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An Efficient Protocol for Callus Induction and Plant Regeneration in Finger Millet (*Eleusine coracana* L.)

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Abstract: Shoot tips of finger millet (*Eleusine coracana*) were inoculated on to Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of auxins [(2,4-D, NAA), cytokinins (KN, BAP) and other growth promoters (Proline, Tryptophan, casein enzyme hydrozylate(CEH)]. The higher degree of embryogenic callus formation observed with 2,4-D was at 2.5 mg L^{-1} in the presence of 0.5 mg L^{-1} of BAP. With NAA, the higher degree of embryogenic callus formation observed was at 3.0 mg L^{-1} in the presence of 1.0 mg L^{-1} of BAP. However, the addition of growth regulators like Tryptophan, Proline and CEH to either 2,4-D or NAA increased greatly the degree of callus formation. Among all the combinations employed the highest degree of callus formation was observed at 2.5 mg L^{-1} of 2,4-D, 0.5 mg L^{-1} of BAP , Proline (500 mg L^{-1}), Tryptophan (250 mg L^{-1}) and CEH (300 mg L^{-1}). A maximum of 97 % of shoot tip explants were successful in inducing callus at this combination of growth regulators. Highest mean number of shoot buds (67%) and good rooting was observed when embryogenic calli was subcultured on MS medium supplemented with 1 mg L^{-1} Kinetin and 0.5 mg L^{-1} NAA. The regenerated plantlets could be transferred successfully to the field with 85% survival.

Key words: MS medium · Callus formation · 2,4-Dichlorophenoxyacetic acid (2,4-D) · Alpha-Naphthalene acetic acid (NAA) · Benzyl amino purine (BAP) · Kinetin (KN)

INTRODUCTION

Finger millet (*Eleusine coracana*) is an annual cereal crop widely grown in arid and semiarid areas of Africa and Asia [1]. The grain is a good source of protein, carbohydrate but contains less amount of fat. It is rich in calcium, phosphorus, iron, cysteine, tyrosine, tryptophan and methionine [2, 3]. Finger millet can be ground and cooked into cakes, puddings or porridge. The grain may also be malted and a flour of the malted grain could be used as a nourishing food for infants [4]. Production of transgenic plants with desired qualities is possible by genetic transformation of the desired genes in to the selected plants through the methodology of tissue culture. Efficient callus formation and regeneration is an important requisite to perform Agrobacterium mediated transformations for producing transgenics. Our present study is aimed to develop heavy metal resistance in finger millet by transferring phytochelatin synthase gene in to it. To achieve our aim we have developed an efficient protocol for callus induction and regeneration in finger millet.

Somatic embryogenisis is the most common method of plant regeneration in all the major species of cereals and grasses [5]. Callus formation and regeneration in fingermillet was reported first from mesocotyl explants [6]. Thiru [7] reported callus formation but no regeneration from different seedling explants. Mohanty [8] reported plant regeneration through shoot bud formation. [9] in a detailed morphogenic and histological study reported formation of apical dome like structures which, upon sub-culture produced multiple shoots. The first in vitro study in finger millet has been reported in which immature inflorescences and shoot apical meristem were induced to form somatic embryos on Murashige and Skoog [10] basal medium containing 2,4-Dichlorophenoxyacetic acid (2,4-D) and Kinetin (KN) [11,12] and also from medium supplemented with picloram and kinetin [13]. The present

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paper describes efficient embryogenic callus formation and regeneration of finger millet.

MATERIALS AND METHODS

Seeds of *Eleusine coracana* were obtained from Regional Agricultural Research Station, Tirupati. The seeds were sterilized and gown on filterpapers in petriplates at room temperature. From one week old seedlings, shoot tips were collected, washed and inoculated on to MS basal medium supplemented with various growth regulators as outlined in the result section [10]. Sucrose at 3% was used as the sole carbon source. The cultures were then incubated in biotron with 16 hr of light cycle in a 24 hr cycle and the temperature regulated at 25±1°C. The culture media consisted of a) callus induction medium and b) regeneration medium.

