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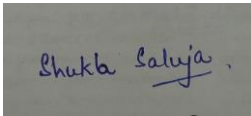
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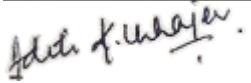
SRI-VIPRA

Certificate of Originality

This is to certify that the aforementioned students from Sri Venkateswara College have participated in the summer project SVP-2317 titled “Micropropagation and Biotechnological Aspects in Improvement in Millets”. The participants have carried out the research project work under my guidance and supervision from 15 June, 2023 to 15th September 2023. The work carried out is original and carried out in an online/offline/hybrid mode.



Shukla Satya,



Aditi K. Umajee.

Signature of Mentor

Acknowledgements

We would like to express our deepest gratitude to all those who have contributed to the successful completion of our Sri-Vipra internship project. Our deepest gratitude to Sri Venkateswara College for hosting the Sri-Vipra program and encouraging young students like us to gain research experience. We would like to thank our esteemed Principal, Prof. K.Chandramani Singh and Prof. C Sheela Reddy for granting us the opportunity to undertake this internship. Your vision and commitment to fostering experiential learning have been a source of inspiration. Our sincere gratitude to Prof. Shukla Saluja for her support and guidance through the course of this project. This journey has been a valuable learning experience, and we would like to extend our heartfelt thanks to our Mentor Dr. Aditi Kothari-Chhajer for their unwavering support, guidance, and mentorship throughout this internship. Her expertise, encouragement, and willingness to share their knowledge has been instrumental in our growth and development.

We would also like to extend our appreciation to the members of the Internship Committee for their dedication and diligence in overseeing and coordinating this internship program. Your efforts have provided students like us with a platform to apply classroom learning in real-world scenarios.

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Introduction

Climate change, water scarcity, natural calamities, increasing populations, inflating food prices, and many other bio-geo-economical aspects are expected to generate grave threat to agriculture and food security worldwide, especially for the poor underprivileged people who live in arid and sub arid regions. These global level issues present a challenge to researchers and scientists to investigate the possibilities of producing, processing, and utilizing other potential alternatives to each issue respectively. Cereal grains are the most important source of the world's food and have a significant role in the human diet. As one of the most important drought-resistant crops, millet is widely grown in the semiarid tropics of Africa and Asia and constitutes a major source of carbohydrates and proteins for people living in these areas. Millets belong to the botanical family Poaceae (Gramineae) and are cultivated primarily for their edible starchy seeds. The term 'millet,' or alternatively, 'minor cereals,' refers to cereals and forage grasses characterized by small-sized seeds, which are useful for both human and animal nutrition as well as forage production. In Western nations, they are primarily employed for avian sustenance. Although millets have begun gaining popularity in North America and Europe as well in recent years, their significance lies in their capacity to thrive in impoverished soils with minimal agronomic inputs, making them a great source of income and food for resource-constrained farmers dwelling in regions with adverse soil and environment conditions.

