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Production of novel bioactive compounds during *in vitro* growth of *Lycopersicon esculentum* Var. Roma

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ABSTRACT

Different explants of Lycopersicon esculentum L. var. Roma were cultured on MS basal medium supplemented with different concentrations of plant growth regulators (PGRs) i.e. BAP, 2,4-D and NAA. Best callogenesis (90%) and regeneration (56%) were recorded for leaf explants with 2.5 mg/l of 2,4-D on MS basal medium. GC-MS detected fifty-four bioactive compounds during in vitro growth of tomato. When compared with the secondary metabolites, detected from field grown tomato plants, it was found that the regenerated plantlets showed much elevated quantity of bioactive compounds. In the field grown tomato plants, only nine secondary metabolites were detected. The results clearly indicated that the production of different metabolites can be either increased or decreased by altering the quality and quantity of plant growth regulators (PGRs) during in vitro growth of tomato.

Key words: Secondary metabolites, bioactive compounds, regeneration, PGRs.

INTRODUCTION

Tissue culture is such a beneficial technique in which plants can be grown by adding on different growth regulators externally and the constitution of growth medium acts as a determining factor for *in vitro* grown of plants [1, 2]. Lycopene (a naturally occurring antioxidant) present in tomato, is very effective to lessen the proliferation of cancerous cells. Due to the presence of vitamin C it is very effective in scurvy. Tomato juice contains iron and potash salts which maintain the blood stream alkalinity and develop resistance to diseases. It provides excellent cure for diarrhea, dysentery, dyspepsia and is also considered as very beneficial to cure all types of nervous abnormalities [3, 4].

GC-MS technique is among those tools which are widely used to analyze and to profile very complicated mixtures of secondary metabolites, amino acids, organic acids, sugars and sugar alcohols, lipophilic compounds and phosphorylate intermediates. GC-MS provides an efficient analysis of chemical mixtures [5, 6].

Greve and Labavitch [7] performed GC-MS analysis to find the synthesis of polysaccharides present in pericarp discs of tomato fruit. An analysis of volatile compounds of tomato using GC-MS was published by Rastogi *et al*., in 1991 detected aldehydes, ketones, esters, alcohols, hydrocarbons, ethers, nitrogen, oxygen, sulfur, phenols, free acids, heterocyclic compounds and lactones [8, 9].

Several widely used vegetables contain rich amounts of vitamins and phenolic compounds. Different vegetables of Solanaceae family including *lycopersicon esculentum* L. (tomato), *Solanum tuberosum* (potato), *Capsicum annuum* (chillipepper) and *Solanum melongena* (eggplant) have been evaluated for their bioactive compound profile using GC-MS [10]. Oliu *et al*. used GC-MS to distinguish both volatile and polar metabolites involved in development and ripening of tomato [11].

MATERIALS AND METHODS

The present study was conducted to estimate the secondary metabolites produced during *in vitro* and *in vivo* growth of *Lycopersicon esculentum* var. Roma by GC-MS. It was also aimed to find the effect of PGRs on the quantity and quality of the metabolites produced during this process. The procedure of experiment was divided into two steps.

During First step, the tomato explants were gathered from Department of Botany, Lahore College for Women University, Lahore. Different types of explants *i.e.* leaf, node, internode (shoot) and bud were sterilized and inoculated on MS basal media [12] with different PGRs (2,4-D, NAA, BAP) under different physical and chemical condition for their *in vitro* growth.

In the second step, extraction of callus was done using double distilled n-hexane for 6 times in ratio of 1g: 10 ml of callus and double distilled n-hexane for the complete extraction of secondary metabolites in the solvent. The samples for each type of callus was mixed with solvent, filtered and were subjected to GC-MS.

GC-MS Analysis

In vitro callus samples were analyzed using a GC-MS-QP 2010 chromatograph, with an injection temperatures, 250ºC and 200ºC, interfaced with Agilent and QP detectors respectively. (Ionization voltage 70eV, m/z scan range 55-950 Da,) and equipped with a DB-5 capillary column (30m×0.25mm, film thickness 0.25µm). The oven temperature was held at 45°C for 1min, then programmed from 45-100°C at a rate of 5°C/min, held for 1min, increased up to 200°C at the rate 10°C/min and was kept at the final temperature for 5min, using Helium as a carrier gas. The injector and detector temperatures were 200°C and 250°C, respectively.

Statistical Analysis

The results obtained were statistically analyzed. The means were separated by Duncan's new multiple range test at 1% level if significance according to the reported method [13].

RESULTS AND DISCUSSION

When MS basal medium was used the %age of seed germination was recorded as 91%, with large texture and dark green in color. Comparatively, the %age of seed germination found was 84% when 2.5mg/l 2,4-D was used, resulted texture and color of seedlings was medium and green, respectively. The physical conditions like temperature (26 ± 2°C), pH (5.75) and photoperiod (16h) were found to be the best for *in vitro* growth of tomato plant (Table 1).

