*International J. Seed Spices 5(2), July 2015:63-66*

# Plant regeneration in coriander (*Coriandrum sativum* L.)

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## Abstract

The present investigation was undertaken with the objective to realizing the regeneration ability of hypocotyl explant of *in vitro* germinated seedlings of coriander and find out suitable combination of plant growth regulators for regeneration in coriander. Various Plant Growth Regulators (PGRs) i.e Auxin (IAA, NAA and 2,4-D), Cytokinines (BAP and kinetins) and Gibberellins ($GA_3$) were used alone or in combinations. Most of the treatments produced good amount of non organogenic callus. Hypocotyl explants incubated on MS medium supplemented with various combination of NAA and IAA showed shoot regeneration from organogenic callus. Regenerated shoots when placed on MS medium supplemented with IBA 0.5 mg/l produced healthy roots. These results are helpful in refinement of regeneration protocol in coriander which is a pre requisite for a crop improvement programme, both for gene insertion and expression.

**Key words**: Auxins, coriander, hypocotyls, organogenesis, PGRs

## Introduction

Coriander is an important seed spice crop belonging to the family *apiaceae* having prime position in flavouring food. In India coriander is grown throughout the sub continent either for green leaf purpose or for seeds. Rajasthan, Gujarat, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Himachal Pradesh are the major coriander growing states in India. Most of the varieties developed so far are based on selection since hybridization is difficult due to small flower size. Use of modern tools of biotechnology for crop improvement in coriander required a reproducible and highly regenerating *in vitro* protocol, both for gene insertion and expression. Limited information is available on response of somatic tissues to differentiate and develop plantlets *in vitro* from callus culture of Coriander (*Coriandrum sativum* L.). There are some reports of regeneration in coriander using hypocotyls explants (Mujib *et al.,* 7; Kim *et al.,* 4; Stephen and Jayabalan 11; Murthy *et. al.,* 9). Most of the workers used 2, 4-D as the source of auxin. There are reports that use of 2, 4-D in the culture medium may lead to somaclonal variations (Torrey 13; Mitra and Steward, 6; Melchers, 5 and Sunderland, 12). Use of either IAA or NAA in culture medium exhibited less mutagenic effect (Sunderland 12; Chand and Roy, 1). The present investigation was undertaken with the objective of realizing the regeneration ability of hypocotyl explant in the presence of auxins, cytokinins, gibberellins alone and in their suitable combination.

## Materials and methods

Clean and healthy seeds of coriander genotype ACr-1 were obtained from seed store of ICAR-National Research Centre on Seed Spices, Ajmer and surface sterilized with 0.1% Mercuric chloride for 4 min and thoroughly washed with sterilized distilled water 4-5 times. These surface sterilized seeds were placed in a sterilized test tube on a filter paper for germination in a BOD at 25°C; the lower half of filter paper was immerged in distilled water. Hypocotyl segment from 10-15 days old germinated seedlings were used as explants and inoculated on MS medium (Murashige and Skoog, 8). MS medium was supplemented with auxins (NAA 0.0, 0.1, 0.5, and 1.0 mg $L^{-1}$, IAA 0.0, 0.1, 0.5, and 1.0 mg $L^{-1}$ and 2,4-D 0.0, 0.1, 0.3, 0.5, 0.8 and 1.0 mg $L^{-1}$), cytokinins (BAP 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg $L^{-1}$, Kn 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg $L^{-1}$), gibberellins ($GA_3$ 0.0, 0.1, 0.3, 0.5, 0.8 and 1.0 mg $L^{-1}$) alone and their combinations (Table 1). Cultures were incubated at 27±1°C under fluorescent light in a 14/10 h photo-period. Light intensity was maintained at 35 µmol $M^{-2}S^{-1}$ at bench level. Observations were recorded periodically on number of explants inoculated and responded, morphology of callus and number of shoots explant$^{-1}$. Number of explants inoculated, number of explants responded, morphology of callus and number of shoots/explants were recorded periodically and final at the 30 days of culture.