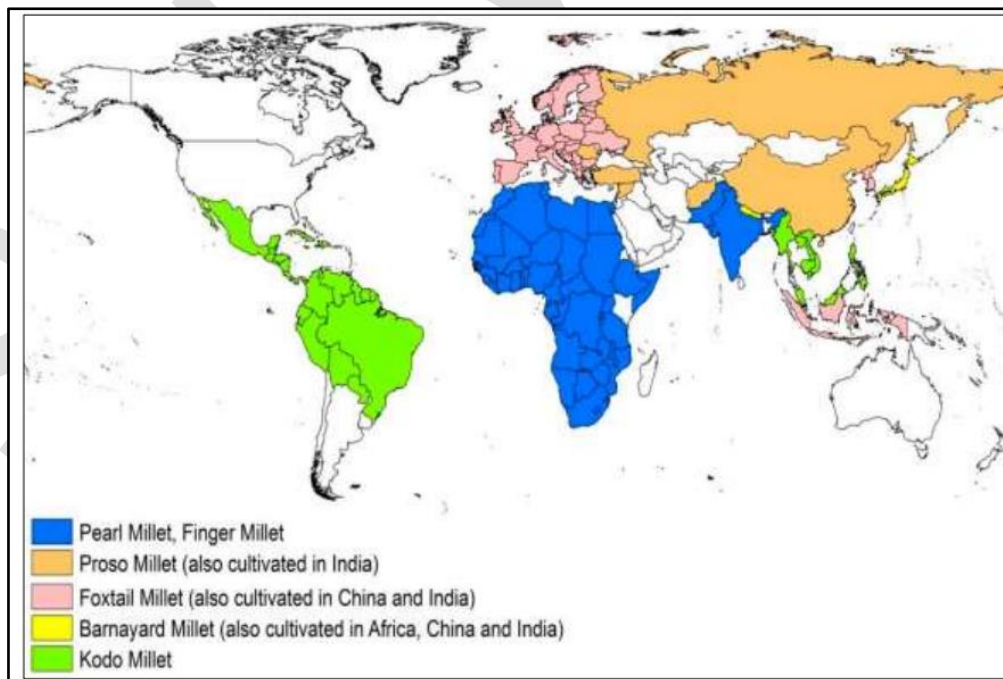


Figure 1: Global Millet Cultivation Scenario (Naresh *et.al.*,2023)

Millets are gaining popularity in recent years due to their nutritional value and ease of cultivation. The year 2023 was termed as “International Year of Millets” by the United Nations Assembly. This brought on more attention than ever to the wide variety of benefits and uses of the millets. Millets are highly nutritious and are regarded to be five times more nutritious than rice and wheat. They are especially rich in dietary fibers, antioxidants, phytochemicals and polyphenols, which contribute broad-spectrum positive impacts to human health. They have high Protein content, Triacylglycerols, Vitamin E, essential amino acids, like leucine, arginine, lysine, and tyrosine Calcium, Low glycemic index, also some species are rich in K, P, Mg, Ca, Na, Zn, and Fe. The demand and consumption of millets have increased in recent times due to awareness created among the public about their enormous health benefits.

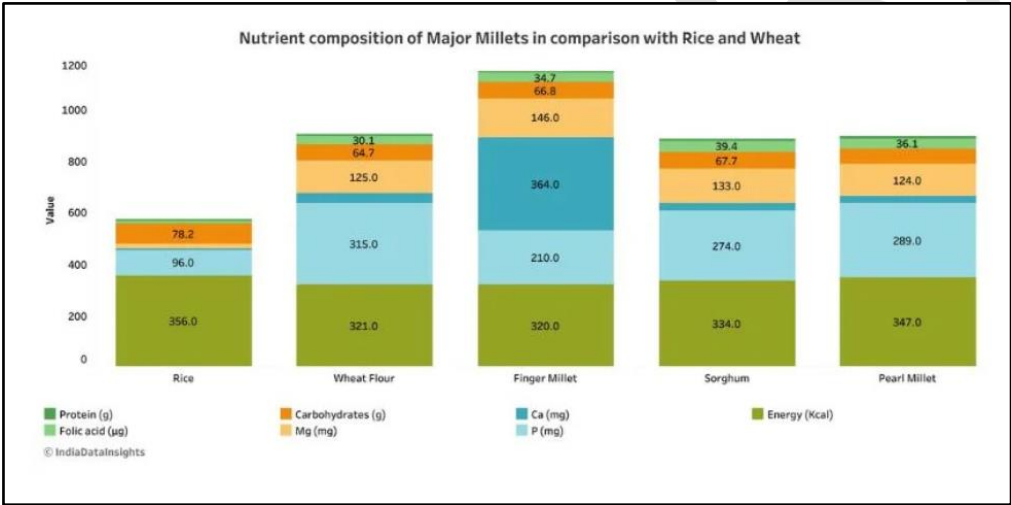


Figure 2: Nutrient composition of millets as compared to major cereals (<https://idronline.org/article/agriculture/millet-cultivation-history-and-trends/>)

