E-ISSN: 2706-8927
P-ISSN: 2706-8919
www.allstudyjournal.com
IJAAS 2019; 1(1): 244-246
Received: 12-05-2019
Accepted: 17-06-2019

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Exploration studies on biochemical markers associated with regeneration potential in *Cuminum cyminum* L.

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Abstract
Embryogenesis and organogenesis have been carried out in Cumin from various explants obtained from *in vitro* raised seedlings. Biochemical analysis was performed with various types of callii obtained, *viz*; embryogenic, non-embryogenic, organogenic and non-organogenic. Total protein content was found to be maximum in embryogenic callus obtained from root explants (19.50 mg/g tissue), while peroxidase activity was higher in non-organogenic callus obtained on TDZ (Thidiazuron) supplemented media from hypocotyl explant (0.15/mg protein).

Keywords: Embryogenic, non-embryogenic, organogenic, non-organogenic

Introductions
Cumin (*Cuminum cyminum* L.) is an annual plant that is not only one of the most popular seed species but also one of the oldest and most cultivated aromatic and herbaceous natural products with numerous medicinal, nutraceutical, and pharmaceutical properties. It is widely used in the beverage, food, liquor, medicine, perfume, and toiletry industries. The objective of this work was to provide a precise and up-to date review of the ethnopharmacology, phytochemistry, and biological activities of cumin.
Cumin (*Cuminum cyminum* L.) belonging to family apiaceae is a popular spice worldwide. Its essential oil component cuminaldehyde (97.7%) makes it important for medicinal purposes with astringenic, carminative, stomachic, antimicrobial and antifertility properties [1]. Wilt, powdery mildew and blight are important diseases of cumin which frequently attack the crop and cause heavy losses in seed yield and quality. No genetic base is available in cumin against resistance to blight and wilt. Exploitation of somaclonal variations or introduction of alien gene could prove useful in such conditions. *In vitro* culture of cells, tissues and organs on chemically defined medium for achieving organogenesis and somatic embryogenesis have generated great interest now a days and attempts are being made to understand the mechanism underlying the processes. It has been known for some time that changes in gene expression occur when the somatic cells embark on embryogenic development as evinced by the increased organic decarboxylase activity, increased enzyme activities in pyrimidine pathway and synthesis of embryo specific proteins in embryogenic cultures of carrot [2]. Although biochemical and molecular approaches have been increasingly used since the protein work reported by Hahne in 1988 [3] events underlying embryogenesis remain poorly understood [4, 5]. Present study aims at the understanding of embryogenic competence associated markers especially peroxidases as a number of reports correlated them with embryogenic potential.

Material and Methods
The proposed study was conducted on cumin genotype; RZ-19. Seeds were procured from SKN College of Agriculture, RAU, Jobner. Seeds were surface sterilized with 0.1% (w/v) $HgCl_2$ for 3 minutes then rinsed 3-4 times with sterile distilled water. Seeds were incubated in dark at 22 ºC. After seven days germinated seedlings were shifted in culture room condition i.e., 16-h photoperiod, 50 µ mol m^{-2} $s,^{-1}$ light intensity and 26 ºC temperature. Roots, hypocotyl segments, cotyledonary leaves and shoot apex of 14 days old seedlings were excised and used as explant for embryogenic and organogenic callus induction. Embryogenic and non embryogenic callus obtained on MS (6)+0.5 mgl^{-1} 2, 4-D (2, 4-Dichlorophenoxy acetic acid) after 35 days of inoculation from all the four explants *viz*., root, hypocotyl, cotyledon and shoot apex, and organogenic and non-organogenic callus obtained on MS + 0.1 mgl^{-1}

TDZ after 40 days of inoculation from hypocotyl explants of cumin were used for biochemical analysis. Samples were homogenized 1gm/3ml of 1M phosphate buffer (6.5 pH) on ice and then centrifuged at 18000 x g for 10 min at 5 ºC. Supernatant was used for protein analysis and enzyme assay.