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## Results and discussion

### *Effect of auxins*

Effect of Auxins (IAA/NAA) on callus induction and organogenesis is presented in Table 1. Maximum responded explant (70%) was observed in treatment $T_1$, $T_2$ and $T_3$ having IAA 0.3, 0.5 and 0.8 mg $L^{-1}$ in the medium. NAA was less pronounced in response. However, in both IAA and NAA (0.5 mg $L^{-1}$) supplemented medium organogenesis was observed (Plate 1 A, B & C). In combination of IAA and NAA, explants inoculated on $T_{31}$ (MS medium supplemented with 1.0 mg NAA and 0.3 mg IAA) showed 100% response which is followed by $T_{26}$, $T_{27}$, $T_{32}$ and $T_{34}$ by showing 90% responded explants. Minimum 50% explants were responded on medium supplemented with NAA 0.3 mg $L^{-1}$and IAA 1.0 mg $L^{-1}$.

**Plate 1 A:** Shoot regeneration on MS medium supplemented with 0.5mg IAA

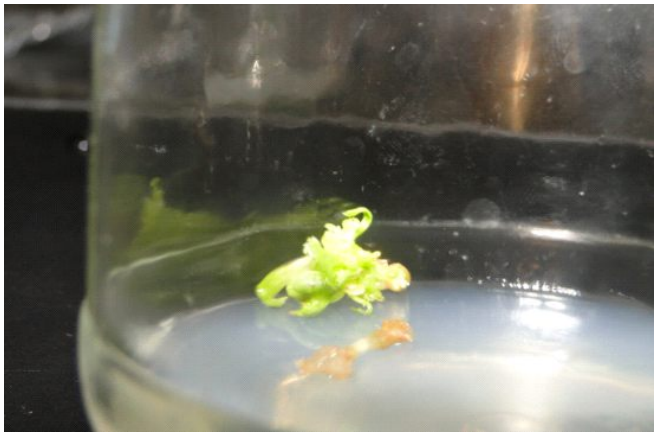

**Plate 1 B:** Shoot regeneration on MS medium supplemented with 0.5mg NAA

### *Effect of cytokinins/ gibberellin*

When cytokinins (BAP and kinetin) were supplemented in MS medium singly in suitable concentration, treatment $T_9$ (Kn 1.0 mg $L^{-1}$) showed 90% responded explants followed by other treatments of this group i.e. $T_7$, $T_8$, $T_{10}$, $T_{11}$ and $T_{12}$ (80%). Adding auxin with cytokinin showed no synergistic effect on organogenesis, however, produced good amount of non-organogenic callus.

### *Effect of PGRs combinations*

When kinetin was supplemented in MS medium with auxin 2, 4-D, a good amount of callus was produced in each treatment however; produced callus was non-organogenic in all the treatment combination. Similarly BAP in combination with $GA_3$produced non-organogenic callus in all the treatments. Regenerated shoots from different treatments were harvested and inoculated on MS medium supplemented with IBA 0.5 mg/l. All the shoots produced good quality roots (Plate 2 A & B). In present study out of several hormonal combinations regeneration was found only on IAA and NAA alone or in combination. Hypocotyl explant proved to be best due to its response to various PGRs and easy to handle in comparison to other explant. It appears to check some more sources of auxins with different combination of one/two type of cytokinins for enhancing the frequency of regeneration in coriander.

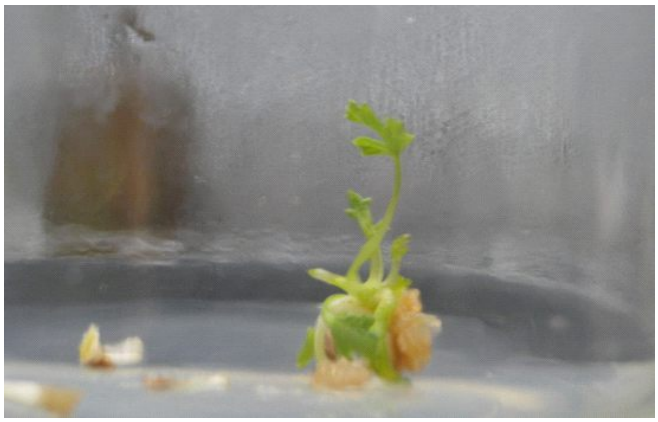

**Plate 1 C** : Organogenic callus on MS medium supplemented with combinations of IAA and NAA each 0.5 mg/l

In a previous report, shoot organogenesis from hypocotyls explants of cumin variety GC 4 was observed on MS and Gamborg's medium supplemented with TDZ at low concentration (0.5-1.0 mg $L^{-1}$) either alone or with NAA (0.1-1.0 mg $L^{-1}$) (Sirajahamad, 10). In present investigation too NAA/IAA alone at low concentration induced organogenesis in coriander.