Millets are being used in various industries apart from food grains, such as the edible oil industry, pharmaceutical industry, and agriculture industry. Cereal oils, including millet oil, are being considered as highly unsaturated oil with the presence of essential fatty acids necessary for human health. Therefore, cereal oils could be commercialized in small quantities. The health benefits of millets are also vast. Phytochemical-rich fractions from foxtail millet seeds have been shown to exhibit antioxidant activity and reduce the viability of breast cancer cells *in vitro* by inducing DNA fragmentation and promoting cell cycle arrest. In addition, because of their important contribution to national food security and potential health benefits, millet grain is now receiving increasing interest from food scientists, biotechnologists, and

nutritionists. They are flexible for growing in diverse conditions, tolerate wide range of soil conditions, are resistant to encroachment of weed, drought tolerant, resistant to salinity, osmotic stress tolerant .

Millets include 10 Genera and at least 14 species. Most of the species have similar nutrient content and have many medicinal and industrial uses.

Table 1. Millet species

| <u>Species</u> | <u>Common Name</u> |
|---------------------------------|--------------------------|
| <i>Brachiaria ramosa</i> | Browntop millet |
| <i>Coix lachryma jobi</i> | Job's tears |
| <i>Digitaria exilis</i> | Hungry rice |
| <i>Digitaria ibura</i> | Fonio or Hungry rice |
| <i>Echinochloa colona</i> | Jungle rice |
| <i>Echinochloa decompositum</i> | Australian millet |
| <i>Echinochloa frumentacea</i> | Japanese barnyard millet |
| <i>Eleusine coracana</i> | Finger millet |
| <i>Eragrostis teff</i> | Teff |
| <i>Panicum miliaceum</i> | Proso millet |
| <i>Panicum miliare</i> | Little millet |
| <i>Paspalum notatum</i> | Bahia grass |
| <i>Paspalum scrobiculatum</i> | Kodo millet |
| <i>Pennisetum glaucum</i> | Pearl millet |
| <i>Setaria italica</i> | Foxtail millet |

The goal of this project is to provide a comprehensive overview of the current state of research in using advanced techniques like tissue culture and biotechnology to improve millet crops. We aim to analyze the existing knowledge and breakthroughs in this field in the past decade, helping to understand the latest trends and challenges. By studying various scientific studies, we want to highlight how these innovative methods can benefit millet farming. This project also explores their potential impact on global food security, sustainable farming and changing climatic conditions. Ultimately, our review aims to be a valuable resource for researchers, policymakers, and anyone interested in millet research, offering insights to guide future studies and agricultural practices.

Material and Methods

Work done on different millets has been reviewed in the current project. Various online tools and software have been used to comprehensively study the existing research data base. The following crops were studied in the current project.

A. Brachiaria ramosa

Botanical name - *Brachiaria ramosa*

Common name - Browntop millet

Family - Poaceae

Origin of cultivation - First domestication of brown top millet probably occurred within the Deccan of south India and it had reached to other parts of India during prehistory period is native to South Asia and is traditionally cultivated as a cereal crop.

Morphological Characteristics - *Brachiaria ramosa* is a tufted perennial grass that is native to tropical and subtropical regions with an erect or ascending growth habit. It can reach a height of 30-150 cm. The leaves of *Brachiaria ramosa* are linear, flat, and glabrous. They are typically 10-30 cm long and 3-10 mm wide.

The inflorescence of *Brachiaria ramosa* is a panicle. The panicle is usually open and spreading, with branches that are 5-20 cm long. The spikelets of *Brachiaria ramosa* are small and arranged in pairs along the branches of the panicle. Each spikelet contains several florets.

The seeds of *Brachiaria ramosa* are small and light brown in color. They are typically 1.5-2.5 mm long and have a narrow shape.

