Journal of Research & Development, Vol. 5 (2005)
ISSN 0972-5407

Regeneration Potential of *Amaranthus hybridus* L. in an *In Vitro* Culture System

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ABSTRACT

Regeneration potential of various explants of *Amaranthus hybridus* was exploited for micropropagation. The explants used included shoot apices, nodal, internodal, leaf and hypocotyl segments. Shoot regeneration and its multiplication was achieved in shoot apices and nodal segments only. Caulogenesis was followed by rhizogenesis in explants of shoot apices. Hypocotyl segments produced only non-regenerative callus, whereas internodal and leaf segments showed no response at all.

Keywords: *Amaranthus hybridus*, shoot regeneration, explants, multiple shoots.

INTRODUCTION

The genus *Amaranthus* belongs to family Amaranthacae and consists of nearly 60 species that can be broadly catagorised into grain, green leaf vegetable and weed types. The main vegetable type of Amaranthus seems to have originated in South and Southeast Asia (Grubben and Van Sloteu, 1981) and then spread through the tropics and temperate zone (Martin and Telek, 1979). *Amaranthus hybridus* which is an annual herb is grown for grain or vegetable production in various parts of world including India. It is also called Green Amaranthus. The harvested *A. hybridus* plant is 50-80% edible, in which only 20-30% of plant is utilized directly as vegetable in United States (Kramer and Kwee, 1977). Its leaves contain 17.4 -38.3% dry matter as crude protein, thus having potential as a protein supplement (Oliveira and de Carvalho, 1975). Its leaf protein contains more lysine than the best high lysine corn and methionine than soyabean meal. Seeds of *A. hybridus* also contain high level of protein. Its cultivation in this region has not received any attention in the past despite the fact that this plant spp. has medicinal value and is also consumed as a vegetable in some parts of Kashmir. So an attempt has been made to investigate the potential for *in vitro* regeneration in different explants of *A. hybridus*.

MATERIAL AND METHODS

Both *in vitro* raised and field grown material was used to carry out tissue culture studies of *A. hybridus*. Plant material and seeds were collected from Chattrahama region of district Srinagar. Explants used for micropropagation included shoot apices, nodal, internodal, leaf and hypocotyl

segments. Shoot apices and hypocotyl segments were used from *in vitro* germinated seeds. Nodal, internodal segments used were obtained from field grown plants, while leaf segments used were *in vitro* raised as well as obtained from field. Seeds and organ explants obtained from field were thoroughly washed with tap water using lab wash and immediately surface sterilized by 0.2% $HgCl_2$ for 15 min and then rinsed 3-4 times with autoclaved double distilled water under laminar air flow before inoculation. Then seeds and different organ explants were inoculated on MS (1962) (x1/2) basal medium supplemented with 3% sucrose and different phytohormones. Medium, before using for inoculations, was autoclaved at a temperature of 121°C and 15 lb pressure for 20-25 min. pH was adjusted between 5.2 and 5.5 and medium was solidified by using 0.8% agar. Cultures were maintained at 25 ± 2°C with 55-65% RH and exposed to 16hr photoperiod provided by cool fluorescent tubes (3000 lux).

RESULTS

Seeds:

30% seed germination was achieved on MS basal medium whereas MS (x1/2) basal medium when supplemented with BAP 2.5μM and 5.0μM, increased seed germination up to 35% and 50% respectively. When BAP was replaced by NAA (5μM), seed germination again increased up to 75% (Fig. 1). Various trials carried out for seed germination and their results are depicted in Table 1.

