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EFFECT OF NUTRIENT LIMITATION ON BIO-SYNTHESIS OF CAPSAICIN

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Abstract

Capsaicin biosynthesis was studied in callus cultures derived from root tip and shoot tip explants of *Capsicum annuum*L. variety Indra. In callus cultures, the activity of two enzymes, viz. PAL and CS increased till 15 or 12 days of subculturing,when these were induced on media, supplemented with $2mgl^{-1}$ 2,4-D and $0.5mgl^{-1}$ kin. Capsaicin content in root-tip derived callus cultures was much higher than that in shoot-tip derived callus cultures. Maximum capsaicin content was estimated(79 mgg^{-1} DW) in nitrogen deficient root-tip derived callus cultures after 15 days of subculturing. When the callus cultures were induced on media, supplemented with NAA, the activity of all the enzymes and capsaicin content was higher than that observed in only 2,4-D and kin. Sucrose and phosphorus depleted media showed a marginal increase for capsaicin content and activity of enzymes in both the root tip and shoot-tip derived callus cultures. Nitrogen depleted (ammonium nitrate and potassium nitrate) medium showed about 6.8 fold increase in capsaicin content and upto 15 and 6 fold increase was observed in activity of CS and PAL enzymes respectively.

1.INTRODUCTION

Chilli (*Capsicum annuum* L.) belongs to the family *Solanaceae*. It was introduced from South Africa in the seventeenth century and is now grown as a cash crop in all parts of India for its pungent fruits. *Capsicum* consists of approximately 20–27 species (Walsh and Hook 2001). Phylogenetic relationships between species were investigated using biogeographical (Tewksbury et al 2006), morphological (Eshbaugh 1975),chemosystematic (Ballardet al 1970), hybridization (Pickersgill 1971) and genetic (Walsh and Hook 2001) data.

The pungency, a commercially important attribute of peppers, is due to the presence of six chemically related compounds viz. capsaicin, dihydrocapsaicin, norcapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin; which constitute, the "capsaicinoids" group (Perucka and Materska 2001). Capsaicin and dihydrocapsaicin account for approximately 90% of capsaicinoids in chilli pepper fruit, are most potent capsaicinoids. Capsaicin has great commercial importance as it is a major component of oleoresin which is exported and is a great foreign exchange earner. It has been reported to show anticancer effect (Moore and Moore 2003) and acts against neurogenic inflammation (Szolcsanyiet al 2004). It has been reported to show protective effects against cholesterol and obesity (Kempaiahet al 2005)

Plant cell and tissue cultures have been established for a myriad of plant species. This technology is being used for the large scale production of specific secondary metabolites which can be used as food additives, nutraceuticals, and pharmaceuticals. (Zhong et al 2001) However, limited knowledge of secondary metabolism and its regulation proves to be an obstacle to the exploitation of the industrial potential of the plant cell and tissue cultures. Developments in transgenic research have opened up the possibility of metabolic engineering of biosynthetic pathways to produce high value secondary metabolites, like capsaicin and vanillin (Ravishankar and

Ramachandra 2002). These approaches together provide the basis for the development of a commercial process in which emphasis is laid on the cultures to increase secondary metabolic activity.

Some of the enzymes of capsaicin biosynthesis are not expressed at adequate levels in *in vitro* cultures of chilli pepper (Ochoa-Alejo and Gomez-Peralta 1993). The influence of 8-methyl nonenoic acid on capsaicin biosynthesis in cell cultures of *Capsicum* spp. has been observed (Prasad et al 2006). The capsaicin biosynthetic pathway has not been fully elucidated at present and thus there is limited information available about the enzymes of the capsaicin biosynthetic pathway in cell and tissue cultures of chilli pepper.