B. Coix lacryma-jobi

Botanical name - *Coix lacryma-jobi*

Common name - Job's Tears/Adlay

Family - Poaceae

Origin of cultivation - Job's tears is native to Southeast Asia and has been cultivated in China, Japan, and Korea for over 2,000 years. It has been used for both food and cultural purposes.

Morphological characteristics - *Coix lacryma-jobi* has hard, bead-like seeds with a diameter of 5-10 mm. Its leaves are long and narrow. Its stem can have anthocyanin coloration, and the wings of the lower glume of the male spikelets can vary in width. The flowers are small and inconspicuous. Its pollen grains are spherical and have the highest vigor at the peak flowering stage.

C. Digitaria exilis

Botanical name - *Digitaria exilis*

Common name - Hungry Rice/Fonio

Family - Poaceae

Origin of Cultivation - The origin of the cultivation of *Digitaria exilis*, also known as fonio, can be traced back to West Africa. It has been cultivated for centuries in countries such as Senegal, Mali, Guinea, and Nigeria. It is considered one of the oldest cultivated grains in Africa.

Morphological characteristics - *Digitaria exilis*, or fonio, is a small-grained cereal crop that is indigenous to West Africa. The plant has a height of about 30-60 cm and has a slender stem with long, narrow leaves. The inflorescence of the plant is a panicle that is 10-30 cm long and has numerous spikelets. The spikelets are arranged in pairs along the branches of the panicle and are about 2-3 mm long. The grains of fonio are small and oblong, measuring about 1.5-2.5 mm in length and 1-1.5 mm in width. The grains of fonio are small and have a yellowish color.

D. Digitaria iburua

Botanical name- *Digitaria iburua*

Common name - Fonio or Hungry rice

Family- Poaceae

Origin of Cultivation - The crop originated in present-day Guinea and Mali (Plateau State, Nigeria).

Morphological characteristics- The plant is an annual with upright stems that range from 45 to 140 cm in height and have 4-5 nodes. Its leaf-blades measure between 15-30 cm in length and are 2-5 mm wide. The inflorescence consists of racemes, typically numbering 4-10, which are digitate and arranged unilaterally. These racemes are 10-15 cm long and have a narrowly winged and angular rachis. Spikelets are either grouped in threes or clustered at each node along the raceme. Fertile spikelets are pedicelled and occur in clusters of 3-5, with unequal pedicels that are pubescent and hairy at the tip. Each spikelet contains 1 basal sterile floret and 1 fertile floret without a rhachilla extension. The spikelets themselves are elliptic or oblong in shape, dorsally compressed, acute at the apex, and measure approximately 2 mm in length. Notably, these spikelets persist on the plant.

E. Echinochloa colona

Scientific name- *Echinochloa colona*

Common name- Jungle Rice

Family- Poaceae

Origin of Cultivation- Jungle rice originated from India but it is now widespread in the tropics and subtropics, sometimes extending beyond 30 °N and 30°S.

Morphological characteristics-

Mature junglerice plants are prostrate or erect and range from about 2 to 3 feet (60-90 cm) in length or height. The species is highly variable. Stems are hairless except for hairs at the stem joints (nodes). Leaves are grayish or dull green and sometimes have banding. Leaves are rolled in the bud. Leaf blades are flat and usually have a hairless upper surface. They are about 1-1/5 to 8 inches (3-22 cm) long and often have purplish banding. These purple bands are what distinguish junglerice from similar looking barnyardgrass, dallisgrass and crabgrass. There are no ligules or auricles. The flower heads are about 1-1/5 to 6 inches (3-15 cm) long, with short, compact branches. It reproduces by seed. Seedling leaves resemble those of the mature plant.

F. Echinochloa frumentacea

Botanical Name: *Echinochloa frumentacea*

Common Name: Barnyard millet

Family: Poaceae

Origin of Cultivation : Japanese barnyard millet was likely domesticated in

Japan or Eastern Asia (Yabuno, 1962). *Echinochloa frumentacea* (syn. *Echinochloa colona*) is found in Pakistan, India, Nepal, and Central Africa. *E. frumentacea* (barnyard millet) is grown in tropical regions including India and Central Africa. It is grown in India from Himalayan region in the north to the Deccan plateau in the south.

