

Effects of Different Hormonal Concentrations on In vitro Regeneration and Multiplication of Pearl Millet (*Pennisetum glaucum* L.)

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Article Received 07-09-2022, Article Revised 24-10-2022, Article Accepted 26-10-2022

Abstract:

An efficient *in-vitro* regeneration and multiplication protocol was developed to check the effects of different hormonal concentrations through somatic embryogenesis of Pearl millet (*Pennisetum glaucum* L.). The explants (seeds) of *P. glaucum* were surface sterilized with different concentrations (30, 50 and 70%) of Sodium hypochlorite to ensure the removal of surface contamination. For the regeneration the explants were inoculated on MS media supplemented with varying concentrations of indole acetic acid (IAA 2 mg/L, 0.75 mg/L, 0.1 mg/L, 0.2 mg/L and 0.3 mg/L) and kinetin (KIN 1.5 mg/L, 0.5 mg/L, 0.5 mg/L, 1 mg/L and 1.5mg/L) respectively. The maximum germination (87.5 %) of explants was obtained by using 70 % Chlorox and minimum germination (62.5 %) of explants was obtained by using 30% Chlorox. The maximum stem length (15.5 cm), roots number (12), roots length (3.96 cm) and leaves length (6.76 cm) was observed on MS medium containing (IAA 2 mg/L and KIN 1.5 mg/L). The maximum leaves number (7) was observed on MS medium containing (IAA 0.75 mg/L and KIN 0.5 mg/L). The minimum stem length (3 cm), roots number (1), roots length (1 cm), leaves number (1) and leaves length (3 cm) was observed on MS medium supplemented with (IAA 0.2 mg/L and KIN 1 mg/L). The maximum Stem length (15.5cm), Roots number (12), Roots length (3.96 cm) and Leaves length (6.76 cm) was observed on MS medium supplemented with (IAA 2 mg/L and KIN 1.5 mg/L) although maximum leaves number was observed on MS medium supplemented with (IAA 0.75 mg/L and KIN 0.5 mg/L). The study revealed an easy and reproducible *in-vitro* regeneration protocol of pearl millet that can provide an efficient plant regeneration which can be further exploited for transgenic applications.

Keywords: Hormones, Peral millet, Regeneration, In-vitro

Introduction

Pearl millet (*Pennisetum glaucum* L.) locally known as “Bajra” is a nutritious course grain cereal. Globally, it is grown on an area of 38.6 million ha with annual production of 29.4 million tons (FAO, 2020). In Pakistan, it is annually grown on an area of 0.35 million hectare with grain production of 0.26 million tons and a yield of 563 kg ha⁻¹ (FAO, 2020). Pearl millet is a very drought resistant crop. Due to its resistance it can grow under extreme environmental condition and therefore, grown in those areas which have insufficient moisture and poor soil fertility (Baker, 2003). Pearl millet is one of the most important cereal crops world-wide. It is a staple food crop for about 90 million people living in the semi-arid tropical regions of Africa and the Indian subcontinent (Gulia *et al.*, 2007). There are approximately 140 species of the genus *Pennisetum* L. It belongs to family Poaceae (Haroun, 2010). Pearl millet is rich in proteins, vitamins, starch, minerals and fibers. Because of its high nutritive values, it is recommended for diabetic and cardiac patients. It is use as a source of bio fuel (Dereje *et al.*, 2012). It helps in lowering the cholesterol level, reducing weight and reducing hunger.

The major problem affecting crops including millets in Asia and Africa are diseases (Bashir *et al.*, 2020). Pearl millet is highly susceptible to Downy mildew disease caused by the fungus *Sclerospora germinicola*, which can cause up to 70% reduction in yield (Arockiasamy *et al.*, 2001). The main insect pest of millet is Chinch bugs. It can damage pearl millet anytime at its growth stage. A reproducible regeneration procedure is essential in studies involving gene transfer for the genetic improvement of this crop plant (Vasil, 1990).

