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Short Communication

In-vitro Plant Regeneration of *Amaranthus paniculatus*

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The genus includes over 60 species found across many parts of the world [1]. Grain Amaranth is a highly economic plant and used as a staple food in some region especially Maxico, central and south America, India and Africa. This crop is given much importance by the most industrialized countries like U.S.A. and Japan. In India *Amaranth* form an important crop of the hilly regions and constitute the main source of the diet of people living in the Himalayan region. A high yield of the grains of this crop can substitute a portion of wheat dependence. Amaranths are consumed because of their high nutritive value due to the presence of lysine and calcium [2], high quantity of riboflavin, ascorbic acid and vitamin-E [3]. Plants also produce secondary metabolites like betalain and anthocyanin [4], Betalian a natural pigment derived from tyrosine [5] and Anthocyanin, a flavonoid [6]. It is a natural food colorant having antiradical and antioxidant. Anthocynin is antiinflamtory, antibacterial / antiviral, antioxidant, anticarcinogenic, antitumor-promoting properties [7]. These therapeutic values have further increased its potential as a crop. Plant tissue culture in Amaranths is limited but it requires refinements callus formation was induced in *A. panieulatus* [8], *A. caudatus*, *A. hypochonriacus*, *A. cruentus* and *A. hybridus* [9]. Tissue culture can be used for micro propagation of its related genotypes, stress resistant plant production and to induce new variants of high protein content or specific amino acids [10].

Seeds of *Amaranthus paniculatus* were procured from local market and seedlings were raised under sterilized conditions. The seeds were pretreated with 0.35 percent fungicide (Benlate) for 15 minutes and rinsed with tap water. Surface sterilization of seeds was done using a 10 percent (w/v) calcium hypochlorite solution. The seeds were rinsed four times with autoclaved distilled water after the first and second sterilization steps for 15-15 minutes. The sterilize seeds were than germinated in culture medium with 2% (w/v) sucrose and 0.5%(w/v) agar. The callus induction was initiated on Murarshige and Skoog (MS), Gamborg and Miller (B5), White's, Nitsch's and Heller's minerals nutrient media. However, for further experimentation MS basal medium was used throughout. The medium was supplemented with the various growth hormones. From seedlings grown under sterilized conditions different explants such as hypocotyls and cotyledons were inoculated on media supplemented with auxin and cytokinin in different concentration and incubated at 25 ± 2°C. The callus so produced was sub cultured 2-3 times for growth and differentiation studies. Auxins used were NAA, IAA, IBA, 2, 4-D alone or in combination. Kinetin (0.05mg/l) was supplemented uniformly in all media as a source of cytokinins.

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Callusing

Effect of auxins

Various concentrations and combinations of auxins, NAA and 2, 4-D along with a uniform concentration of Kinetin (0.05mg/l) induced callusing in cotyledon explants. A combination of Kinetin (0.05mg/l), NAA (1mg/l) and 2, 4 -D (5mg/l) produced profuse callus (Table 1). Callusing was observed in all the nutrient media but hypocotyls explants produced good callusing with 0.05mg/l Kinetin and 1mg/l NAA in B5 medium. However, best response was observed in MS medium with Kinetin (0.05mg/l), NAA or IBA (1 to 5mg/l) and 2,4-D (1 to 5mg/l) (Table 2).

Table 1 Different combinations of auxin and cytokinins showing fresh weight and dry weight in mg

Different combinations of auxins and cytokinins	Fresh weight (mg.)	Dry weight (mg.)
MS + 0.05 K + 1 NAA	251	17
MS + 0.05 K + 2 NAA	380	25
MS + 0.05 K + 5 NAA	607	47
MS + 0.05 K + 1 2,4-D	175	10
MS + 0.05 K + 2 2,4-D	230	14
MS + 0.05 K + 1 NAA + 1 2,4-D	475	34
MS + 0.05 K + 1NAA + 2 2,4-D	575	43
MS + 0.05 K + 1NAA + 5 2,4-D	1655	121

Effect of Cytokinins

It was seen only on MS medium**.** In all the taken combinations NAA (0.5mg/l) gave best results with Kinetin (4mg/l) in hypocotyls explants whereas, IAA (0.5mg/l) gave best results with lower concentration of Kinetin (1 mg/l). BAP (4mg/l) responded best with anyone of NAA or IAA in 5mg/l concentration (Table 3)

Differentiation

Differentiation of roots, shoots and inflorescence was observed on MS, B5 and Nitsch media only.