Morphological Characteristics : *Echinochloa frumentacea* is an annual plant growing to 1.8 m (6ft) by 0.2 m (0ft 8in). It is in flower from July to September, and the seeds ripen from September to October. The species is hermaphrodite (has both male and female organs) and is pollinated by Wind.

G. Eleusine coracana

Botanical Name: *Eleusine coracana*

Common Name: Finger millet

Family: Poaceae

Origin of Cultivation : Finger millet was domesticated in western Uganda and the Ethiopian highlands (Figure 2) at least 5000 years ago before introduction to India approximately 3000 years ago (Dida *et.al.*, 2008, 2014). Finger millet is predicted to have been domesticated in Uganda and the Ethiopian Highlands (Dida *et.al.*, 2008).

Morphological Characteristics : Finger millet is a robust, tufted, tillering annual grass, up to 170 cm high, with erect, slender stems rooting at the lower nodes. The shallow root system is fibrous and strong, difficult to pull out. Stems and leaves (up to 75 cm long and 2 cm broad) are usually green. The inflorescence is a panicle with 4-19 finger-like spikes that resembles a fist when mature, hence the name finger millet. The spikes bear up to 70 alternate spikelets, carrying 4 to 7 small seeds.

H. Eragrostis teff

Botanical Name: *Eragrostis teff*

Common Name: Teff

Family: Poaceae

Origin of Cultivation : Tef cultivation as a cereal food grain is restricted to Ethiopia, except in very small quantities in Eritrea and recently, in the USA, the Netherlands and Israel. However, the plant is known

elsewhere in the world such as in South Africa, India, Pakistan, Uganda, Kenya and Mozambique mainly as a forage or pasture crop.

Morphological Characteristics: Morphologically, the tef plant root system is thin and fibrous (thread-like) rarely emerging from nodes above the base, and growing 4–8 cm deep under field conditions (Ebba, 1969). The stems are mostly erect (ascending), but creeping or bending or elbowing (geniculate) in some cultivars, and jointed with hollow internodes separated by nodes. Each spikelet bears a pair of unequal sized glumes at the base and a number of florets (3–17) above. Each floret in turn comprises a tri-nerved lemma, a two-nerved bow- or boat-shaped palea, three stamens (arising from the ovary base and having very fine and slender filaments bearing length-wise opening anthers at the apex), and an ovary or a pistil. The ovary consists of two or, in a few exceptional cases; three styles each ending in a plumose (feathery) yellowish white stigma.

I. *Panicum miliaceum*

Botanical Name: *Panicum miliaceum*

Common Name: Proso millet, Broomcorn millet

Family: Poaceae

Origin of Cultivation :

The homeland of cultivation of proso millet (*Panicum miliaceum*) is considered to be China, from where it spread to Central, East and South-West Asia, Transcaucasia. Broomcorn millet (*Panicum miliaceum* L.) is one of the earliest domesticated crops in the world. Weedy broomcorn millet [*Panicum ruderalis* (Kitag.) Chang or *Panicum miliaceum* subsp. *ruderalis* (Kitag.) Tzvel] is thought to be the descendant of the wild ancestor or the feral type of this cereal. (Chumxiang *et.al.*, 2021).

Morphological Characteristics :

Proso millet is an erect annual grass up to 3-4 ft tall, usually free-tillering and tufted, with a rather shallow root system. Its stems are cylindrical, simple or sparingly branched, with simple alternate and hairy leaves. The inflorescence is a slender panicle with solitary spikelets. Its compact panicle droops at the top like an old broom, hence the name broom corn. Its round seeds are about 1/8 inch wide and covered by a smooth, glossy hull. Seeds may be cream, yellow, orange-red, or brown in colour.

