HAAK in a Test Tube

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ABSTRAT

Various explants viz. Shoot tips, nodal, hypocotyl, root and leaf segments from in vitro raised seedlings of Haak (*Brassica oleracea* L. var acephala L.) were cultured on MS medium supplemented with different growth regulators either alone or in combinations. Shoot tips when cultured on BAP (5μM) and IBA (10μM) supplemented medium produced multiple shoots. Micro cuttings rooted directly when cultured on IBA (5μM) enriched medium. Hypocotyl segments produced callus with a combination of BAP (5μM) and IBA (10μM) followed by its differentation into shoots and roots on the same medium. However, direct shoot induction was oboserved when Kn (10μM) was used alone in hypocotyl segments. Nodal explants produced multiple shoots and roots under the influence of NAA (10μM) supplemented MS medium followed by their elongation on the basal medium. Multiple shoots and roots were also produced when leaf and root explants were cultured on IBA (10μM) and BAP (10μM) + NAA (5μM) supplemented media, respctively.

Keywords: Haak, acephala, shoot tip, nodal segment, leaf segment, root segment, differentiation, multiple shoots.

Abbreviations: MS — Murashige and Skoog; BAP —6 benzyl amino purine; IBA —Indole butyric acid; NAA — Napthalene acetic acid; Kn — Kinetin.

INTRODUCTION

Haak (*Brassica oleracea* L. var. acephala.L.) belongs to family Brassicaceae, which consists of over 150 species of annual or biennial herbs several of which are cultivated as oil seed crops or vegetable crops. Haak is grown in some parts of India especially in Kashmir. It is commonly propagated through seeds and is regarded as the hardest cole crop, which withstands drought or temperatures of -10° C to 15°C. It is rich in fats, proteins, carbohydrates, fibre, minerals and vitamins (Anonymous, 1948). Presence of isothiocyanate, sulforaphane and other compounds in plants of family Brassicaceae have strong cancer preventing properties (Anonymous, 2001).

A number of species from family Brassicaceae have been exploited for tissue culture purposes (Elmshener *et al.*, 1978; Genteby and Cocking, 1977; Horak, 1972; Horak *et al.*, 1971 and Landa and Lustinec 1971). But till date no study has

been made regarding the assessment on in vitro morphogenetic potential of Haak (*B. oleracea* L. var.acephala L.). Since this plant grows abundantly in Kashmir and is almost daily consumed by every household, it becomes imperative to assess the morphogenetic response of its different vegetative parts to different phytohormones in an in vitro culture system so that a complete protocol for its clonal propagation is worked out for its future research strategies and in vitro conservation. An attempt was, therefore, made to work out the possibility of using in vitro technique for clonal propagation and multiplication, which have proved to be successful.

MATERIAL AND METHODS

Authentic seeds of Haak (*Brassica oleracea* L. var. acephala L.) after overnight soaking were sterilized with $HgCl_2$ (0.1%) for 5-7 minutes followed by three times rinsing with autoclaved double distilled water on laminar air flow. The sterile seeds were then inoculated on MS (1962) medium supplemented with BAP (10μM) and NAA (5μM). After one week 5 cm long seedlings were produced (Fig. 1). Various explants like shoot tips, nodal segments, hypocotyl and leaf segments from these in vitro raised seedlings were inoculated on MS medium supplemented with 3% sucrose and different growth regulators. The rest of methodology followed was same as in one previous communications.

RESULTS

Effects of various concentrations of growth hormones on different explants of var. acephala Haak are summarized in Table 1. Shoot tips and hypocotyl segments when cultured under the combined effect of BAP (5μM) and IBA (10μM) produced 6 –12 healthy shoots followed by their simultaneous rooting after 4- weeks (Fig.2). The regeneration of shoots and roots was direct in shoot tips and indirect through callus in hypocotyl segments on the same growth adjuvants (Fig. 3). One cm long shoot tips also regenerated roots directly without multiplication in presence of IBA (5μM) after 4 weeks (Fig.4). Hypocotyl segments exhibited direct shoot regeneration when cultured in presence of Kn (10μM).

Nodal segments revealed direct multiple shoot and root induction on medium augmented with NAA (10μM) whereas leaf segments regenerated indirectly under the influence of IBA (10μM) after 4 weeks of culture period. It was followed by their elongation on hormone free medium (Fig. 5 and 6).

Indirect regeneration of shoots and roots from callus was also observed when root segments were cultured in presence of BAP (10μM) and NAA (5μM) combination after 4 weeks. (Fig.7).

g. 1-7: In vitro culture of different explants of Haak (*B. oleracae* var. acephala L.)