Table 2 Response of callus initiation and growth of varying auxin concentration in Heller's, Nitsch's, White's and B5 media

Media supplemented with different concentrations of auxins (mg/l)	Cotyledon	Hypocotyl
Nitsch's medium		
N + 0.05 K + 1 NAA	+	+
N + 0.05 K + 2 NAA	+	+
N + 0.05 K + 5 NAA	+	+
N + 0.05 K + 1 NAA + 1 2,4-D	+	+
N + 0.05 K + 1 NAA + 2 2,4-D	+	+
N + 0.05 K + 1 NAA + 5 2,4- D	+	+
White's medium		
W + 0.05 K + 1 NAA	-	-
W + 0.05 K + 2 NAA	-	-
W + 0.05 K + 5 NAA	+	+
W + 0.05 K + 1 NAA + 1 2,4-D	+	+
W + 0.05 K + 1 NAA + 2 2,4-D	+	+
W + 0.05 K + 1 NAA + 5 2,4- D	+	+
B5 medium		
B5 + 0.05 K + 1 NAA	+	+++
B5 + 0.05 K + 2 NAA	+	+
B5+ 0.05 K + 5 NAA	+	++
B5+ 0.05 K + 1 NAA + 1 2,4-D	++	++
B5+ 0.05 K + 1 NAA + 2 2,4-D	+	++
B5+ 0.05 K + 1 NAA + 5 2,4- D	+	++
Heller's medium		
H + 0.05 K + 1 NAA	-	+
H + 0.05 K + 2 NAA	+	+
H + 0.05 K + 5 NAA	+	++
H + 0.05 K + 1 NAA + 1 2,4-D	+	+
H + 0.05 K + 1 NAA + 2 2,4-D	+	+
H + 0.05 K + 1 NAA + 5 2,4- D	++	++

+Callus initiation; ++Normal callusing; +++Good callusing; ++++Very good callusing

Table 3 Response of callus initiation and growth of varying anxin concentration in MS medium

MS medium supplemented with different concentrations of auxins (mg/l)	Cotyledon	Hypocotyl
MS + 0.05K +1 NAA	++	++
MS + 0.05K +2 NAA	++	+++
MS + 0.05K +5 NAA	+++	+++
MS + 0.05K +1 IAA	++	++
MS + 0.05K +2 IAA	++	+++
MS + 0.05K + 5 IAA	+++	+++
MS + 0.05K + 1, 2,4-D	++	+++
MS + 0.05K + 2, 2,4-D	+++	+++
MS + 0.05K + 5, 2,4-D	++++	++++
MS + 0.05K + 1NAA + 1, 2,4-D	++	+++
MS + 0.05K + 1NAA + 2, 2,4-D	+++	+++
MS + 0.05K + 1NAA + 5, 2,4-D	++++	++++
MS + 0.05K + 5NAA +1, 2,4-D	+++	+++
MS + 0.05K + 5NAA +2, 2,4-D	++++	++++
MS + 0.05K + 5NAA +5, 2,4-D	++++	++++
MS + 0.05K + 5IBA +5, 2,4-D	++++	++++

+Callus initiation; ++Normal callusing; +++Good callusing; ++++Very good callusing

Differentiation from hypocotyls

Normal rooting and shooting were observed on Nitsch's medium along with 2,4-D (1, 2 and 5mg/l) + NAA (1mg/l) + K (0.05mg/l) (Table 4). B5 medium when supplemented with NAA (1mg/l) and Kinetin (0.05 mg/l) good rooting was observed. Higher concentration of 2,4-D did not promote rooting in hypocotyls although initiation of roots was observed at 2,4-D (1mg/l), NAA(1mg/l) and K(0.05mg/l) combination (Table 4). MS medium along with auxin and kinetin responded the best towards differentiation. 2,4 - D (5mg/l), NAA(1mg/l) and K (0.05mg/l) gave very good response towards rooting but shooting was normal (Table 4).