J. *Panicum miliare*

Botanical Name: *Panicum miliare*

Common Name: Little millet

Family: Poaceae

Origin of Cultivation: It was domesticated in India (De Wet *et al.*, 1983a). It is important minor millet in low and moderate-rainfall areas. In India, this millet is grown primarily in the drought-prone arid and semi-arid zones and used mostly for food purposes especially by people of economically weaker sectors. Besides India, it is cultivated in Nepal, Western Burma, and also in some African countries.

Morphological Characteristics: There are two races of little millet (*Panicum miliare*), namely, Nana and Robusta (House *et al.*, 2000). Plants in race nana vary from 60 to 170 cm in height. The inflorescence is 14–15 cm long, erect, open, and highly branched. These branches sometimes droop at maturity. Plants in the race Robusta are 120–190 cm tall. The inflorescence is 20–45 cm long, opening compact, and highly branched.

K. Paspalum notatum

Botanical Name : *Paspalum notatum*

Common Name : Bahiagrass

Family : Poaceae

Origin of Cultivation: *Paspalum notatum* Flüge (bahiagrass) stands out as forage, which is one of the most important native pasture species of southern Brazil, Uruguay, northeastern Argentina, and southern Paraguay (Quarin *et.al.*, 1984). This grass is the main herbal product of most grasslands of Rio Grande do Sul (Nabinger and Dall’Agnol, 2008), and tends to expand gradually for being resistant to trampling and growth stimulation by grazing (Weiler *et.al.*, 2015).

Morphological Characteristics: *Paspalum notatum* is a perennial rhizomatous grass, up to 1 m high, notable for its prominent, dual V-shaped inflorescences (6 cm long). Its root system can be up to 2 m deep. Several commercial cultivars are available. It is mostly used for pasture. The spikelets occur in two rows and are two-flowered, 2.7-4 mm long, hairless, elliptical in shape and shiny. Lower glume is absent, and the upper glume is as long as the spikelet, and similar in texture to the lower lemma.

L. Paspalum scrobiculatum

Botanical name : *Paspalum scrobiculatum*

Common Name : Kodo Millet

Family : Poaceae

Origin of Cultivation: Kodo millet is indigenous to the Indian subcontinent and known to be in cultivation since time immemorial, and is mentioned in Brihad Sanhita. It has originated in tropical Africa, and was first introduced and domesticated in India (Malleshi and Hadimani, 1994). The crop is believed to be domesticated in southern Rajasthan and Maharashtra states of India some 3000 years ago (Kajale, 1977; Vishnu-Mittre, 1977).

Morphological Characteristics: Plants are slender to stout, up to 90 cm tall, and often root from the lower nodes. Leaf blades are linear, glabrous or pubescent, up to 40 cm long, with the basal leaf sheaths glabrous or pilose. Inflorescences are composed of rarely more than 5 racemes (3-7) that are alternately arranged on a short to elongated primary axis.

M. *Pennisetum glaucum*

Botanical Name: *Pennisetum glaucum*

Common Name: Pearl Millet

Family: Poaceae

Origin of Cultivation : Archaeological evidence indicates the use of *P. glaucum* as a wild cereal along northern Ghana and the south-western fringes of the Sahara as far back as 3000 years. Race *typhoides* could be the oldest form of cultivated pearl millet (de Wet *et.al.*, 1992) and also the progenitor of West African cultivated races that probably reached India about 2500 years ago (Rao *et.al.*, 1963).

Morphological Characteristics : Pearl millet is an erect grass that has a summer annual cycle of between 75 and 120 days depending on environmental conditions. Usually, it is of fast growth and reaches an average height of 1.5 to 3 m. The plant develops compact cylindrical panicles that are 2 to 3 cm wide and 15 to 60 cm long, capable of producing between 500 and 2000 seeds per panicle (Durães *et.al.*, 2003; Taylor, 2016). Its seeds are oval shaped, similar to a pearl, from which it gets its name. The grains are 3 to 4 mm long.

N. *Setaria italica*

Botanical Name : *Setaria italica*

Common Name : Foxtail Millet

Family : Poaceae

Origin of Cultivation : It is an ancient crop of south eastern Europe and China. Its ancestor *S. viridis* could have been taken into cultivation at Eurasian range.