The present investigation was therefore planned to study the capsaicin biosynthesis in the somatic cell cultures of *Capsicum annuum* Lvariety. The objective of this study was to increase capsaicin production in callus cultures by inhibiting cell growth by limiting three major nutrients i.e. sucrose, nitrogen and phosphate. The effect of nutrient limitation on capsaicin production in callus cultures was related to two enzymes viz. phenylalanine ammonia-lyase and capsaicin synthetase which determine the entry of phenylalanine into phenylpropanoid pathway. Capsaicin production in callus cultures obtained from different explant sources (shoot tip and root tip) was observed in different compositions of tissue culture media with the objective to study the activity of capsaicin synthesizing enzymes, phenylalanine ammonia lyase (PAL) and capsaicin synthetase (CS) in *Capsicum* in response to tissue culture media.

2. MATERIALS AND METHODS

2.1 Plant Material

The experimental material consisted of *Capsicum annuum* L viz. Indra and this was procured from the Department of Vegetable Science, Punjab Agricultural University, Ludhiana.

2.1.1 Collection and preparation of plant material

Shoot tips and root tips were taken for *in vitro* culturing. The viable seeds of *Capsicum annuum*L. were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) under sterile conditions with gentle agitation for 7 min. The seeds were then rinsed thrice with double distilled water. The sterilized seeds were germinated aseptically on Murashige and Skoog's (1962) basal medium in jars. The seeds were incubated at 25±2ºC in growth rooms with continuous light (daylight flouroscent tubes; 18/6 hours light/dark conditions). Seven day old seedlings were used for preparation of explants for culturing. Shoot and root segments were cut using a sharp sterilized scalpel blade, into pieces of 1 cm were then used as explants.

2.2 Composition of medium

The MS medium containing different supplements was used to study its effect on callus induction and proliferation. The following combinations of plant growth regulators were studied:
MS medium.

Notation	2,4-D	Kin	NAA
M1	2.0	0.5	-
M2	2.0	0.5	2.0

2.3 Callus cultures

2.3.1 Callus Establishment and Maintenance

Shoot and Root segments (each 1cm in length) were taken for raising callus. These segments were inoculated on 50 ml MS medium supplemented with 3% sucrose, $2mgl^{-1}$ 2,4-D and 0.5 mgl^{-1} kin and solidified with 1% agar (callusing medium). Friable callus that formed at the cut ends of the explants was maintained on the fresh callusing medium by regular subculturing at 4-week intervals. Callus cultures were incubated at 25±2ºC in growth room with continuous light (day light flourescent tubes; 18/6 hours light/dark photoperiod).

2.3.2 Culturing of Callus on Different Media Compositions

The friable callus (30 days) was subcultured on the following different media compositions:

a. MS medium containing 3% sucrose, $2mgl^{-1}$ 2,4-D and 0.5 mgl^{-1} Kin and solidified with 1% agar (control)
b. Control without sucrose
c. Control without nitrogen (potassium nitrate and ammonium nitrate)
d. Control without phosphate

The enzyme activities (PAL and CS activities) and capsaicin content of these calli were determined after every 3 days (taking the day of subculturing as 0 day) for a period of 21 days.

2.4 Biochemical analysis

2.4.1 Capsaicin (Bajaj, 1980)

Capsaicin was quantitatively analyzed in calli by colorimetric method. Capsaicin was extracted from 0.5 g of the material to be analyzed in 5ml of ethyl acetate. After 24 hours, extract was filtered through Whatman No. 1 paper to remove suspended impurities and 1 ml of sodium nitrite-sodium molybdate reagent (0.5 M sodium nitrite and 0.025 M sodium molybdate in aqueous solution) was added. The components were thoroughly mixed. After 15 min, 2ml 1N NaOH was added. Absorbance was read at 430 nm in Spectronic 20 spectrophotometer (Bausch and Lomb) against reagent blank within 30 min of color development. Blank was prepared by replacing chromogenic reagent with distilled water.

Standard curve was prepared by using capsaicin in the concentration range of 20-100 µg/ml.

2.4.2 Assay of Enzyme Activity

2.4.2.1 Phenylalanine ammonia-lyase (Hadwidger and Schwochau, 1971)

Extraction :0.5 g (fresh mass) calli was homogenized in a chilled mortar with 5ml 0.05 M boric acid-borax buffer (pH 8.8). This and subsequent extractive operations were carried out at 4ºC. The homogenate was centrifuged at 20,000 xg for 10 min at 4ºC. The supernatent was used for PAL assay.