Tissue culture is an important area of biotechnology that can be used to improve the productivity of plants. It enhances availability of plant stock with desired traits. The concept of tissue culture was first given by Haberlandt in “1902”. Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial prepared nutrient medium static or liquid under aseptic conditions often to produce the clones of the plants. The resultant clones are true to type of the selected genotype. The technique depends mainly on the concept of totipotency of plant cells which refers to the ability of a single cell to develop into whole plant. Micropropagation technique

provides a rapid reliable system for a production of large number of genetically uniform disease free plantlets under in-vitro condition. Micropropagation procedures for multiplication of desired or selected genotypes of *P. glaucum* L. are available (Arockiasamy et al., 2006; Oldach et al., 2001). The optimization of regeneration method is, therefore, necessary for different millet types in order to increase the efficiency of transformation. Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial prepared nutrient medium static or liquid under aseptic conditions often to produce the clones of the plants. The resultant clones are true to type of the selected genotype. The technique depends mainly on the concept of totipotency of plant cells which refers to the ability of a single cell to develop into whole plant. The present study was aimed to optimize the standard protocol for the micro-propagation of pearl millet in *in-vitro* conditions.

Materials and Methods

Optimization of culture condition and growth room:

The optimum conditions i.e. 25±2 °C temperature, 16-18 hours of photoperiod was maintained. For sterilization UV was kept on for 15-20 minutes followed by surface sterilization of explant using chlorox with different concentrations.

Plant material and MS media preparation: Healthy seeds of Pearl millet (*P. glaucum* L.) were obtained from local market in Muzaffarabad on the basis of their morphological features (Figure 1). Optimum temperature conditions and white light 7000 lux were maintained.



Figure 1. Hybrid seed of Pearl millet (*P. glaucum* L.) used for the study.

For the preparation of MS media measured concentration of IAA, kinetin stocks solutions, and 30g sucrose was added and pH of solution was maintained at 5.7- 5.8. Then for solidification of media 6g agar was added

followed by addition of one liter distilled water. Then media was boiled and poured into the test tubes and sterilized using autoclave.

Sterilization of Seeds: Surface sterilization of all the glassware and surgical instruments was done by exposing them to UV light for 15-20 min to avoid bacterial and fungal contamination. Then surface sterilization of explants was done by washing with 30, 50 and 70% sodium hypochlorite thrice and then with distilled to ensure removal of contamination.

Culturing of explants for regeneration: For organogenesis seeds were used as explants were cultured on MS medium. By changing concentrations of hormones 5 types of media were prepared;

1. IAA= 2 mg/L, Kinetin= 1.5 mg/L
2. IAA= 0.75 mg/L, Kinetin= 0.5 mg/L
3. IAA= 0.1 mg/L, Kinetin= 0.5 mg/L
4. IAA= 0.2 mg/L, Kinetin= 1mg/L
5. IAA= 0.3 mg/L, Kinetin= 1.5 mg/L

All the concentrations were used with three replications for each medium and were maintained in growth room at 25±2 °C with 16-18 hours of photoperiod.

Data collection: Data was collected after every 24 hours. Contaminated plants were removed and data has been compiled by noting different parameters i.e. regeneration experiment, number of healthy and contaminated explants, mean, standard deviation and percentages of germination. Also different parameters viz. stem length, number of roots, root length, number of leaves, and leaves length was recorded.

Results

Percentage of germination: The study findings revealed that the maximum germination (87.5%) of explants was obtained by using 70% Chlorox and minimum germination (62.5%) of explants was obtained by using 30% Chlorox (Table 1).

Regeneration parameters: The study was carried out to analyze the effect of different media supplemented with varying concentration of growth regulators on regeneration of pearl millet (*P. glaucum* L.) through *in vitro* culture. It is recognized that different concentration of hormones that are present in the media results in differential response of the explants (seeds) (Figure 2). The MS media is supplemented with different concentration of hormone that was kinetin (KIN) and indole acetic acid (IAA). The germination of inoculated explants was different at different media.

Table 1. Germination % age of Pearl millet (*Pennisetum glaucum* L.).

