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HIGH FREQUENCY *IN VITRO* PLANT REGENERATION FROM IMMATURE INFLORESCENCE (FROM) IN FINGER MILLET (*ELEUSINE CORACANA* (L.) GAERTN

SATISH KUMAR
Mata Basanti Devi School of Biosciences and Biotechnology, Agra, (U. P.) India-282 007
Corresponding author : E-mail: kumarsragi@gmail.com

The small millets are quite important in areas of their cultivation as dryland crops, as well as for tribal and hill agriculture. Finger millet-*Eleusine coracana*, has been the most cultivated and useful small millet crop in India, especially for its nutraceutical qualities. An attempt had been made to develop in vitro regeneration protocol using immature spikes. The immature inflorescence pieces of different sizes were cultured on MS medium supplemented with various growth regulators-2,4-D, 2,4,5-T, *p*CPA, IAA, Kn and BAP, either alone or in various combinations. Among the various growth regulators investigated, 2,4-D (2 mg/l) with Kn (0.5 mg/l) gave maximum callusing response. Effect of developmental stage of immature inflorescence was also studied by collecting immature spikes of different sizes. In cultivar PR-202, a combination of BAP (0.2 mg/l and 2,4-D (2 mg/l) had been found to be very responsive in very young immature spikes (1.25 cm). Successful plant regeneration was obtained from maintained organogenic callus when sub-cultured on BAP supplemented medium.

Key words: *Eleusine coracana*, Nodular callus, Organogenesis, Plant regeneration, Staple food.

Introduction

The millet grains are well known for their superior quality. Finger millet is an important staple food in parts of East and central Africa and India, particularly in Karnataka, Tamil Nadu, Andhra Pradesh, Madhya Pradesh, Maharashtra. India and China are the Major producers of millet crops, contributing 40% of millet production in Asia[1]. Finger millet is a robust, free tillering, tufted annual grass. Nutritional quality of finger millet is comparable to wheat and rice. It is the richest source of calcium and phosphorous. A number of edible and highly nutritious food items are prepared from finger millet. In most of the tissue culture studies in finger millet, seed and mature embryos have been used as potent explants. Among immature explants, spikes are less preferred because of very short time period for availability of spikes in juvenile stage. Moreover, contamination had been a big barrier in establishing axenic cultures. The present investigation aims to develop a highly morphogenic pathway of rapid plant regeneration using immature inflorescence

explants. Role of various auxins and cytokinins alone or in various combinations has been explored on callus induction and plant regeneration. In finger millet, plant regeneration takes place via organogenesis or direct shoot formation[2].

Material and Methods

Collection of plant material and sterilization-

Eleusine coracana (L.) Gaertn. Belonging to grass family- Poaceae, is a small genus comprising nine species[3]. The genus *Eleusine* exhibits characteristic feature of possessing secund spikes of overlapping spikelets and an ornate grain enclosed by a free pericarp. Shoot segments of field grown plants of cultivar PR-202 containing un-emerged juvenile inflorescence were collected for culture of immature inflorescence. Whole shoot segments of 2-5 cm containing immature inflorescence were washed thoroughly with running tap water followed by washing with 5% solution of liquid detergent-Extran (Merck, India). Plant material was washed with distilled water 3-5 times and then sterilize with 70% alcohol for 30 sec. Further sterilization with 0.1% $HgCl_2$ was carried out under Laminar Airflow hood. Plant material was sterilized with freshly prepared aqueous solution of mercuric chloride for 3- 5 minutes followed by rinsing with autoclaved distilled water several times.

Preparation of explants and nutritional supplementation-

The immature inflorescences were used at different stages of development, picked by measuring different sizes. In all experiments the immature inflorescences were collected when it is completely covered by leaves. All the different sizes of inflorescences were averaged into two categories (i) av. Size 1.25 cm, and (ii) av. Size 3 cm. Explants were cultured on MS[4] medium containing 3% sucrose and solidified with 1% agar in Erlenmeyer flasks. Nutrient medium was sterilized by autoclaving at 15 psi for 20 minutes. Nutrient medium was supplemented with various concentrations of auxins (2,4-D, 2,4,5-T, *p*CPA, IAA, NAA) and cytokinins (BAP and Kn).