Morphological Characteristics : Characteristically a small, erect or decumbent plant up to 1.5 m tall, with branched culms. Leaf -blades are linear-lanceolate, up to 29 cm long but usually shorter, and glabrous. Panicles are densely flowered, spike-like in appearance, erect or slightly nodding at maturity, and usually less than 10 cm long. Branches of the panicle are usually less than 1 cm long, bearing a cluster of several spikelets, each subtended by one to three bristles that exceed the 2.0-2.5 mm long spikelets in length.

Methodology for Micropropagation Studies

Plant material and explant preparation: The explant material was collected and surface sterilized. The explants underwent a thorough washing procedure using tap water. After that, the explants underwent three water rinses before undergoing a washing step using a 20% tween 20 solution. Afterward, the explants were immersed in 70% alcohol for a duration of 30 seconds. Subsequently, they were washed using a 0.1% solution of mercuric chloride (HgCl_2) for a duration of 30 seconds. This was followed by three consecutive rinses with distilled water.

MS basal medium (Murashige and Skoog) was prepared with 3% sucrose. 100 μL of 2,4-D and 100 μL of BAP were supplemented to the two batches of medium. pH was adjusted to 5.8 before addition of agar (0.8%, g/l) to the medium. The medium along was autoclaved at 121 psi for 20 min. After autoclaving, about 40 ml of medium was poured in each sterilized conical flask in laminar air flow and the medium was left for solidification. After solidification of the MS medium, the surface sterilized explant was inoculated in the conical flask. The cultured flasks were kept in growth chamber under controlled photoperiod (16/8 hour), temperature and humidity conditions.



Labeling Flask



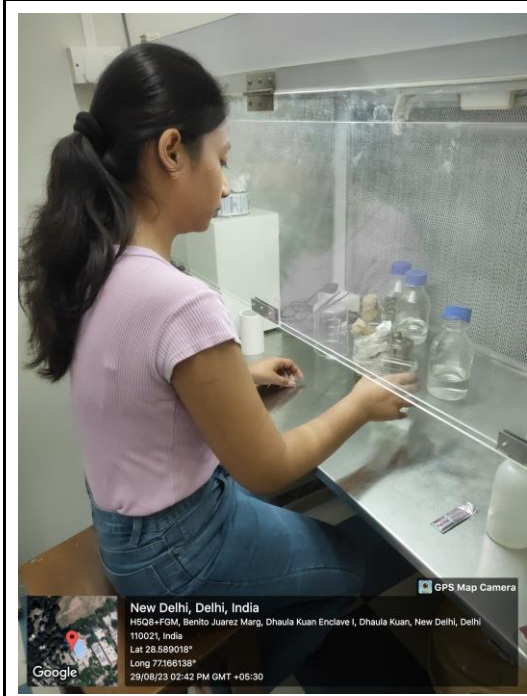
Media Preparation



Pouring of Medium



Pouring of Medium



Sterilization of explant material



Culturing of Explant on Sterile media



Culturing Explant material



Culture Flask

Literature Review

The study involved by collecting research papers and review articles from authors worldwide using different databases. The research and review data was collected over the period of 2007-2023. Data on micropropagation, genetic modifications and improvements in different species of millets.

Results and Discussion

In our micropropagation experiment, we were able to successfully regenerate new tissues from the cultured explants material. The studies are still underway.

Along with this, our literature review also yielded impressive results. In the last decade sophisticated research has been done either to develop a more efficient protocol for *in vitro* regeneration or to study the effect of various factors on somatic embryogenesis or to study inheritance of *in vitro* characters. V Satyavathi *et.al.* in 2016 studied the reciprocal differences of *in vitro* characters in pearl millet which arise due to unequal contribution of cytoplasmic determinants from male and female gametes to zygote. Recently, Khan *et.al.*, in 2022 developed an efficient *in vitro* regeneration and multiplication protocol to study effects of different hormonal concentrations through somatic embryogenesis of pearl millet. *In vitro* regeneration has been done of foxtail millets to study the effects of cefotaxime, amino acids and carbon source on somatic regeneration of foxtail millet (Satish.L *et.al.*, 2015). A study on the effect of activated charcoal in developing an efficient protocol for micropropagation of *Setaria italica* was reported by Rathinapriya.P *et.al.*, 2018.