Sterilization % age	Total seeds	Seedling	Contaminated	Germination % age
Chlorox 30 %	8	5	3	62.5 %
Chlorox 50 %	8	6	2	75 %
Chlorox 70 %	8	7	1	87.5 %



Figure 2. Different stages of Pearl millet (*Pennisetum glaucum* L.) regenerations in different media.

The maximum stem length (15.5 cm) was recorded on MS medium supplemented with IAA 2 mg/L and KIN 1.5 mg/L while minimum (3 cm) at the medium that contained IAA 0.2 mg/L and KIN 1 mg/L (Figure 3). Similarly the maximum roots number (12) was observed on MS medium supplemented with IAA 2 mg /L and KIN 1.5 mg/L whereas minimum (1) at the medium that contained IAA 0.2 mg/L and KIN 1 mg/L (Figure 4). Furthermore, the maximum roots length (3.96 cm) was recorded on MS medium supplemented with IAA 2 mg/L

and KIN 1.5 mg/L while minimum (1 cm) at the medium that contained IAA 0.2 mg/L and KIN 1 mg/L (Figure 5). The maximum leaves number (7) was observed on MS medium supplemented with IAA 0.75 mg/L and KIN 0.5 mg/L and minimum (1) at the medium that contained IAA 0.2 mg/L and KIN 1 mg/L (Figure 6). The maximum leaves length (6.76 cm) was observed on MS medium supplemented with IAA 2 mg/L and KIN 1.5 mg/L and minimum (3 cm) at the medium that contained IAA 0.2 mg/L and KIN 1 mg/L (Figure 7).

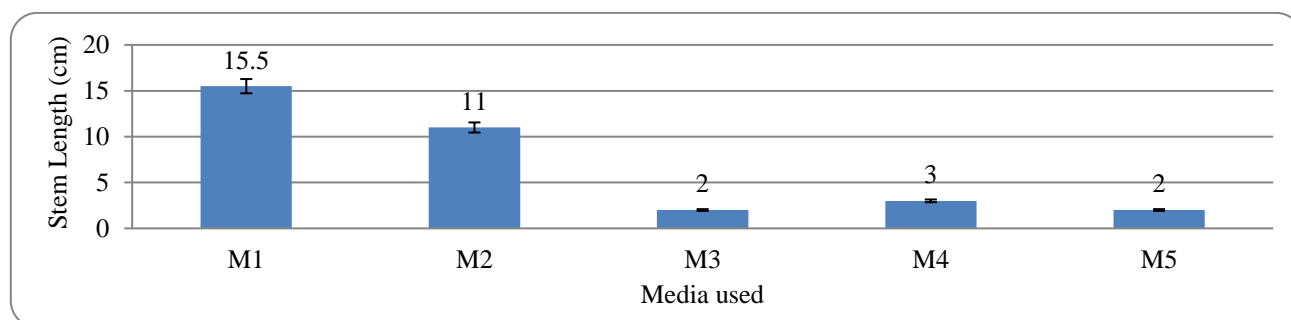


Figure 3. Effect of different media on regeneration of stem length (cm).

Media 1 IAA=2 KIN=1.5 (mg/L); **Media 2** IAA=0.75 KIN=0.5 (mg/L); **Media 3** IAA=0.1 KIN=0.5 (mg/L); **Media 4** IAA=0.2 KIN=1 (mg/L); **Media 5** IAA=0.3 KIN=1.5 (mg/L)

