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In Vitro Culture of *Brassica oleraceae* var. Gongylodes (Knol Khol)

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ABSTRACT

Various explants from in vitro raised seedlings of *B.oleraceae* var. gongylodes (Knol khol) were cultured on MS medium supplements with different concentrations of growth regulators. Shoot tips and nodal segments when cultured on auxin cytokinin combination i.e. IAA(1mg/l) + BAP(1mg/l) resulted in multiple shoot formation. Isolated microshoots grown in presence of IBA (1mg/l) resulted in root regeneration as well as shoot elongation. Similar results were observed on medium containing BAP (1mg/l) alone. However, friable callus formation from petiole explants was observed on IBA (1&3mg/l) supplemented medium which incase of leaf segments resulted in profuse root induction.

Keywords: Knol khol, shoot tips, nodal segments, petiole, leaf segments, callus, phytohormones regeneration.

Abbreviations: MS-Murashige and Skoog; BAP-6 benzylamino-purine; IAA-indole acetic acid; IBA-indole butyric acid.

INTRODUCTION

Brassica oleraceae var. gongylodes belongs to family Brassicaceae. Nearly 150 species belong to this family. Some of these are consumed as vegetables and some cultivated for oil. These vegetables are rich in vitamins and minerals but in terms of carbohydrate content they come next to cereals. Knol khol, also called kohlrabi, is propagated through seeds. In this species of Brassicaceae no head is formed but the short stem is transformed into a juicy mass of edible tissue which stands out of the ground. It is large spherical and turnip like, white or purple in color with large leaf scars. It is much used for human consumption in several countries including India but in United States it is used chiefly for stock feed. Kohlrabi is an early spring or fall crop as it does not like the heat of summer. In India it is widely grown in Maharashtra, U.P, Punjab and Kashmir (Sharma, 1996). Not

much relevant tissue culture work has been carried on Knol khol as other species of the family have been. As it is much used for human consumption in our state, a study has been made regarding its in vitro culture of various organs so that a complete protocol is developed for its microclonal propagation, *in vitro* conservation and other allied studies.

MATERIAL AND METHODS

Seeds of knol khol (*B. oleraceae* var. gongylodes) were washed with lab detergent(Cedpol) containing few drops of Tween -20(wetting agent)under running tap water for 5-10 minutes. These washed seeds after overnight soaking were surface sterilized by 0.1% $HgCl_2$ for 5-7 min. and finally rinsed 3-4 times with autoclaved double distilled water. Sterilized seeds were inoculated on MS (1962) medium supplemented with 3% sucrose. pH of the medium was adjusted to 5.6 and 0.8% agar was used as jelling agent. The cultures were maintained at 23±2°C with 55-65%RH and exposed to 16h photoperiod provided by cool fluorescent tubes(3000 lux).

RESULTS

Varied morphogenetic responses observed on in vitro culture of seeds and different explants excised from seedlings of *B.oleraceae* var. gongylodes and cultured on MS medium supplemented with different concentrations and combinations of growth regulators are summarized in Table 1. The seeds of knol khol when cultured on basal medium resulted in full-fledged seedling formation (Fig.1a). Excised shoot tips and nodal segments from these seedlings when cultured on medium supplemented with IAA (1mg/l)+ BAP (1mg/l) resulted in multiple shoot formation and root regeneration (Fig.1b &1c). Similar results were observed when IAA (2mg/l) + BAP(2mg/l) was used in the medium(Fig.1d). Isolated microshoots grown in presence of IBA (1mg/l)exhibited adventitious root regeneration ,condensed stem formation as well as shoot elongation (Fig.2a). Similar response was observed on BAP (1mg/l)supplemented medium(Fig.2b). Petiole explants responded only to friable callus formation when cultured on medium containing IBA (1&3mg/l)(Fig.2c).In contrast to this root induction was observed when leaf segments were cultured on same IBA concentrations (Fig.2d).

DISCUSSION

Present findings reveal high morphogenetic potential of various explants of *B. oleraceae* var.gongylodes. Multiple shoot formation was observed by culturing shoot tips