Numerous other studies have been conducted in the field, such as the investigation titled "Optimization of various growth regulator doses for *in vitro* regeneration of *Echinochloa frumentacea* from Caryopsis" (Dosadet *et.al.*, 2015.). In a study conducted by Anju *et.al.*, 2016 various concentrations of 2,4-D in conjunction with 0.5 mg/l BAP or kinetin were examined to assess their effectiveness in inducing callus formation in different finger millet cultivars. The study also aimed to establish an efficient plant regeneration strategy for finger millet (*Eleusine coracana*) by somatic embryogenesis. A recent investigation conducted in 2021 revealed that the differential response observed *in vitro* is influenced by the genotype and the endogenous phytonutrient content. The study found significant variability among the tef genotypes in terms of the number of internodes per plant, culm length, and plant height. However, there were no apparent differences between plants generated from seeds and those obtained through *in vitro* methods with regards to these three traits (Plaza-Wüthrich *et.al.*, 2015).

For *Coix Lacryma-jobi* multiple studies have been done in the field of micropropagation. YuYang *et.al.*, in 2012 through multiple experiments concluded that Kinetin is a major factor affecting the differentiation of the *C. lacryma-jobi* spears and generation of virus-free plantlets. They also reported that aerial root was the best explant for inducing embryonic callus and embryoid. In a recent report, Bernardo *et.al.* in 2020, reported that the most effective double sterilization procedure for *C. lacryma-jobi* is using 1% NaOCl (v/v) for 15 minutes. Longest shoot growth was obtained using 1 ppm 2,4-D and BAP. Ash additives had a growth-promotive effect. Li *et.al.*, in 2021 published the first report on the morphology, vigor, optimal germination conditions for pollen microspores and anther culture of *C. lacryma-jobi*. The optimal conditions for pollen germination was 10~15% sucrose + 0.01% boric solution at 25 for 5~7 hrs. Although the callus induction rates from anther culture were in significant difference among different *Coix* cv, calluses could be successfully induced from all the 7 cv in the medium of MS+2, 4-D 1~2 mg/L+KT 1.5 mg/L, and some of which could be induced to short cluster buds in the medium of MS+IAA 0.5 mg/L+KT 2 mg/L.

Ntui *et.al.* in 2010 reported the development of a plant regeneration protocol for *Digitaria exilis*. Multiple mediums were used and the best result was obtained on Murashige and Skoog (MS) medium supplemented with 2 mg l⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) and 1 g l⁻¹ casamino acid. Weiler *et.al* in 2014 used the plant material from a commercial seed lot of *Paspalum notatum* var. sauræ (cultivar Pensacola). For chromosome duplication in seedlings, the seeds were deglumed and germinated on sterile petri dishes. XiaoQing *et.al.* in 2012 used the mature seed of *Paspalum notatum* Flugge. cv. Taiyanghua as the explant material. The dry-and-loose pellet callus were induced in the subculture medium with 16 g/L agar, and the wet-and-sticky pellet callus in 8 g/L agar. The two types of pellet callus were salt-stressed for 30 d. Eight pairs were associated with the salt-tolerance of somatic mutants, and two pairs, WXE-30 and WXE-246, were suitable for identification of the mutant with salt tolerant in *Paspalum notatum*.

The inclusion of these exemplary papers greatly contributed to the successful execution of our micropropagation experiment. We also noticed that millets have received lesser research attention universally, this emphasizes the need for research on deciphering nutritional traits present in millets and to develop strategies for biotechnological genetic advancements in millets.

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