Assay of PAL : The assay mixture contained 0.2 ml enzyme extract, 1ml 10mM L-phenylalanine and 2.5 ml 0.05 M boric acid-borax buffer (pH 8.8). The reaction mixture was incubated for 1 hour at 37°C. The reaction was then stopped by adding 0.1 ml of 5 M HCl. The acidifiedmixture was extracted twice with 7.5ml diethyl ether. The ether extracts were pooled and evaporated to dryness under a stream of air. The residue was dissolved in 5ml 0.05 M NaOH. The cinnamic acid formed at the end of the reaction was estimated by measuring the absorbance at 290 nm in a Beckman DU 7 spectrophotometer. Standard curve was prepared by using trans-cinnamic acid in the concentration range of 5-35 µg/ml.

2.4.2.2 Capsaicinsynthetase(Collins et al 1995).

Extraction : One gm of callus was homogenized in 10 ml of 0.1M potassium phosphate buffer, (pH 6.8) with 100 mg ascorbic acid and 5 mM -mercaptoethanol. The homogenate was centrifuged at 10000 xg for 30 minutes at 4ºC. The supernatant was used as enzyme extract.

Assay : The reaction mixture contained 0.5M potassium phosphate buffer pH 6.8, 1µM each of $MgCl_2$ and ATP, 5µM each of vanillylamine, 8-methyl-nonanoic acid and CoA in 1 ml of enzyme extract. The reaction mixture was incubated for 2 hour at 37ºC and terminated by 0.5 N HCl. The reaction mixture was taken into chloroform and later evaporated and resuspended in 100µl of methanol. Methanol fraction was used for determining capsaicin levels. The specific activity of CS was expressed in terms of unit of capsaicin produced per mg protein per hour.

3. RESULTS AND DISCUSSION

3.1 Activity of PAL Enzyme

The shoot tip derived callus cultures showed a steep increase in PAL activity till 12 days of subcuturing and then decrease till 21 days. (Plate 1 {A-C}). In control medium the maximum activity of PAL enzyme attained was 87 µg cinnamic acid formed $h^{-1}mg^{-1}$protein at 12D (Table 1). M1 with sucrose and phosphorous deficiency showed about 2.5 fold increase in PAL activity (121 µg cinnamic acid formed $h^{-1}mg^{-1}$protein at 15D) and (130 µg cinnamic acid formed $h^{-1}mg^{-1}$protein at 12D) respectively. However, the nitrogen stressed medium (Plate 1 {D}) showed about 4.8 fold increase in PAL activity (234 µg cinnamic acid formed $h^{-1}mg^{-1}$ protein at 15D) (Table 1).

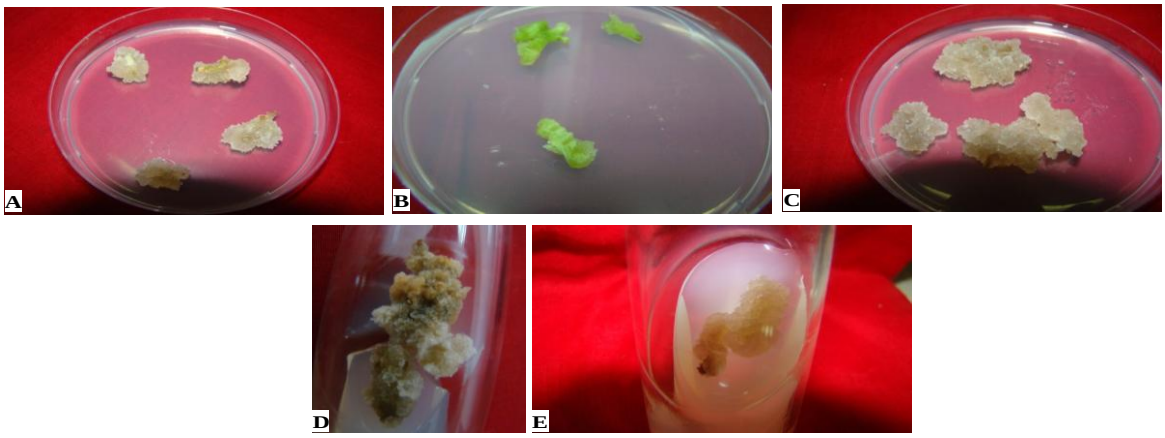

Plate 1 (A-E): Callus induction from shoot-tip explants of Indra variety

In case of shoot tip derived callus cultures, there was maximum increase in PAL activity on 6th and 12th days of