Aseptic conditions and explant culture-

Aseptic conditions were set to avoid contamination and to obtain axenic cultures by providing UV radiation to nutrient medium and other material. Cultured explants were incubated in growth room provided with suitable growth conditions of temperature and light. Cultures were incubated at 26±1°C temperature. A photoperiod of 16 hr. and 8 hr. dark was regulated by a timer.

Primary cultures and plant regeneration-

Culture response was measured after 4-6 weeks of incubation. Immature inflorescence derived nodular callus was cultured on plain MS medium and MS+ GA_3 (1 mg/l) for plant regeneration. Under in vitro conditions, regenerated plantlets were having a few weak and short roots. These roots were unable to hold plantlets successfully in the soil and also found inefficient to absorb nutrients from soil. The regenerated shoots were carefully taken out and after cutting the minor roots, cultured on half MS medium without any plant growth regulator. After 4-6 weeks, a cluster of long, healthy roots was obtained.

Field transfer-

Well regenerated and well rooted plantlets were transferred to the pots containing 1:1 ratio of garden soil and manure. The pots were kept under field conditions. The plantlets successfully grew and after 70-80 days, fertile spikes emerged from the plants.

Result and discussion

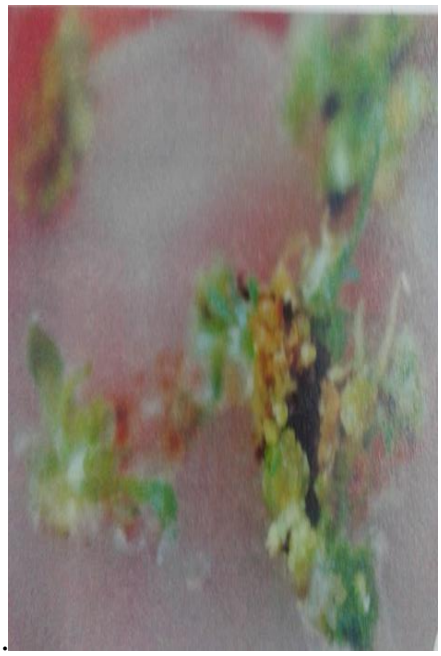
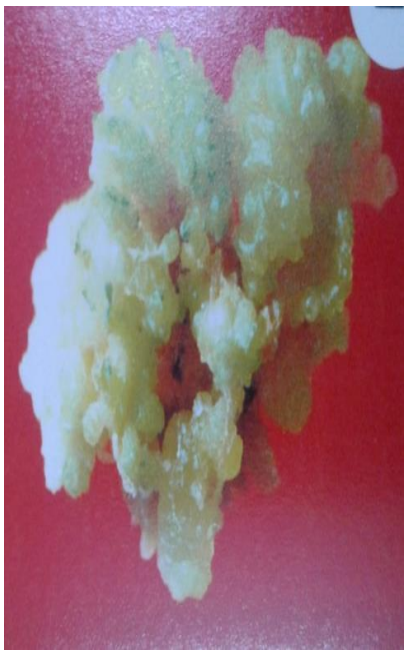
Immature inflorescence segments cultured on MS medium supplemented with 2,4-D (2

Auxins (mg/l)		Cytokinin (mg/l)		No. of explants responded	% Response	Fresh weight (mg) Mean ± SD
	0		0	NR	00	----
2,4-D	1		0	18	60.0	79±2.7
	2		0	7	23.3	85±2.6
	3		0	6	20.0	73±1.9
	1	Kn	0.5	17	56.6	103±4.3
	2		0.2	20	66.6	93±3.2
	2		0.5	17	56.6	142±4.1
	1	BAP	0.5	19	63.3	173±5
	2		0.2	25	83.3	148±3.7
	2		0.5	13	43.3	137±3.9
2,4,5-T	1		0.2	12	40.0	37±1.2
	1		0.5	29	96.6	81±1.7
	2		0.2	15	50.0	99±1.6
	2		0.5	12	40.0	103±2
*p*CPA	1	Kn	0.2	NR	00	---
	1		0.5	28	93.3	102±2
	2		0.2	19	63.3	65±1.1
	2		0.5	5	16.6	47±0.9
	1	BAP	0.5	15	50.0	116±5
	2		0.2	19	63.3	63±3.1
	2		0.5	27	90.0	102±3.7
IAA	1		0.5	12	40.0	59±1.5
	2		0.2	6	20.0	68±1.9
	2		0.5	14	46.6	47±1