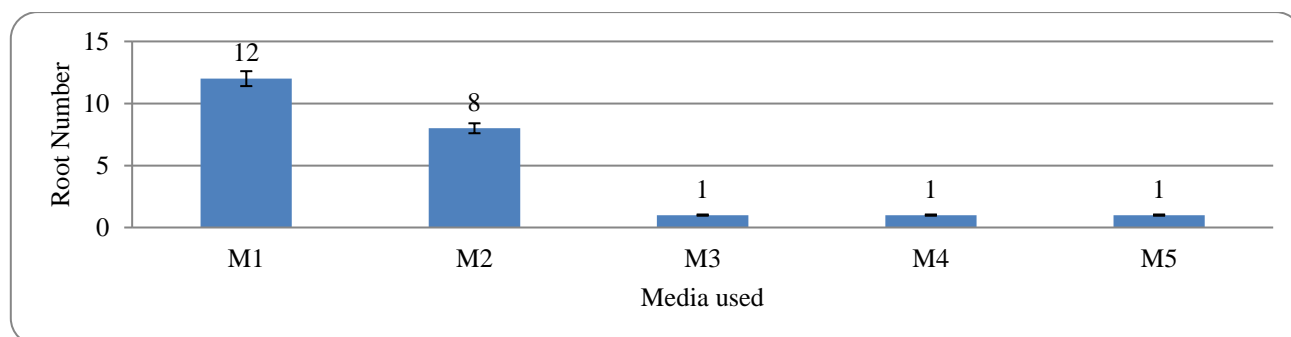


Figure 4. Effect of different media on regeneration of roots number.

Media 1 IAA=2 KIN=1.5 (mg/L); **Media 2** IAA=0.75 KIN=0.5 (mg/L); **Media 3** IAA=0.1 KIN=0.5 (mg/L); **Media 4** IAA=0.2 KIN=1 (mg/L); **Media 5** IAA=0.3 KIN=1.5 (mg/L)

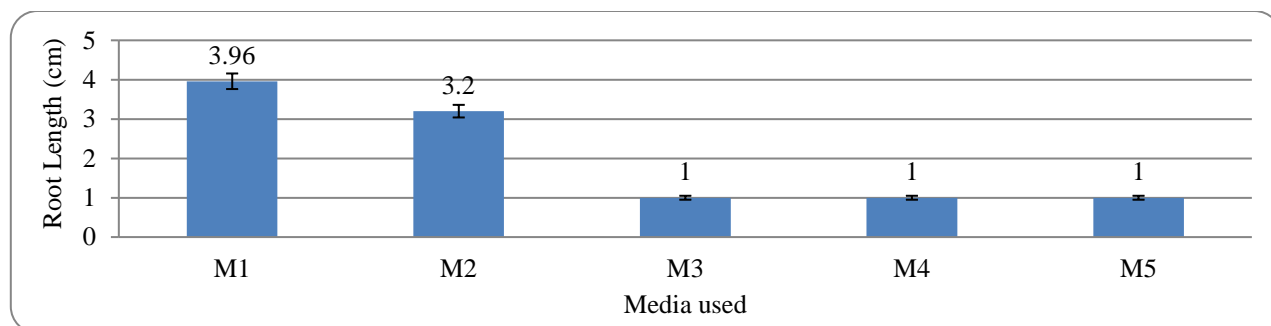


Figure 5. Effect of different mediums on regeneration of root length (cm).

Media 1 IAA=2 KIN=1.5 (mg/L); **Media 2** IAA=0.75 KIN=0.5 (mg/L); **Media 3** IAA=0.1 KIN=0.5 (mg/L); **Media 4** IAA=0.2 KIN=1 (mg/L); **Media 5** IAA=0.3 KIN=1.5 (mg/L)

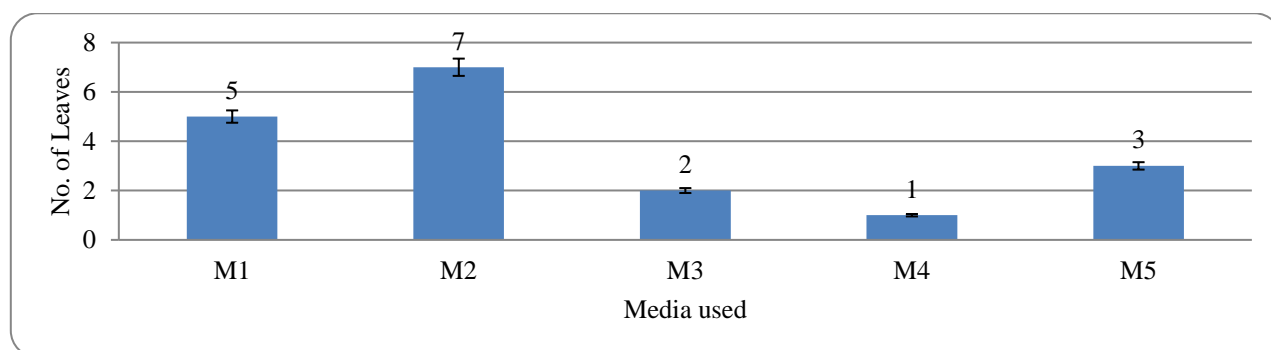


Figure 6. Effect of different mediums on regeneration of number of leaves.