subculturing. (Table1). The calli which were induced on M1 media, after subculturing on control medium showed increase in enzyme activity, while in nitrogen stressed medium they showed about 6 fold increase in PAL activity. The specific activity was (212 µg cinnamic acid formed $h^{-1}mg^{-1}$protein) maximum on nitrogen stressed medium

Table: 1 PAL activity in shoot-tip and root-tip -derived callus cultures of *Capsicum annuum*L. var Indra cultured on 2mgl^{-1} 2,4-D and 0.5mgl^{-1} kin and after various days of subculturing on different media compositions.

	Control		Nitrogen Deficiency		Phosphorous Deficiency		Sucrose Deficiency	
No. Of Days	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	35±1.3	49±2.8	35±1.3	49±2.8	35±1.3	49±2.8	35±1.3	49±2.8
3	38±1.4	53±3.5	76±1.4	106±0.7	64±1.1	89±0.6	60±0.3	85±0.8
6	65±2.1	80±2.3	130±2.8	182±0.8	87±1.3	121±1.4	75±0.6	106±1.1
9	73±0.7	84±0.7	152±5.7	204±1.3	92±2.1	128±2.3	83±0.8	117±2.2
12	77±1.4	87±1.3	212±1.4	234±0.4	105±2.5	130±2.1	86±0.3	121±2.3
15	53±2.8	59±0.6	208±9.9	230±0.6	110±2.3	115±1.4	69±2.8	97±1.1
18	34±1.1	38±2.1	189±0.6	169±2.8	98±1.3	86±0.7	43±2.8	61±4.2
21	32±2.8	35±0.3	98±0.9	88±1.3	93±0.7	79±0.7	39±3.5	56±1.1

After introduction of NAA, when the shoot tip derived calli were induced on M2 media, maximum PAL activity was observed at 15 days of subculturing. In M2 control medium, the PAL activity was maximum (108 µg cinnamic acid formed $h^{-1}mg^{-1}$ protein) which was higher than that in calli induced on M1 media. As shown in (Plate 1{E}), the PAL activity of cultures in sucrose and phosphorous stressed media increased only marginally 157 and 168 µg cinnamic acid formed $h^{-1}mg^{-1}$protein respectively, but in nitrogen stressed medium, it increased by 3.69 fold (240 µg cinnamic acid formed $h^{-1}mg^{-1}$protein) (Table 2).

Table: 2 PAL activity in shoot tip and root-tip -derived callus cultures of *Capsicum annuum* L. var Indra cultured on 2mgl^{-1} 2,4-D, 2mgl^{-1}NAA and 0.5mgl-1 kin and after various days of subculturing on different media compositions.

	Control		Nitrogen Deficiency		Phosphorous Deficiency		Sucrose Deficiency	
No. Of Days	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	65±2.5	67±2.8	65±2.5	67±2.8	65±2.5	67±2	65±2.5	67±2
3	67±2.8	69±3.5	130±1.4	132±2.1	117±2.8	119±3	112±5.7	114±2.8
6	101±2.8	103±0.8	222±0.8	224±1.1	158±1.3	160±2	139±2.8	141±1.1
9	105±1.4	107±0.7	232±1.1	234±1.1	166±0.7	168±2	153±3.7	155±4.2
12	108±0.7	110±1.4	240±1.4	242±4.2	168±0.7	170±2	157±2.7	159±2.5
15	73±3.4	75±1.1	235±4.2	237±1.4	149±2.8	151±1	125±2.8	129±1.4
18	48±1.3	50±1.3	191±1.4	193±2.8	111±2.8	113±4	78±1.4	81±1.1
21	44±0.8	46±1.1	101±2.8	103±2.8	99±1.1	101±1	72±2.8	74±3.1