Differentiation from callus

In Nitsch's medium 2,4 -D (2 or 5 mg/l) combined with NAA(1mg/l) and Kinetin(0.05mg/l) normal shooting and rooting was observed (Table-4). On B5 medium very good shooting was observed even after a week when NAA (1mg/l) and Kinetin (1mg/l) were used as supplements. A good rooting was also observed and inflorescence was also observed. Good rooting response was obtained in 2,4-D (1mg/l) + NAA (1mg/l) +K (0.05mg/l) combination (Table 4). On MS medium a combination of 2,4-D (1mg/l) + NAA(1mg/l) + K (0.05mg/l) gave very good rooting and shooting. Callus in *A. paniculatus* could be induced from hypocotyls and cotyledons. Induction

started from epidermal and sub epidermal parenchymatous cell of explants and originated throughout the surfaces. Callus outgrowths were first observed on either or both ends of the explants. Callus growth initiated on the surface or cut ends of explants during the *in-vitro* propagation of sessile Joyweed (*Alternanthera sessilis*), a member of the Amaranthaceae family [11]. In present study the callus formation proceeded from the cut ends to the center of the hypocotyls segments until the explant became a mass of cells. Sub culturing was done after 40 days on the same medium. Earlier reports have demonstrated that hypocotyls segments and stem sections are more responsive for callus induction in *Amaranthus* spp. [12] but in the present study hypocotyl segments and cotyledons were used as explants.

Table 4 Response of callus initiation and growth of varying cytokinin concentration in MS medium

Medium supplemented with different concentrations of cytokinins in mg/l	Cotyledon	Hypocotyl
1 K + 0.5 NAA	++	+++
2 K + 0.5 NAA	++	++
4 K + 0.5 NAA	+++	++++
1 BAP + 0.5 NAA	+++	+++
2 BAP + 0.5 NAA	++	+++
4 BAP + 0.5 NAA	++	++++
1 K + 0.5 IAA	+++	++++
2 K + 0.5 IAA	++	+++
4 K + 0.5 IAA	++	+++
1BAP + 0.5 IAA	++	++++
2 BAP + 0.5 IAA	+	++
4 BAP + 0.5 IAA	++	+++

+Callus initiation; ++Normal callusing; +++Good callusing; ++++Very good callusing

Table 5 Differentiation response of callus and explants of seedling under different regime of auxins and cytokinins

Media supplemented with different concentrations of auxin	Callus		Hypocotyl and Cotyledons	
	Shoot	Root	Shoot	Root
Nitsch's medium				
N + 0.05 K +1 NAA	-	-	-	-
N + 0.05 K +2 NAA	-	-	-	-
N + 0.05 K +5 NAA	-	-	-	++
N + 0.05 K +1 NAA + 1, 2,4 –D	++	+	++	++
N + 0.05 K +1 NAA + 2, 2,4 –D	++	++	++	++
N + 0.05 K +1 NAA + 5, 2,4 –D	++	++	++	-
B5 medium				
B5 + 0.05 + 1 NAA	++++	+++	+	+++
B5 + 0.05 + 1 NAA + 1, 2,4-D	-	-	-	+
B5 + 0.05 + 1 NAA + 2, 2,4-D	-	-	-	-
B5 + 0.05 + 1 NAA + 5, 2,4-D	-	-	-	-
MS medium				
MS + 0.05 K +1 NAA	-	-	-	-
MS + 0.05 K +1 NAA + 1, 2,4-D	++	+++	++	+++
MS + 0.05 K +1 NAA + 2, 2,4-D	+++	+++	++	+++
MS + 0.05 K +1 NAA + 5, 2,4-D	++++	++++	++	++++