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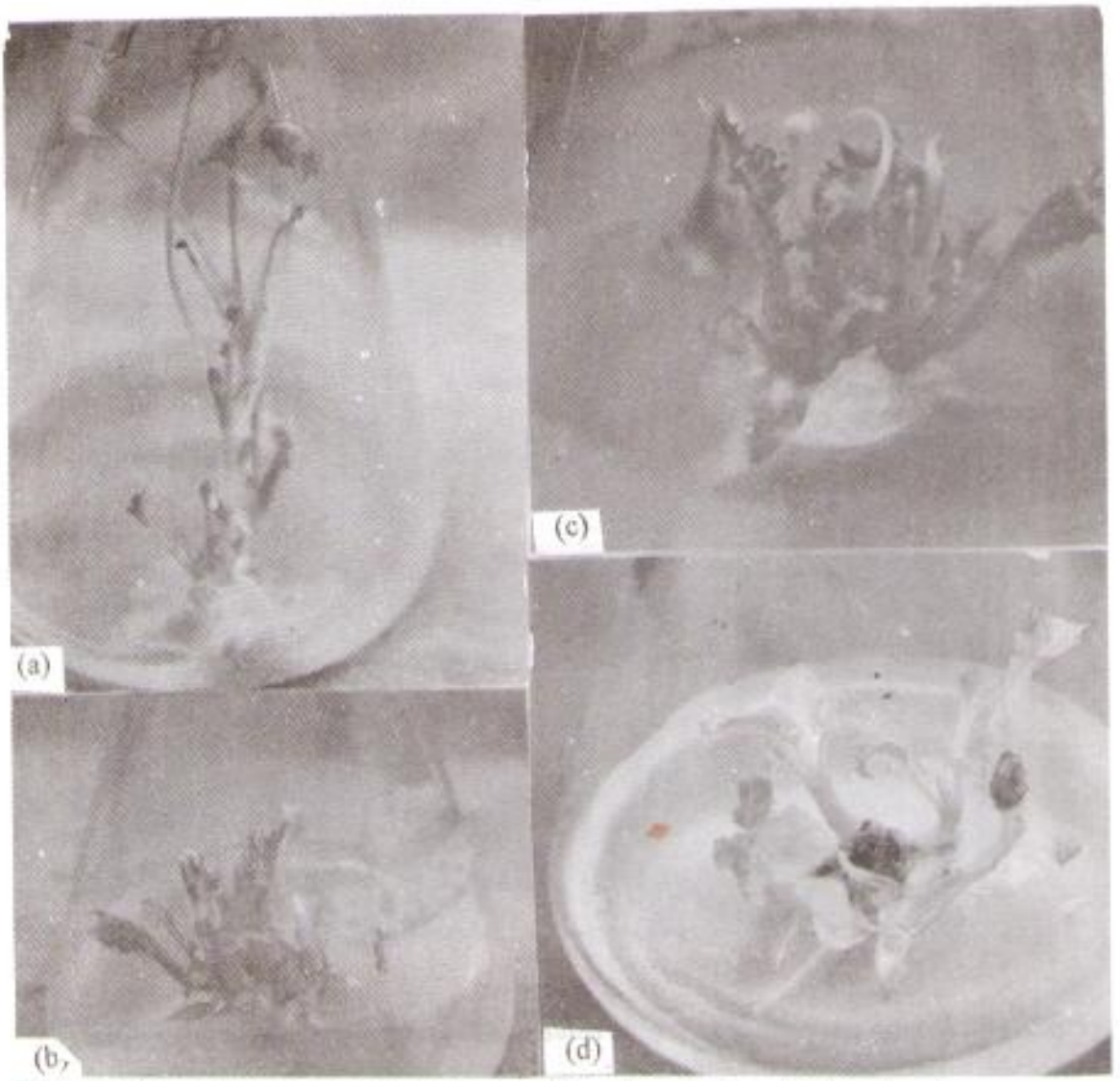

Fig.1 (a-d) ***In vitro*** **regeneration from cultured explants of** ***Brassica oleraceae*** **var.gongylodes.**

(a) *In vitro* seed germination and seedling formation on MS basal medium (after 2 weeks)

(b) Multiple shoot formation and root regeneration from shoot tip explants on MS+ IAA(1mg/l) +BAP (1mg/l) (after 4 weeks).

(c) Shoot multiplication and root regeneration from nodal segments on MS+ IAA (1mg/l) +BAP (1mg/l) (after 4 weeks).

(d) Multiple shoot and root regeneration from shoot tip explants on MS+ IAA (2mg/l) +BAP (2mg/l) (after 4 weeks).