The callus cultures derived from root tip exhibited maximum activity at 12 days of subculturing, on M2 media. In the case of *Capsicum* species, the expression levels of several genes of phenylpropanoid pathway (PAL, CA4H, COMT) is positively correlated with capsaicinoid accumulation (Curry et al 1999). Our current records also justify this statement. Protein and nucleic acid contents of callus during the growth showed an inverse relationship with PAL (Jhasiet al 2012). This explains why the activity of PAL decreased after callus growth in control medium and increased when no growth was observed in nitrogen deficient media, because of less production of proteins.

3.2 Activity of CS Enzyme

Shoot tip derived callus cultures showed concomitant increase in CS activity in nitrate depleted medium suggesting a switch in cell activity to the metabolism of nitrogen free compounds such as phenolics.

Callus cultures in M1 media exhibited about 9.23 fold increase in control medium. However 15, 9.73 and 4.46 fold increase on nitrogen, phosphorous and sucrose stressed media respectively was observed (Table 3). A linear increase in CS activity was observed from day 3 to 15 of subculturing. Thereafter, it decreased to negligible amounts at day 21 of subculturing.

Table: 3 CS activity in shoot tip and root-tip-derived callus cultures of *Capsicum annuum*L. var Indra cultured on 2mgl^{-1} 2,4-D and 0.5mgl^{-1} kin and after various days of subculturing on different media compositions.

	Control		Nitrogen Deficiency		Phosphorous Deficiency		Sucrose Deficiency	
No. Of Days	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	30±2.1	102±4	30±2.1	102±3.5	30±2.1	102±3.5	30±2.1	102±3
3	68±2.1	105±3	22±4.2	230±2.8	172±0.7	241±1.4	123±2.3	190±1
6	73±0.8	112±1	248±1.3	300±2.1	214±0.6	254±1.4	129±1.4	200±1
9	114±4	146±1.4	265±2.5	302±0.9	216±1.4	267±2.8	131±1.3	204±2
12	120±2	177±3.5	273±0.7	314±2.3	223±2.8	273±0.6	132±4.2	208±1
15	126±6	277±0.9	268±1.4	451±1.4	292±1.4	279±2.8	134±1.3	267±1
18	116±5	166±1.4	262±1.4	264±0.3	169±0.8	240±1.4	75±1.9	238±1
21	106±2	160±2.1	209±2.5	230±1.4	146±1.9	195±2.3	63±2.3	173±1

Callus cultures from root tip explants showed increased CS activity as compared to shoot tip calli. Root tip cultures in nitrogen stressed M1 media showed maximum activity (451 µg capsaicin h^{-1}mg^{-1} protein at 15D) as compared to control medium (277 µg capsaicin h^{-1}mg^{-1} protein at 12D). Sucrose and phosphorous stressed root tip cultures caused only 2.75 and 3.65 fold increase in CS activity by day 18 and 12 respectively (Table 3).

After NAA was added, the calli were induced on M2 media and after subcuturing the activity of enzyme followed same pattern but comparatively lesser fold increase in the CS activity was observed. Shoot tip derived callus cultures under control and nutrient deficient media showed enhanced CS activity. About 1.5 fold increase in CS activity was observed at 15 days of subculturing. The increase in CS activity was much (57 µg capsaicin h^{-1}mg^{-1} protein at 15D) in nitrogen depleted media (Table 4).

Table: 4 CS activity in shoot tip and root-tip-derived callus cultures of *Capsicum annuum*L. var Indra cultured on 2mgl^{-1} 2,4-D, 2mgl^{-1}NAA and 0.5mgl^{-1} kin and after various days of subculturing on different media compositions.