Media 1 IAA=2 KIN=1.5 (mg/L); **Media 2** IAA=0.75 KIN=0.5 (mg/L); **Media 3** IAA=0.1 KIN=0.5 (mg/L); **Media 4** IAA=0.2 KIN=1 (mg/L); **Media 5** IAA=0.3 KIN=1.5 (mg/L)

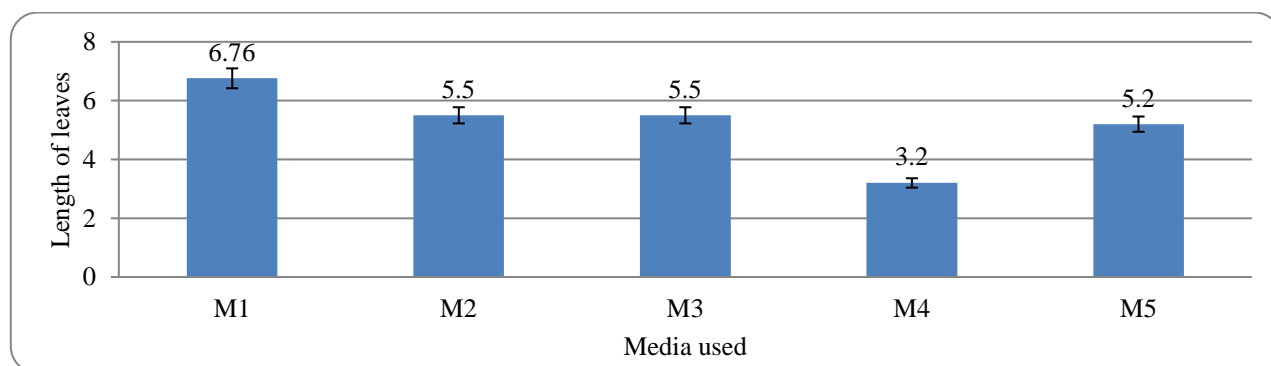


Figure 7. Effect of different mediums on regeneration of length of leaves (cm).

Media 1 IAA=2 KIN=1.5 (mg/L); **Media 2** IAA=0.75 KIN=0.5 (mg/L); **Media 3** IAA=0.1 KIN=0.5 (mg/L); **Media 4** IAA=0.2 KIN=1 (mg/L); **Media 5** IAA=0.3 KIN=1.5 (mg/L)

Discussion

This protocol involves the use of explants (seeds) were cultured on MS medium supplemented with different concentrations of growth regulators (auxin and cytokinin) for inducing high frequency of plant regeneration. Interaction of auxins and cytokinins plays vital role in cell division, growth, development, differentiation and the formation of plant organs (TB Jha et al., 2007; Purkayastha et al., 2010; Shrivastava et al., 2008). Explants obtained from mature seeds were

considered good source material for biotechnological studies because of easy storage, accessibility, and large amounts of homogeneous quality explants (Z-G Li et al., 2012). The plant regeneration through *in vitro* culture is considered to be one of the most promising ways for multiplying a selected variety true to its type.