RESULTS AND DISCUSSION

Callus Induction: Different concentrations and combinations of various growth regulators were added to MS medium for effective callus induction. When compared to NAA, 2,4-D was found to be better for callus induction. The degree of callus formation was improved when 2,4-D (2.5 mg L^{-1})was supplemented with 1 mg $L^{-1}$of BAP. However, the highest degree of callus formation and highest per cent (97%) of explants that induced callus was achieved with 2,4-D (3 mg L^{-1})+ BAP(0.5 mg L^{-1}) supplemented with Proline (500 mg L^{-1}), Tryptophan (250 mg L^{-1}) and CEH (300 mg L^{-1}) (Table 1). Auxins play important role in callus induction. The induction of callus in cereals and millets is commonly achieved by 2,4-D [14,15]. Our results revealed that a combination of auxins (2,4-D) and low concentrations of cytokinins (BAP) are better inducers of callus than auxins alone. Supplementation of proline, tryptophan and CEH (casein enzyme hydrozylate) have further enhanced the efficiency of callus formation and the percent of explants that have given rise to callus. 2,4-D and KN have also been used for callus induction and somatic embryogenesis in the related species Eleusine indica [16].Similar observations were noted by Kothari and Chandra [17] and Latha *et al* [12] in finger millet.

Plantlet Regeneration from Embryogenic Callus: The well developed embryogenic calli were transferred to regeneration medium for shoots and roots formation. Among different combinations and concentrations of

Table 1: Callus induction from shoot tips of Finger millet in the presence of different growth regulators

Plant growth regulators (mg L^{-1})	% of explants induced callus formation	Degree of callus formation
2,4-D		
0.5	12%	+
1	25%	+
1.5	35%	+
2	60%	+++
2.5	82%	+++
3	70%	+++
4	64%	++
NAA		
0.5	20%	+
1	35%	+
1.5	56%	+++
2	68%	+++
2.5	72%	+++
3	69%	++++
4	56%	++
2, 4-D + KN		
0.5 + 0.25	26%	+
1.0 + 0.5	35%	+
1.5 + 0.75	55%	++
2.5 + 0.5	85%	++++
3.0 + 1.0	90%	++++
4.0 + 1.0	60%	++
2, 4-D + BAP		
0.5 + 0.25	20%	+
1.0 + 0.5	45%	+
1.5 + 0.75	64%	++
2.5 + 0.5	92%	+++
3.0 + 1.0	85%	++++
4.0 + 1.0	55%	++
NAA+ KN		
0.5 + 0.25	15%	+
1.0 + 0.5	25%	+
1.5 + 0.75	35%	+
2.5 + 0.5	75%	+++
3.0 + 1.0	88%	++++
4.0 + 1.0	54%	++
NAA+ BAP		
0.5 + 0.25	16%	+
1.0 + 0.5	32%	+
1.5 + 0.75	55%	++
2.5 + 0.5	78%	+++
3.0 + 1.0	80%	++++
4.0 + 1.0	60%	++
2,4-D +BAP+ Proline + CEH + Tryptophan		
2.0 +0.5+500 +300 +250	92%	+++
3.0 +0.5+ 500+ 300 + 250	97%	++++
NAA + Proline + CEH* + Tryptophan		
2.0 +0.5+500 +300 +250	75	+++
3.0+ 0.5+500+ 300 + 250	88%	++++