Table.1. Induction of nodular callus from immature inflorescence in *Eleusine coracana* (cultivar, PR-202) cultured on MS medium supplemented with different auxins alone or in combination with cytokinins. *(Number of explants cultured/treatment-30; culture duration-4 weeks)*

mg/l) alone formed maximum amount of compact, shiny green, hard nodular callus. A combination of Kn (0.2, 0.5 mg/l) and 2,4-D (1,2 mg/l) remarkably enhanced the fresh weight of nodular callus. Kn (0.5 mg/l) and 2,4-D (1 mg/l) gave best response of induction of nodular callus (Fig. 1). The developmental stage of immature inflorescence has been reported to affect the culture response. In the present investigation, the inflorescences of 1.25 cm gave good response of induction and enlargement of nodular callus (Table.1). Previously, the immature inflorescence has been reported to be suitable for high frequency plant regeneration in *E. coracana*[5,2], in *Echinochloa* sp.[6] and *Paspalum*[7].

Immature inflorescence induced primary nodular callus was sub-cultured on

1.  2. 

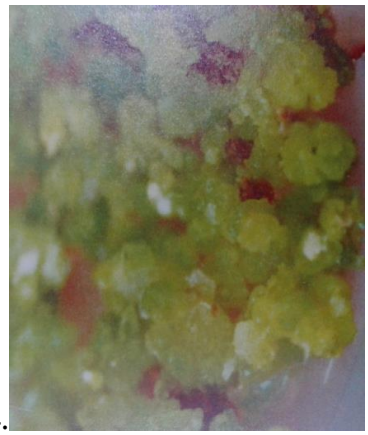
3.  4.  5. 

PLATE.1. *In vitro* growth response of immature inflorescence in *Eleusine coracana*, cultured on MS medium supplemented with 2,4-D (2 mg/l) and BAP) 0.5 mg/l).
Fig. 1. Primary nodular callus induced from immature inflorescence (1.25 cm)
Fig. 2. Compact organogenic callus after subculturing on MS + 2,4-D (0.2 mg/l)
Fig. 3. Direct shoot formation from immature inflorescence
Fig. 4. Long term maintenance of nodular callus
Fig. 5. Plantlets regenerated from nodular callus derived from immature inflorescence

MS medium supplemented with low level of 2,4-D. initial 100 mg of nodular callus increased approx. 3 times on MS+ 2,4-D (0.1 mg/l) medium, within 4 weeks (Fig. 2).

In wheat [8], reported formation of multiple shoots through micro-tillering on auxin and cytokinins supplemented medium. Earlier[9-12], have reported formation of nodular callus and plant regeneration on 2,4-D supplemented medium. Yemets *et. al.*, (2013)[13] reported high frequency nodular callus formation by using 2,4-D (2 mg/l) in combination with low level of Kn (0.4 mg/l) from cultured seeds of finger millet.

Nodular callus induced from immature inflorescence was cultured on regeneration medium supplemented with different plant growth regulators- GA_3, NAA, IAA, IBA, PAA (0.5, 1, 2 mg/l). Maximum number of shoots was obtained on GA_3 (1 mg/l) added medium (fig. 3). No significant regeneration response was observed on the MS medium supplemented with other auxins. Immature inflorescence (3 cm) exhibited direct shoot formation from the region of florets without any intervening callus stage.

Plant regeneration via organogenesis and multiple shoot formation in finger millet and other millets has been earlier reviewed[2,9,14,15]. Recently, Kashyap *et. al.*, (2018)[16] have successfully reported plant regeneration via organogenesis on 2,4-D and BAP supplemented medium. Sharma *et. al.*, (2017)[17] also reported callus induction from mature embryo cultures in finger millet. Regeneration of plantlets was mostly favoured when hormone supplementation is kept low in the medium. High frequency plant regeneration from nodular callus on MS medium supplemented with moderate to low level of BAP has been successfully achieved[17]. In Proso millet, Bankar and Gadakh(2017)[18] also reported shoot formation on MS medium fortified with BAP. The plantlets regenerated on MS medium supplemented `with GA_3 (1 mg/l) were provided with healthy root system and were not needed to transfer on rooting medium. They were directly transferred to field.

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