	Control		Nitrogen Deficiency		Phosphorous Deficiency		Sucrose Deficiency	
No. Of Days	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	25±2.8	69±2.8	25±2.8	69±2.8	25±2.8	69±2.8	25±2.8	69±2.8
3	31±2.3	71±2.8	50±2.3	123±3.7	41±2.8	91±2.8	38±1.3	79±0.8
6	35±0.3	73±2.8	53±1.3	129±0.7	43±3.1	95±0.6	40±0.1	81±0.8
9	37±1.1	75±2.8	55±0.8	131±2.8	45±0.8	98±0.9	41±0.4	83±0.8
12	39±0.7	79±0.8	57±0.3	133±2.8	47±1.1	101±0.8	43±2.8	86±1.4
15	41±0.4	81±0.8	61±0.4	132±0.8	51±0.3	103±0.7	42±0.8	88±1.2
18	35±0.8	83±1.4	63±1.3	134±1.1	53±0.8	105±1.1	39±0.7	91±1.4
21	31±0.7	84±0.4	65±0.6	135±0.8	55±0.4	109±1.4	35±0.8	93±1.4

Higher levels of 2, 4-D were found to inhibit callus proliferation while lower concentration allowed morphogenesis to occur (Malik et al 2003) and caused a negative effect on activity of enzyme. The best callus growth was obtained when 2mgl^{-1}2, 4-D and 0.5mgl^{-1} kin were used in medium (Hogue et al 1990). To investigate the regulation of capsaicin biosynthesis, the promoter of CS was fused to GUS, this test showed GUS expression in

leaves and roots (Kim et al 2009). It follows that the CS activity can be observed in roots.

3.3 Capsaicin Content

In Indra capsaicin accumulation was (29 mgg^{-1} DW at 12D) in control media. In nitrogen stressed medium increased 6.81 fold (75 mgg^{-1}DW at 15D). On the other hand, in phosphate and sucrose stressed media, it increased only 4.09 and 2.6 fold respectively (Table 5). Root tip derived callus cultures showed maximum value of capsaicin accumulation at 15 days of subculturing(Plate 2{A}).

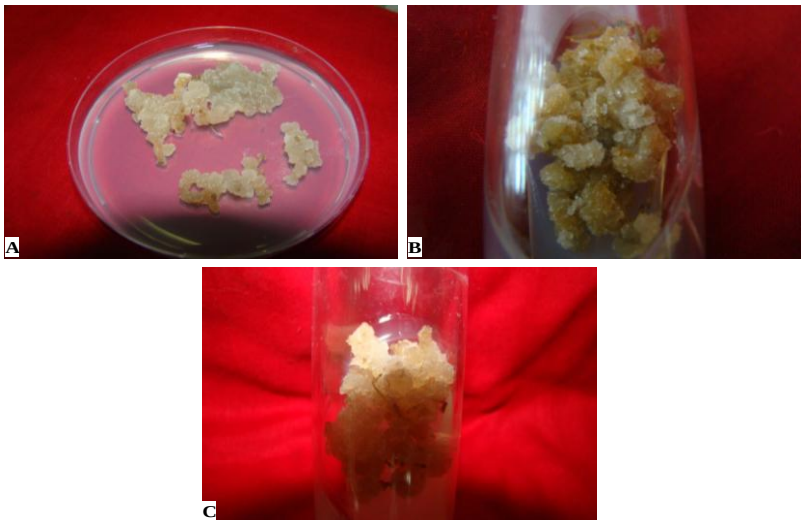

Plate 2 (A-C) : Callus induction from root-tip explants of Indra variety

Capsaicin accumulation in root tip derived callus cultures was higher than that in shoot tip derived callus cultures. Capsaicin accumulation in root tip derived callus cultures was about 1.6 fold more than in shoot tip derived callus cultures. Drastic differences in pattern of capsaicin accumulation in callus cultures derived from two explants sources reflected different status of metabolic activity in the cells of shoot tip and root tip. In shoot tip cells, there was a slow accumulation of capsaicin as nutrients are consumed in other metabolic activities. In control there is a rapid accumulation of capsaicin till 12 days of subculturing in root tip derived callus cultures. In shoot tip cells, the secondary metabolic pathway is less active. As callus cultures derived from shoot tip has lesser capacity to synthesize capsaicin than those from root tip, indicates the source of capsaicin synthesis in callus culture. It has been indicated the highest concentration of capsaicin is found in ovary and lowest concentration can be found in seeds. (Supalkovaet al 2007). Likewise in our current research, it has been investigated that the capsaicin content may vary in different parts of *Capsicum*.