Shoot tip culture:

Shoot tips excised from *invitro* raised seedlings were inoculated on MS medium augmented with different growth regulators. Various types of morphogenetic responses recorded are depicted in Table 2. Shoot tips cultured on MS (x1/2) + BAP (2.5 μM) showed initiation of multiple shoots followed by stunted growth pattern. On raising BAP concentration to 5μM there was elongation of main shoot which in turn regenerated multiple adventitious roots at basal end of shoots to give complete plantlets on same medium (Fig. 2). All these plantlets showed *in vitro* development of inflorescence after 10 weeks (Fig. 3). Initiation of multiple shoots also took place on MS (x1/2) + BAP (10μM) + NAA (10μM) which was also followed by stunted growth pattern. In contrast, a combination of 2,4-D (5μM) + BAP (10μM) resulted in multiple shoot formation and its subsequent elongation (Fig. 4).

Nodal and internodal segments:

Field grown nodal and internodal segments were inoculated on MS medium supplemented with different growth regulators (Table 3). Nodal segments when inoculated on MS (x1/2) + BAP (5μM) and MS (x1/2) + BAP (10μM) + NAA (10μM) showed initiation of shoot multiplication followed by stunted growth of shoots in both the cases (Figs. 5&6). Internodal segments cultured in presence of all the phytohormones and their combinations that were used for nodal segments showed no response at all.

Hypocotyl culture:

Hypocotyl explants produced various degrees of non-regenerative friable callus (Table 4).

Leaf culture:

Leaf segments from both field grown plants and *in vitro* raised plantlets were cultured on MS medium with different growth regulators viz BAP, NAA, 2,4-D but showed no response at all.

Table 1. *In vitro* germination of *Amaranthus hybridus*

Medium	Nature of Response**	Percentage response*
MS basal medium	Germination slow, normal seedling development	30
MS (x1/2) + BAP (2.5µM)	Germination slow, seedlings developed callus at basal end of hypocotyl.	35
MS (x1/2) + BAP (5µM)	Germination normal but slow.	50
MS (x1/2) + NAA (5µM)	Normal seed germination.	75
MS + BAP (10µM) + NAA (10µM)	Seed germination slow.	25

* 10 replicates per treatment
** data scored after 4 weeks of culture period

Table 2. Morphogenetic response of shoot tips of *A. hybridus* to various phytohormones

Medium*	Nature of Response**	Percentage response	Mean shoot No. (± SD)
MS basal medium	No response	-	-
MS (x1/2) + BAP (2.5µM)	Multiple shoots, stunted growth of shoots	30	3±0.5
MS (x1/2) + BAP (5µM)	Shoot elongation and its growth to form full shoot followed by thin, long and branched root formation, formation of inflorescence.	50	-
MS + BAP (10µM)	No response	-	-
MS + BAP (10µM) + NAA (10µM)	Initiation of shoot multiplication followed by stunted growth	40	4±0.6
MS + 2,4-D (5µM) + BAP (10µM)	Shoot multiplication and elongation	80	8±0.6

* 10 replicates per treatment
** data scored after 4 weeks of culture period

Table 3. Morphogenetic response of nodal and internodal segments of *A. hybridus* to various phytohormones.

Medium*	Nature of Response**	Percentage response
MS basal medium	No response	-
MS (x1/2) + BAP (2.5μM)	No response	-
MS (x1/2) + BAP (5μM)	Initiation of shoot multiplication followed by stunted growth of shoots	50
MS + BAP (10μM)	No response	-
MS + BAP (10μM) + NAA (10μM)	Initiation of shoot multiplication followed by stunted growth	70
MS + NAA (2.5μM)	No response	-

* 10 replicates per treatment
** data scored after 4 weeks of culture period

Table 4. Response of hypocotyl segments of *A. hybridus* to various phytohormonal concentrations and combinations

Medium[a]	Nature of response[a]	Degree of callus formation[b]	Percentage response
MS (x1/2) + BAP (2.5μM)	Light green friable callus formed at the cut ends of the explant	+	100
MS + BAP (10μM) + NAA (5μM)	Dark brown friable callus formed at the ends of the explant	+	100
MS + 2,4-D (5μM) + BAP (10μM)	Light green friable callus formed at the cut ends/base of the explant	++	100

a. data recorded at the end of 4 weeks of culture period/ 10 replicates per treatment
b. + low; ++ moderate