+Callus initiation; ++Normal callusing; +++Good callusing; ++++Very good callusing

The callus produced was amorphous, creamy white in colour (when induction was done on high auxin concentration) while it was brown (on high Kinetin supplemented media). The callus was fragile. Out of all growth substances used medium supplemented with NAA and 2,4-D was most suitable. Four major interacting processes for growth of callus namely growth, regressive changes, differentiation and pattern formation [13]. During callus formation three major changes take place i.e., induction division, differentiation in the cells of explant. This involves various physiological changes which lead to dedifferentiation in the outer cells. 2,4-D is found to increase in RNA content followed by cell division in the quiescent cell [14] and the nucleolus is the site of action of growth substances [15]. Different explants on the basis of their growth factor requirement into four categories which are auxin requiring, cytokinin requiring, auxin and cytokinin requiring and requiring complex natural extracts [16]. *Amaranthus paniculatus* falls under auxin requiring for callus induction. However, cytokinins showed synergistic effect. Among auxins a combination of NAA (1mg/l) and 2,4-D (5mg/l) showed synergistic effect.

The synergistic effect of BAP and synthetic auxins of callus induction has been reported in *Amaranthus* spp. using hypocotyls or stem sections withy BAP and low doses of NAA or 2,4-D [17]. In *A. gangeticus*, a higher BAP: NAA ratio was needed for optimal callus growth in stem explants [18]. In the present study high BAP or K: NAA ratio gave good callusing whereas low BAP or K:IAA ratio was not so effective for callusing. NAA, BAP and 2,4-D achieved the most rapid callusing in *Amaranthus paniculatus* [19]. As far as regeneration is concerned growth regulator supplementation induced differentiation from callus. On Nitsch's medium roots and shoots were differentiated in combination of NAA (1mg/l) and 2,4- D (1,2,5 mg/l) while on B5 medium best shooting was observed only in NAA (1mg/l) and K (0.05mg/l) with very less rooting. In MS medium profuse rooting and shooting was

observed in a combination of NAA and 2,4-D. High cytokinin: auxin ratio promoted shooting.

In the initiation of organogenesis, a relative concentration of auxin and cytokinin is involved but it is difficult to determine the balance of growth regulators at the place of organ formation and it is also a challenge to find out that the balance is due to residual hormones in the primary explants or the endogenous hormones synthesized by the callus itself [20]. As MS basal medium alone induced root formation *in A. tricolor* which indicates that *A. tricolor* may have a sufficient level of endogenous auxin and that exogenous application may have resulted in a supra optimal concentration that resulted in inhibition of root formation [21].

SUMMARY

A research on *Amaranthus paniculatus* L. agris (fao) was undertaken to investigate the morphogenesis of the plant through tissue culture techniques. The genus *Amaranthus* (Amaranthaceae) has attracted great interest in the past year as an agronomic crop in various parts of the world. In fact, *Amaranthus i*s an underutilized crop and a cheap source of proteins, minerals, vitamin A and C and is a promising vegetable. Hence, it is an agriculturally valuable crop but tissue culture technique for this crop remains limited. A combination of plant growth regulators (PGRS) was used to induce callus formation from hypocotyls. The callus formation was faster with combination of Murashige and Skoog medium (MS) + 0.5mg/l Kinetin + 1to 5 mg/l NAA or IAA and 2 and 5 mg/l 2,4-D. Rooting and shooting was best observed in MS medium followed by B5 and Nitsch medium when media were supplemented with 5mg/l 2,4-D, 1mg/l NAA and 0.05mg/I K. The callus underwent differentiation of inflorescence under B5 and MS media at different auxin and cytokinin concentration levels. Plants raised were excellently acclimatized in natural environment with 89 percent survival rate.

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