Callus morphology is an important trait for assessment of morphogenic potential of callus. Fragile callus generally proved non organogenic while green compact callus is an indicative of organogenic callus. In present investigation almost all type of callus morphology was observed. In green compact callus some shoot buds like

**Plate 2 A:** regenerated shoots placed on MS medium supplemented with IBA 0.5 mg/l for root induction

**Plate 2 B:** Rooted regenerated shoot of coriander

**Table 1 :** Effect of PGRs alone and in combinations on morphogenic potential of hypocotyls callus of coriander var ACr 1

| T | PGR combinations | % explants responded | Remark | T | PGR combinations | % explants Responded | Remark |
|---|---|---|---|---|---|---|---|
| $T_0$ | MS medium without PGR | 00 | * | $T_{35}$ | IAA 0.3mg + BAP 1.0mg | 60 | *** |
| $T_1$ | IAA 0.3mg | 70 | *** | $T_{36}$ | IAA 0.5mg + BAP 1.0mg | 60 | *** |
| $T_2$ | IAA 0.5mg | 70 | **** | $T_{37}$ | IAA 0.8mg + BAP 1.0mg | 60 | *** |
| $T_3$ | IAA 0.8mg | 70 | *** | $T_{38}$ | NAA 0.3mg + BAP 1.0mg | 50 | *** |
| $T_4$ | NAA 0.3mg | 10 | ** | $T_{39}$ | NAA 0.5mg + BAP 1.0mg | 70 | *** |
| $T_5$ | NAA 0.5mg | 50 | *** | $T_{40}$ | NAA 0.8mg + BAP1.0mg | 50 | *** |
| $T_6$ | NAA 0.8mg | 40 | **** | $T_{41}$ | Kin 0.5mg + 2,4-D 0.5mg | 80 | *** |
| $T_7$ | Kin 0.5mg | 80 | *** | $T_{42}$ | Kin 0.5mg + 2,4-D 0.8mg | 90 | *** |
| $T_8$ | Kin 0.8mg | 80 | *** | $T_{43}$ | Kin 0.5mg + 2,4-D 1.0mg | 80 | *** |
| $T_9$ | Kin 1.0mg | 90 | *** | $T_{44}$ | Kin 0.8mg + 2,4-D 0.5mg | 80 | *** |
| $T_{10}$ | BAP 0.1mg | 80 | *** | $T_{45}$ | Kin 0.8mg + 2,4-D 0.8mg | 60 | *** |
| $T_{11}$ | BAP 0.5mg | 80 | *** | $T_{46}$ | Kin 0.8mg + 2,4-D 1.0mg | 90 | *** |
| $T_{12}$ | BAP 1.0mg | 80 | *** | $T_{47}$ | Kin 1.0mg + 2,4-D 0.5mg | 100 | *** |
| $T_{13}$ | 2,4-D 0.5mg | 50 | *** | $T_{48}$ | Kin 1.0mg + 2,4-D 0.8mg | 70 | *** |
| $T_{14}$ | 2,4-D 0.8mg | 80 | *** | $T_{49}$ | Kin 1.0mg + 2,4-D 1.0mg | 80 | *** |
| $T_{15}$ | 2,4-D 1.0mg | 80 | *** | $T_{50}$ | BAP 0.1mg + $GA_3$ 0.1mg | 50 | *** |
| $T_{16}$ | $GA_3$ 0.1mg | 60 | *** | $T_{51}$ | BAP 0.1mg + $GA_3$ 0.3mg | 80 | *** |
| $T_{17}$ | $GA_3$ 0.3mg | 70 | *** | $T_{52}$ | BAP 0.1mg + $GA_3$ 0.5mg | 10 | *** |
| $T_{18}$ | $GA_3$ 0.5mg | 70 | *** | $T_{53}$ | BAP 0.5mg + $GA_3$ 0.1mg | 80 | *** |
| $T_{19}$ | NAA 0.3mg + IAA 0.3mg | 60 | *** | $T_{54}$ | BAP 0.5mg + $GA_3$ 0.3mg | 60 | *** |
| $T_{20}$ | NAA 0.3mg + IAA 0.5mg | 50 | *** | $T_{55}$ | BAP 0.5mg + $GA_3$ 0.5mg | 70 | *** |
| $T_{21}$ | NAA 0.3mg + IAA 0.8mg | 50 | *** | $T_{56}$ | BAP 1.omg + $GA_3$ 0.1mg | 80 | *** |
| $T_{22}$ | NAA 0.3mg + IAA 1.0mg | 60 | *** | $T_{57}$ | BAP 1.0mg + $GA_3$ 0.3mg | 80 | *** |
| $T_{23}$ | NAA 0.5mg + IAA 0.3mg | 70 | *** | $T_{58}$ | BAP 1.0mg + $GA_3$ 0.5mg | 80 | *** |
| $T_{24}$ | NAA 0.5mg + IAA 0.5mg | 80 | **** | $T_{59}$ | kin 0.1mg + $GA_3$ 0.1 mg | 60 | *** |
| $T_{25}$ | NAA 0.5mg + IAA 0.8mg | 80 | *** | $T_{60}$ | kin 0.1mg + $GA_3$ 0.3 mg | 60 | *** |
| $T_{26}$ | NAA 0.5mg + IAA 1.0mg | 90 | *** | $T_{61}$ | kin 0.1mg + $GA_3$ 0.5 mg | 00 | * |
| $T_{27}$ | NAA 0.8mg + IAA 0.3mg | 90 | *** | $T_{62}$ | kin 0.1mg + $GA_3$ 0.8 mg | 70 | *** |
| $T_{28}$ | NAA 0.8mg + IAA 0.5mg | 60 | *** | $T_{63}$ | kin 0.5mg + $GA_3$ 0.1 mg | 70 | *** |
| $T_{29}$ | NAA 0.8mg + IAA 0.8mg | 50 | *** | $T_{64}$ | kin 0.5mg + $GA_3$ 0.3 mg | 00 | * |
| $T_{30}$ | NAA 0.8mg + IAA 1.0mg | 70 | *** | $T_{65}$ | kin 0.5mg + $GA_3$ 0.5 mg | 00 | * |
| $T_{31}$ | NAA 1.0mg + IAA 0.3mg | 100 | *** | $T_{66}$ | kin 0.5mg + $GA_3$ 0.8 mg | 00 | * |
| $T_{32}$ | NAA 1.0mg + IAA 0.5mg | 90 | *** | $T_{67}$ | kin 1.0mg + $GA_3$ 0.1 mg | 00 | * |
| $T_{33}$ | NAA 1.0mg + IAA 0.8mg | 80 | *** | $T_{68}$ | kin 1.0mg + $GA_3$ 0.3 mg | 00 | * |
| $T_{34}$ | NAA 1.0mg + IAA 1.0mg | 90 | *** | $T_{69}$ | kin 1.0mg + $GA_3$ 0.3 mg | 00 | * |
| | | | | $T_{70}$ | kin 1.0mg + $GA_3$ 0.8 mg | 00 | * |