Table: 5 Capsaicin content in shoot tip and root-tip -derived callus cultures of *Capsicum annuum*L. var Indra cultured on 2mgl^{-1} 2,4-D and 0.5mgl^{-1} kin and after various days of subculturing on different media compositions.

	Control		Nitrogen Deficiency		Phosphorous Deficiency		Sucrose Deficiency	
No. Of Days	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	11±0.7	20±0.3	11±0.7	20±0.3	11±0.7	10±0.3	11±0.7	20±2
3	15±0.4	21±0.4	37±0.6	49±0.1	30±1.8	41±0.7	24±2.8	34±1
6	20±0.9	26±0.1	52±0.8	59±0.4	37±2.8	46±0.9	27±0.9	38±3
9	28±0.7	29±0.7	55±0.9	65±0.8	39±1.1	49±0.8	28±0.6	40±4
12	29±0.9	32±0.4	65±0.1	67±0.4	41±2.8	50±2.8	29±0.1	41±4
15	26±0.9	39±0.4	75±1.3	79±0.8	45±0.7	49±2.8	27±0.6	45±2
18	21±0.4	25±0.3	55±1.3	65±1.3	34±0.7	40±0.6	18±0.9	37±1
21	19±0.4	24±1.3	41±0.7	38±0.8	30±1.3	34±0.3	16±0.7	29±1

After addition of NAA, the callus cultures derived from shoot tip exhibited maximum value of

capsaicin accumulation at 9 or 12 days of subculturing. Capsaicin accumulation in phosphate and sucrose stressed media showed about 2 fold increase but in nitrogen stressed medium a 3.3 fold increase in capsaicin accumulation was observed (Table 6).

In callus cultures derived from root tip of *Capsicum annuum* L., phosphate and sucrose stress (Plate 2{C}) led to marginal increase in capsaicin accumulation in control M4 medium. The nitrogen stress (Plate 2{B}) led to a 2 fold increase in capsaicin accumulation (Table 6).

Table: 6 Capsaicin content in shoot tip and root-tip -derived callus cultures of *Capsicum annuum* L. var Indra cultured on 2mgl^{-1} 2,4-D, 2mgl^{-1} NAA and 0.5mgl^{-1} kin and after various days of subculturing on different media compositions.

	Control		Nitrogen Deficiency		Phosphorous Deficiency		Sucrose Deficiency	
No. Of Days	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
0	18±0.7	23±0.8	18±0.7	23±0.8	18±0.7	23±0.8	18±0.7	23±0.8
3	19±2.8	24±0.9	37±1.1	46±0.4	27±0.6	33±0.4	26±0.7	31±0.7
6	25±1.3	30±0.8	49±0.7	57±0.6	33±0.1	39±0.6	30±0.7	35±0.8
9	27±3.7	31±0.6	52±0.4	61±0.4	35±2.8	41±0.6	32±0.1	37±0.8
12	28±0.9	33±1.1	60±1.1	69±1.3	36±0.3	42±0.6	34±0.7	39±2.8
15	23±1.3	28±1.1	58±0.3	67±1.3	34±2.8	40±0.1	29±0.4	35±0.3
18	18±1.1	23±0.4	50±1.3	58±0.4	28±0.9	34±0.6	22±2.8	28±0.1
21	17±0.3	22±2.8	30±0.1	38±0.8	26±0.9	32±0.3	20±1.1	26±0.4

This effect of the nutrients has been associated with an alteration in the growth rate of callus cultures. Nutrient limitation reduces growth and therefore, reduces protein synthesis and thus increases accumulation of secondary metabolites. Addition of 4mm tyrosine to 100% P and 100% K stress led to 8.4 fold & 7.5 fold higher capsaicin production as compared to control respectively (Pandhair and Gossal 2009).

Both suspended and immobilized cells have the ability to accumulate capsaicin and yields can be increased by nutrient limitation (Hall and Yeoman 1991).

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