Fig. 1. *Invitro* seed germination of *Amaranthus Hybridus.*

Fig. 2. Response of shoot tips of *Amaranthus hybridus* to MS (x1/2) + BAP (5μM)

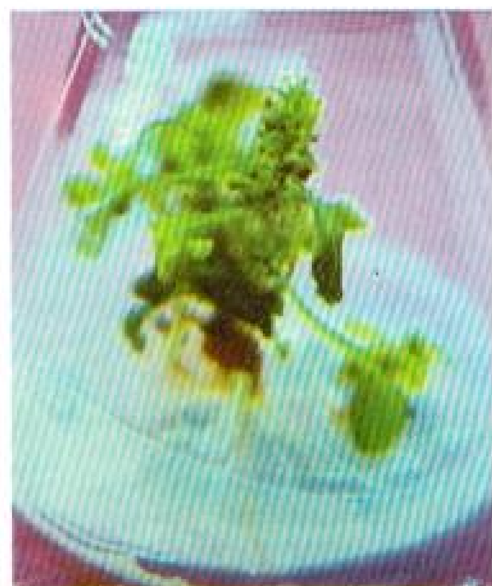

Fig. 3. *Invitro* plantlet formation with inflorescence.

Fig. 4. Response of shoot tips of *Amaranthus hybridus* to MS (x1/2) + 2,4-D (5μM) + BAP (10μM)

Fig. 5. Initiation of shoot multiplication followed by stunted growth of shoots on MS (x1/2) + BAP (5μM)

Fig. 6. Initiation of shoot multiplication followed by stunted growth of shoots on MS (x1/2) + BAP (10μM) + NAA (10μM)

DISCUSSION

The present study was carried out in order to explore the possibility of *in vitro* propagation of this medicinally important vegetable plant. The preliminary tissue culture study carried out showed that plant has a potential for *in vitro* propagation and plantlet regeneration. Out of the various explants used shoot apices showed maximum potential of shoot multiplication followed by nodal segments while other explants - internodal segments, hypocotyl and leaf segments - did not show any shoot multiplication potential.

Scanning of literature reveals that no tissue culture work has been carried out on *A. hybridus* and present brief study is thus the first report of its kind on this plant species. In the present study maximum shoot number was obtained on MS medium enriched with 2,4-D (5µM) + BAP (10µM) using shoot tips as explants. However, nodal segments revealed the potential of shoot multiplication either with BAP alone or when it was used with NAA. These results reveal that the behaviour of regeneration phenomenon shown by the plant is more or less akin to other medicinal plants like *Lupinus polyphyllus* (Kamili *et al.*,2003; Tyub *et al.*,2004), *Lavandula viridis* (Dias *et al.*, 2002), *Artemisia pallens* (Benjamin *et al.*, 1990), *Dioscorea floribunda* (Sengupta *et al.*, 1984), *Plectranthus vetiveroid* (Sivasubramanium *et al.*, 2002) and *Adhateda baddomei* (Sudha and Seeni, 1994).

In hypocotyl segments of *Amaranthus hybridus* only callus formation was recorded on auxin (NAA/ 2,4-D) + cytokinin (BAP) combination which shows similarity of behaviour to *in vitro* processes with other medicinal plants viz. *Dioscorea annua* (Sengupta *et al.*, 1984), *Artemisia pallens* (Benjamin *et al.*,1990).Leaf and internodal segments of *A. hybridus* failed to show any response of *in vitro* growth when cultured on different phytohormonal regimes which express that these explants do not behave like such explants of some other medicinal plants worked out earlier e.g. *Leptadenia reticulate* (Hariharan *et al.*, 2002), *Artemisia annua* (Kamili *et al.*, 2001) and *Piper longum* (Sarasan *et al.*, 1993).

In conclusion present investigation forms an important preliminary step for micropropagation of *Amaranthus hybridus*. Extension of these studies will prove highly beneficial for future strategies in tissue culture studies on propagation and conservation of this plant.

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