*CEH: casein enzyme hydrozylate

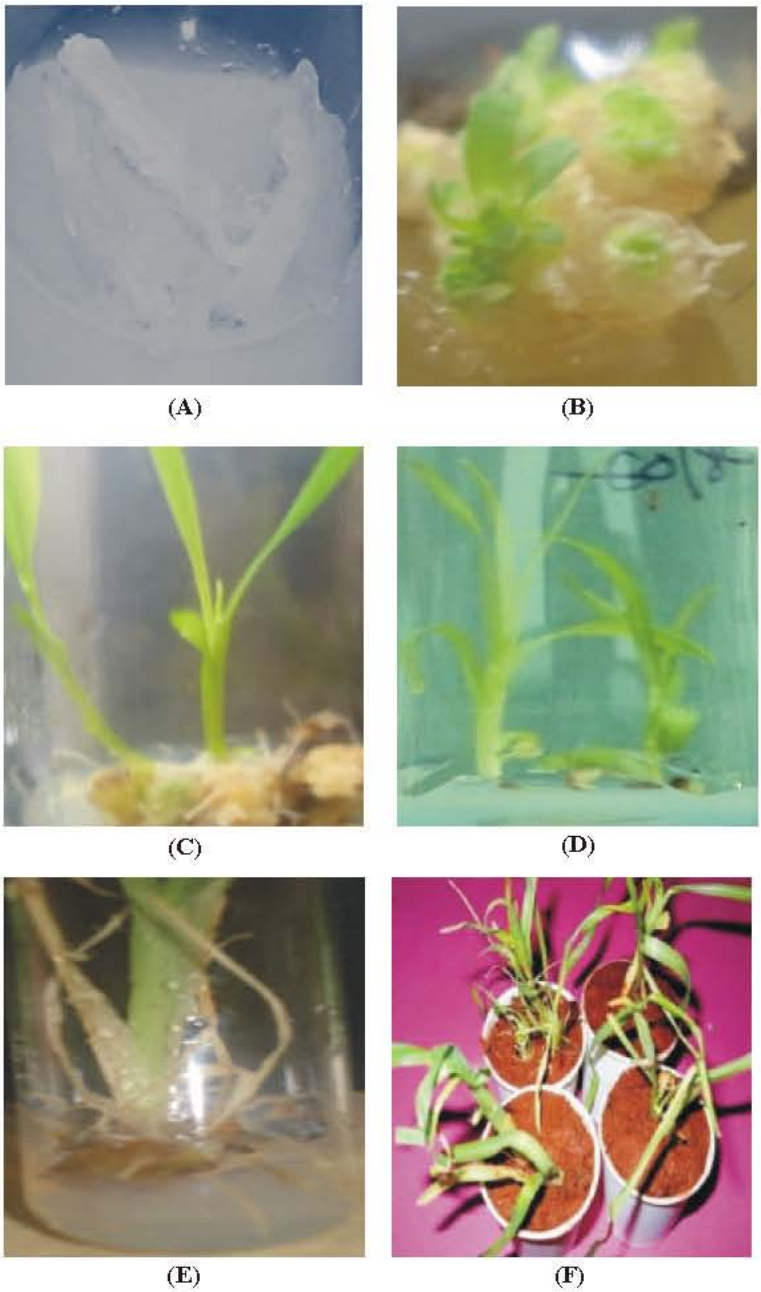

Fig. 1: Callus induction and plant regeneration from shoot tips of *Eleusine coracana*
A-Callus intiation on MS + 2.5 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BAP.
B-Callus formation and shoot buds induction on MS +2,4-D (3 mg L^{-1}) +BAP(0.5 mg L^{-1}) + Proline (500 mg L^{-1}) + CEH (300 mg L^{-1} + Tryptophan (250 mg L^{-1}); .
C, D and E- shoot growth and root formation from embryogenic calli on MS supplemented.
with 1 mg L^{-1} Kinetin and 0.25 mg L^{-1} NAA.F - Regenerated plants transferred to pots.

growth regulators employed, the highest mean number of shoots (67%) and roots (39 %)were formed when MS medium was supplemented with KN (1 mg L^{-1})and NAA (0.25 mg L^{-1}) as shown in Table 2. Cytokinins play important role in shoot growth. In our study KN with low concentration of NAA resulted in high mean number of shoots and good rooting. Similar reports were noted by others in finger millet [18, 19]. KN and BAP have also

Table 2: Effect of growth regulators on shoot and root formation from embryogenic callus on MS medium (each treatment had 3 replicates).

Phytoharomones mg L^{-1}	No .of shoots/ plantlets (Mean ± SE)	No. of Roots (Mean ± SE)
KN+NAA		
0.5+0	38.3 ±4.2	33.6±3.2
1.0+0	59.5± 6.4	24.5±2.5
2.0+0	42.1± 2.1	38.6±7.2
0.5+0.025	48.3±6.2	18.1±3.1
1.0+ 0.25	67.3±7.2	39.8±5.2
2.0+ 0.55	32.5±5.4	26.5 ± 6.4
BA+NAA		
0.5+0	44.6±3.8	20.7±7.5
1.0+0	62.6 ±7.2	26.4±3.5
2.0+0	56.8 ±8.1	30.6±2.4
0.5+0.025	56.6±4.2	19.7 ± 6.5
1.0+ 0.25	64.3 ± 8.2	33.9±8.3
2.0+ 0.55	41.6 ±3.5	23.2±1.6
KN +2, 4-D		
0.5+0.025	34.0 ± 0.5	22.6±2.1
1.0+0.25	58 ± 0.5	38.6±2.1
2.0+0.75	12.5±2.8	28.6±2.1
BA+2,4-D		
0.5+0.025	26±2.1	20.6±2.1
1.0+ 0.25	55±.3.6	32.2±1.5
2.0 + 0.75	41.53±4.2	18.6±2.7

been used for efficient shoot induction and plant regeneration in pearl millet [20-23].

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