* No response, ** No callus, *** Non organogenic callus, **** Organogenic callus

structures were visible initially but could not convert in to shoot. The explants showing regeneration produced small amount of callus at cut end of hypocotyls. Unlike other crop species coriander is very difficult to regenerate and considered recalcitrant species. Redifferentiation from dedifferentiated tissue is very difficult in this crop. There is meager report of somatic embryogenesis in coriander like other crops of *apiaceae* family.

Plant growth regulators have been found to be important factors affecting the rate of regeneration of *de novo* shoot buds from excised explants. Shoot differentiation appears to be a function of cytokinin because auxin alone did not initiate shoot development. The combined use of cytokinin and auxin improved the efficiency of regeneration although this depended on the combinations and concentrations employed. In present investigation however, auxin alone is more effective than in combination with cytokinins. Gayatri *et.al.,* (2) and Ignacimuthu *et al*., (3) reported Plant regeneration with the use of NAA (0.5**µ**M) through somatic embryogenesis from mature leaf explants of *Eryngium foetidum*. L. is an aromatic plant grown as a leafy vegetable commonly known as spiny coriander belongs to the family umbelliferae. The regenerated shoots were rooted and elongated on MS medium supplemented with 0.1 mg $l^{-1}$ IAA and 1.0 mg $l^{-1}$ $GA_3$. These plantlets were hardened and transferred to the soil.

In present investigation fairly good number of PGR combination has been tried. Hypocotyl explant proved to be best due to its response to various PGRs and easy to handle in comparison to other explant. It appears to check some more sources of auxins with different combination of one/two type of cytokinins for enhancing the frequency of regeneration in coriander.

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Received : February 2015; Revised : April 2015; Accepted : May 2015.