The explants (seeds) of *Pennisetum glaucum* were surface sterilized with different concentrations of chlorox (30, 50 and 70%). The maximum germination (87.5 %) of explants was obtained by using 70 % Chlorox and

minimum germination (62.5 %) of explants was obtained by using 30% Chlorox. The maximum growth was observed on MS medium supplemented with IAA (2 mg/L) and KIN (1.5 mg/L). The maximum stem length (15.5 cm) was recorded on MS medium supplemented with (IAA 2 mg/L and KIN 1.5 mg/L) and minimum (3 cm) at the medium that contained (IAA 0.2 mg/L and KIN 1mg/L). The maximum leaves number (7) was observed on MS medium supplemented with (IAA 0.75 mg/L and KIN 0.5 mg/L) and minimum (1) at the medium that contained (IAA 0.2 mg/L and KIN 1 mg/L). Jalaja *et al.* (2016) reported 80% shoot regeneration on MS medium supplemented with the combination of 0.2 mg/l auxin (NAA) and 0.5 mg/l cytokinin (Kinetin) with 30 g/l. The regeneration on NAA and BAP was also observed but the percentage of regeneration was less. They observed 70% roots regeneration on MS basal medium supplemented with 0.2mg/l IAA. Arockiasamy *et al.* (2006) observed maximum shoot regeneration via organogenesis on MS medium supplemented with 4.43 μ M of BAP and 4.64 μ M of kinetin from the calli. The best root regeneration was recorded on MS medium supplemented with 0.49 μ M IBA. Rao (2013) reported that the explant proliferate best shoots (40-70%) on the MS medium supplemented with Kinetin (0.4 mg/l) and best root regeneration was observed on MS medium supplemented with 2.5 mg/l IAA. Sharma *et al.* (2012) observed best multiple shoots formation on MS medium supplemented with BAP (4.44 μ M) and IAA (2.85 μ M). The best rooting 60 to 80% was obtained on MS medium supplemented with 4.90 μ M of IBA and KIN (0.23 μ M). Rishi *et al.* (2012) reported best root induction on MS medium supplemented with 0.5 μ M/l of auxin. Jalaja *et al.* (2016) observed high frequency of shoots and roots regeneration on Murashige and Skoog nutrient agar medium supplemented with 2mg/l 2, 4-D and 0.2 mg/l NAA, 2 mg/l Kinetin and 30 g/l sucrose. The highest number of multiple shoots developed in medium containing 8.8 μ M Cytokinin (W Li *et al.*, 2002). Multiple shoots was also recorded at this concentration (Al-Abed *et al.*, 2006). Parmar *et al.* (2014) observed maximum Shoot regeneration and multiplication on MS medium supplemented with Cytokinin (1 mg/l). The maximum number of roots (3-4) with mean length (3.58 cm) were obtained on MS medium supplemented with Auxin (2.5 mg/l) and minimum root induction of *Oroxylum indicum* L. was observed on MS medium supplemented with Auxin (0.5 mg/l). P Jha *et al.* (2009) observed multiple shoots induction on MS medium containing either BA or kinetin (4.4, 8.8, 17.6, or 26.4 μ M) and maximum rooting was observed on MS medium supplemented with 0.8% activated charcoal. Varalaxmi *et al.* (2010) observed maximum regeneration potential of 40 shoots per calli on regeneration medium containing 1.0 mg/l Kin. Shoot developed were efficiently rooted within 15 days on the medium containing NAA. Wala *et*

al. (2003) observed multiple shoots on MS medium supplemented with BA (2.21 μ M). The shoots were rooted either on half strength of MS basal medium or on the one supplemented with NAA (0.53 μ M). The maximum germination (87.5 %) of explants was observed by using 70 % Chlorox and minimum germination (62.5 %) of explants was obtained by using 30% Chlorox. The maximum Stem length (15.5cm), Roots number (12), Roots length (3.96 cm) and leaves length (6.76 cm) was observed on MS medium supplemented with (IAA 2 mg/L and KIN 1.5 mg/L) although maximum leaves number was observed on MS medium supplemented with (IAA 0.75 mg/L and KIN 0.5mg/L). The minimum Stem length (3 cm), Roots number (1), Roots length (1), Leaves number (1) and Leaves length (3 cm) was observed on MS medium supplemented with IAA 0.2 mg/L and KIN 1 mg/L.

Authors' Contribution

All the authors have equal contribution in this manuscript

Conflict of Interest

The authors have no potential conflict of interest.

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