Protein Estimation

For precipitation of protein 0.2 ml of supernatant was mixed with 10% TCA and kept at 4 ºC for 12 hrs. It was then centrifuged at 2000 x g for 20 min. Pellet so formed was dissolved in 0.4 ml 1N NaOH and made up to 2 ml with distilled water. Protein was estimated in all the samples [7].

Peroxidase Assay

To 3.5 ml phosphate buffer (pH 6.5), 0.2 ml sample enzyme extract and 0.1 ml freshly prepared o'dianisidine solution (1mg/ml methanol) was added in a cuvette. Incubated assay mixture to 28 ºC-30 ºC and then added 0.2 ml, 0.2 MH_2O_2, mixed and immediately placed in spectrophotometer. Started stop watch and read initial absorbance at 430 nm, then at every 30 sec intervals up to 3 min. Increase in absorbance against time was plotted. From the linear phase, read the change in absorbance per min. and expressed enzyme activity in terms of increased absorbance per unit time per mg protein.

Results

Embryogenic and Non-embryogenic callus obtained on MS + 0.5 mgl^{-1} 2, 4-D from all four explants of ecotype RZ-19 was taken for biochemical study. EC and NEC were differentiated morphologically. Organogenic and non-organogenic callus obtained on MS medium supplemented with 0.1 mgl^{-1} TDZ were also analyzed biochemically. Total protein content was found remarkably higher in embryogenic callus obtained from roots, followed by hypocotyl segments. Peroxidase activity was found to be much higher in embryogenic callus from hypocotyl than that of roots. Non-embryogenic callus contained lowest protein content. Peroxidase activity was also lower in non-embryogenic callus. Organogenic and non-organogenic callus showed non-significant difference in terms of total protein content and peroxidase activity (Table 1, 2).

Table 1: Peroxidase activity in different types of calluses obtained from hypocotyl segments of cumin.

Callus	A/min	Enzyme Activity/mg protein	Protein mg/gm tissue
Embryogenic callus	0.0976 + 0.0047	0.068 + 0.0034	11.216 + 0.12
Non Embryogenic callus	0.0223 + 0.0049	0.052 + 0.019	3.43 + 0.91
Organogenic callus	0.1036 + 0.0064	0.141 + 0.010	8.18 + 0.95
Non Organogenic callus	0.1133 + 0.0032	0.150 + 0.036	6.17 + 1.41

Table 2: Peroxidase activity in Embryogenic callii obtained from different explants of cumin on MS + 0.5 mgl-1 2, 4-D

Explants	A/min	Enzyme Activity/mg protein	Protein mg/gm tissue
Root	0.055 + 0.012	0.023 + 0.0037	19.50 + 6.61
Hypocotyl	0.097 + 0.0047	0.068 + 0.0034	11.216 + 0.12
Cotyledon	0.037 + 0.007	0.028 + 0.0098	10.62 + 1.56
Shoot apex	0.044 + 0.003	0.047 + 0.0065	7.29 + 0.49

Values are mean of five experiments.

Discussion

A number of scientists reported peroxidases as early indicator of somatic embryogenesis and organogenesis as well. Presence of peroxidases indicates both leaf and root morphogenesis before primordia becomes visible [8, 9]. Twice as much protein and increased peroxidase activity in embryogenic callus as compared with non-embryogenic callus has been reported from citrus and lettuce [10, 11]. Our results also corresponded to these investigations. Total protein content was found three times higher in embryogenic callus than non-embryogenic callus. Among all four explants also protein contents were found to be associated with their embryogenic competence i.e., more protein in root and hypocotyl callus than cotyledon and shoot apex callus. Peroxidase activity was also significantly higher in hypocotyl explant. Organogenic and non organogenic callus could not be differentiated on this respect. Both types of calluses showed high protein and peroxidase activity. This may probably be associated with presence of TDZ.

Conclusion

Present study indicates more values of proteins in embryogenic callus and organogenic callus suggesting their role in regeneration potential of callii. Peroxidase activity was higher in organogenic and non organogenic callus obtained on TDZ, suggesting the triggering of some proteins due to the action of TDZ. Isolation and identification of individual proteins using other analytical approaches would be required to more clearly define their role in somatic embryogenesis and organogenesis.

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