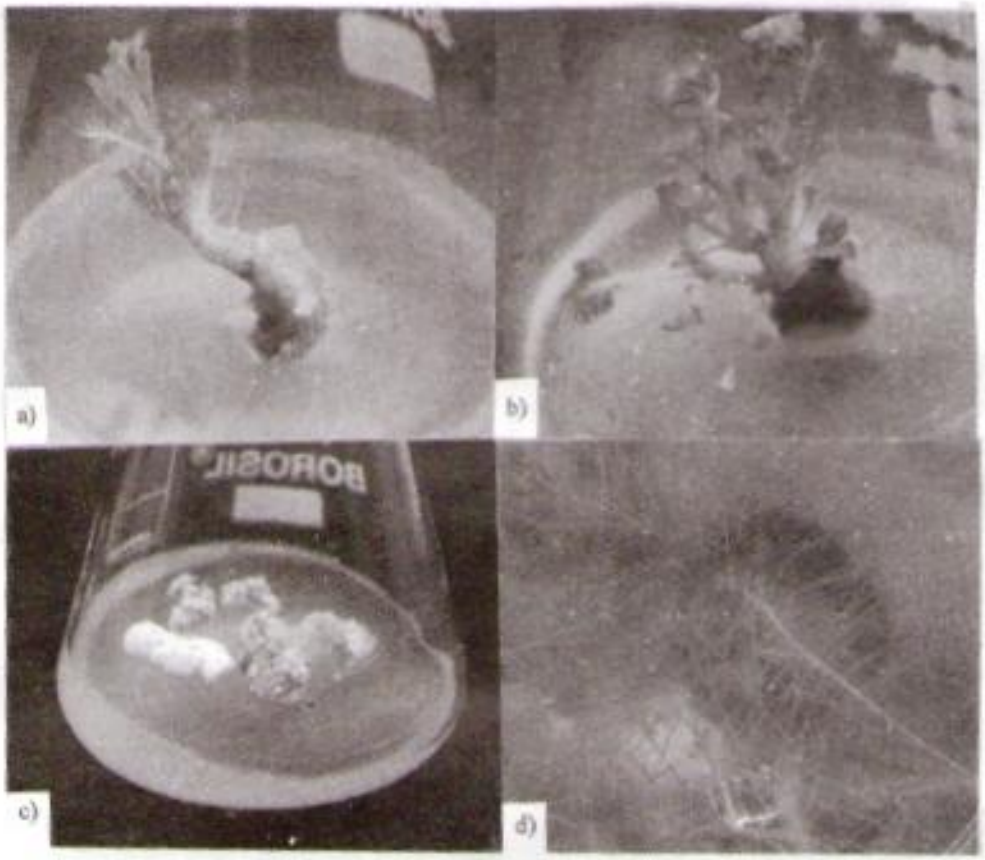

Fig.2 (a-d) ***In vitro*** **response of cultured explants of** ***Brassica oleraceae*** **var.gongylodes.**

(a) Shoot growth, condensed stem and adventitious root formation in isolated microshoots on MS+ IBA(1mg/l)(after 4 weeks)

(b) Shoot growth, condensed stem and adventitious root formation in isolated microshoots on MS+BAP(1mg/l)(after 4 weeks)

(c) Friable callus formation in petiole explants on MS+IBA(3mg/l)(after 4 weeks)

(d) Profuse root regeneration in leaf explants on MS+IBA(3mg/l)(after 4 weeks)

Table 1. Morphogenetic response of different explants of *Brassica oleraceae* var. gongylodes to various phytohormonal concentrations and combinations.

MEDIUM	EXPLANT	NATURE OF RESPONSE*	DEGREE OF CALLUS FORMATION	%age RESPONSE
MS basal	Seeds	Seedling formation	-	90
MS + IAA(1mg/l)+BAP (1mg/l)	Shoot tips	Multiple shoot formation and root regeneration	-	100
-do-	Nodal segments	-do-	-	100
MS +IAA(2mg/l)+ BAP (2mg/l)	Shoot tips	Multiple Shoot formationa and root regeneration	-	100
- do-	Nodal segments	-do-	-	100
MS +IBA(1mg/l)	Microshoots	Shoot growth and adventitious root formationa at cut end	-	60
MS+BAP(1mg/l)	Microshoots	-do-	-	80
MS+ IBA(1mg/l)	Petiole	Friable callus formation	+	80
MS+ IBA(3mg/l)	Petiole	-do	+++	100
MS +IBA(1mg/l)	Leaf	Root regeneration	-	60
MS+ IBA (3mg/l) Seeds	Leaf	-do-	-	80

*Mean of 10 replicates; data scored at the end of 4 weeks of culture period.
-no growth, +low, +++high.

and nodal segments on medium supplemented with combination of auxin IAA(1mg/l) and cytokinin BAP (1mg/l). This is in accordance with the observations of Loudon *et.al* (1989) in Brassica species, Chung and Jee(1988) in flowering cabbage and Lollio and Olsen (1989) in *B. oleracaea* var. acephala and *B. capitata* who achieved the similar results on auxin cytokinin combination. Similar results were observed when the concentration of auxin and cytokinin was raised to 2mg/l each.

Maximum root regeneration from leaf segments was observed on medium supplemented with IBA (1&3 mg/l). This is in conformity with the findings of Murata and Orton (1987) in *Brassica* species who also observed root induction on auxin IAA (2-5mg/l). Shoot development and root formation from microshoots was observed in presence of BAP (1mg/l). These results are favoured by the reports of Kamili *et.al* (2001) on *B.oleraceae* var. acephala who observed the similar results on MS medium supplemented with BAP(1mg/l). Callus formation was recorded from petiole explants when auxin alone was present in the medium. These findings are again in agreement with those of Murata and Orton (1987) in Brassica species. Present results indicate that all the explants of *B. oleraceae* var. gongylodes posseses the potentiality to regenerate and produce plants in multiples which can be exploited for large scale production of the crop keeping in view the increasing population trend of India in general. Furthermore, the micropropagation studies can also prove beneficial for conserving the germplasm through in vitro techniques and for genetic transformation studies.

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