1. Seed germination on MS basal medium
2. Multiple shoot formation from shoot tip explants and their proliferation on MS + BAP (5μM) + IBA (10μM).
3. Indirect shoot and root formation from shoot tip explants on MS + BAP (5μM) + IBA (10μM).
4. Direct root regeneration from shoot tip explants on MS + IBA (5μM).
5. Shoot and root regeneration from nodal explants on MS + NAA (10μM).
6. Shoot and root regeneration from leaf segment on MS + IBA (10μM).
7. *Shoot and root regeneration from root explants on MS + BAP (10μM) + NAA (5μM).*

DISCUSSION

The present investigations were carried out to assess the morphogenetic potential of various explants of Haak (*B. oleracea* L. var. acephala L.). In present findings maximum shoot and root regeneration was achieved by culturing shoot tip explants on MS medium supplemented with BAP (5μM) and IBA (10μM), which is in accordance with the findings of Loudon *et al.* (1989) in *Brassica* spp. Observations on shoot tips producing roots directly without undergoing multiplication on MS + IBA (5μM) medium are again in conformity with those of Loudon *et al.* (1989) but are in contrast to observations of Murata and Oroton (1987) in *Brassica* spp. who observed complete shoot and root regeneration with NAA (13μM).

Present studies revealing indirect regeneration by culturing hypocoty. segments on BAP (5μM) and IBA (10μM) supplemented medium are in disagreement with those of Lillo and Oleson (1989) who observed only callus formation on 2,4-D (4.5μM) + NAA (0.5μM) + BAP (2.2μM) in *Brassica oleracea* var. acephala and capitata. Subsequent subculture of this callus on BAP (4.4μM) + GA_3 (0.3μM) enriched medium lead to differentiation of shoot and root.

Multiple shoot and root formation were also observed by culturing nodal explants on NAA (10μM) fortified medium which is quite contrary to the findings of Loudon *et al.* (1989) in *Brassica* Spp. who achieved root and shoot regeneration by using a combination of 2,4-D (4.3μM) + BA (2.2μM) + NAA (0.5μM). Complete regeneration of plantlets was also observed in leaf segments when cultured in presence of IBA (10μM) alone which is again in contrast to findings of Hosoki *et al.* (1989) who used the combination of NAA (0.5μM) + Zeatin (5μM) in *Brassica oleracea* var. acephala but is in accordance with the observations of Murata and Oroton (1987) in *Brassica* spp. and Chung and Jee (1988) in flower cabbage.

Culture of root segments on BAP(10μM) and NAA (5μM) lead to indirect shoot and root regeneration which is contrary to the observations of Lillo and Oleson (1989) in *Brassicu oleracea* var. acephala and capitata but in accordance with the observations of Chung and Jee (1989) in flower cabbage. It is concluded from the present studies that morphogenetically all the explants of the Haak, used are highly responsive in terms of shoot and root regeneration and hence will serve as basis for future research in such a direction.

Table 1. Morphogenetic response of different explants of *Brassica oleracea* var. acephala Haak to different phytohormones

Medium	Explant	Nature of response *	Degree of callus induction	%age of shoot/root regeneration
MS basal medium (Control)	Shoot tips	-	-	-
MS+NAA(10μM)	-do-	Friable callus formation at the basal end followed by white hairy root and shoot regeneration	++	60
MS +2, 4-D (10μM)	-do-	Friable callus induction at the basal end followed by explant elongation	++	-
MS+ BAP (10μM)	-do-	Compact callus formation followed by shoot and root regeneration	++	60
MS+IBA (5μM)	-do-	Direct root formation at the basal end	-	30
MS+BAP(5μM) + IBA (10μM)	Shoot tips and hypocotyl segments	Compact callus formation followed by multiple shoot and root regeneration	+	100
MS+Kn (10μM)	Hypocotyl segments	Shoot and root formation	-	50
MS+BAP(10μM)	-do-	Shoot and root formation	-	60
MS+ BAP (10μM) + NAA(5μM)	Nodal segments	Compact callus formation followed by shoot regeneratioin	++	70
MS+IAA (5μM)	-do-	Compact callus formatioin, no differentiation	+	-
MS+IBA (5μM)	Leaf segments	Compact callus formation followed by shoot and root regeneration	++	60

Table 1 contd.

Medium	Explant	Nature of response *	Degree of callus induction	%age of shoot/root regeneration
MS+IBA (10μM)	-do-	Compact callus formation, Multiple shoot and root formatioin	+	100
MS+ BAP (10μM)+NAA (10μM)	Root segments	Compact callus formatioin followed by shoot and root regeneration (Indirect)	++	50

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

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