Table 1 Effect of Different Physical Factors on *in vitro* growth of *Lycopersicon esculentum* L

Different Growth Parameters	Best Growth Conditions	*In vitro* growth rate (% mean)	
		No. of Cultures involved in Callogenesis	No. of Cultures involved in Regeneration
Photoperiod (2000-3000 lux)	16 h	92±0.23[a] LSD Value =1.90	79±0.41[a] LSD Value =1.45
Sucrose conc. g/l	34	95±0.70[a] LSD Value =1.60	79±0.43[a] LSD Value =1.51
Temperature Ranges (°C)	26± 2	89±0.68a LSD Value =1.91	62±0.31[a] LSD Value =1.57
pH Ranges	5.72	92±0.41[a] LSD Value=1.32	75±0.93[a] LSD Value=1.98

The mean with different letter in each column are significantly different according to Duncan's multiple range test(0.005p value) ± = Standard error

Effect of different concentrations of 2,4-D, NAA and BAP (2 to 3.5mg/l) on callogenesis of *Lycopersicon esculentum* L. var. Roma in MS basal medium was recorded using several explant types *i.e.*, leaf, internode, node

and buds. The best results of callogenesis (90%) and regeneration (56%) were obtained with leaf explant on MS basal medium supplemented with 2.5mg/l of 2,4-D (Fig.1, Table.2).

Fig.1. (a) Showing maximum Callogenesis and; (b) Showing maximum Regeneration using leaf explant and MS basal media fortified with 2,4-D

(**a**) (**b**)

Table 2: Callogenesis and Regeneration of *Lycopersicon esculentum* L. on MS basal medium supplemented with different PGRs using Leaf as Explant

No of Culture:30

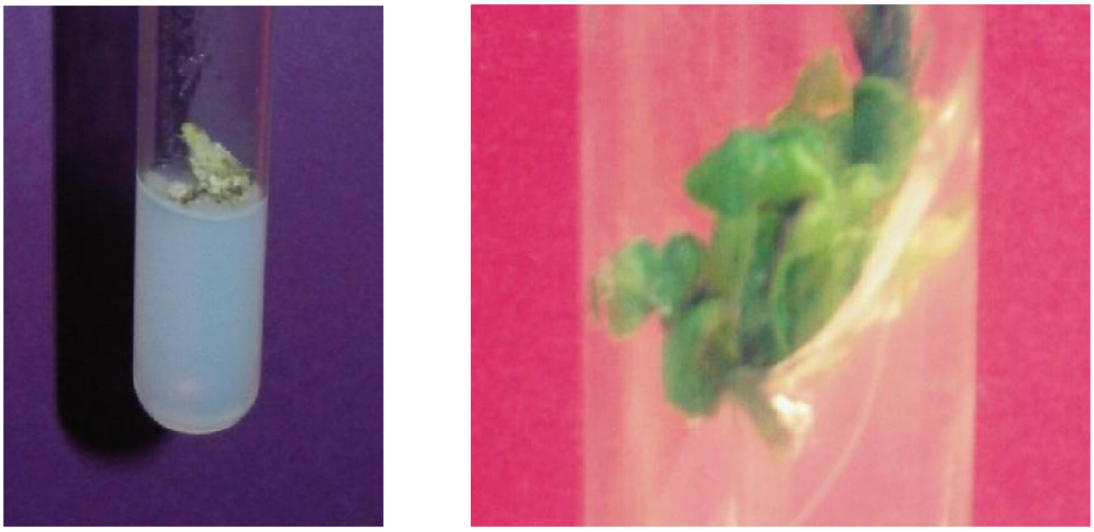
Best Explant used	Best PGR used	Conc.of PGR in MS medium	Best regeneration (% mean)	Best callogenesis (% mean)	Callus growth	Callus color & texture	LSD Value
Leaf	2,4-D (mg/l)	2.5	56±0.98[a]	90±0.28[a]	+++	Yellowish Green, Loose	1.96
Leaf	NAA (mg/l)	3.5	--	80.5±0.57[a]	+++	Greenish White, Compact	1.37
		2.5	40±0.19[b]	--	+++	Greenish Brown, Loose	
Leaf	BAP (mg/l)	2	20±0.82[c]	40±0.98[b]	+++	Dark Green, Compact	1.89

The mean with different letter in each column are significantly different according to Duncan's multiple range test (0.005p value) ± = Standard error

The present study supported the results of other authors [14] where the response of *in- vitro* grown tomato plants were detected using leaf discs and hypocotyls as explant for regeneration and callus induction on MS basal medium supplemented with different PGRs. Highest callogenesis from leaf discs (82%) and from hypocotyls (57.2%) was observed using MS basal medium supplemented with 2mg/l (BAP), 2mg/l (NAA), 4mg/l (kin) and 2mg/l (IAA).

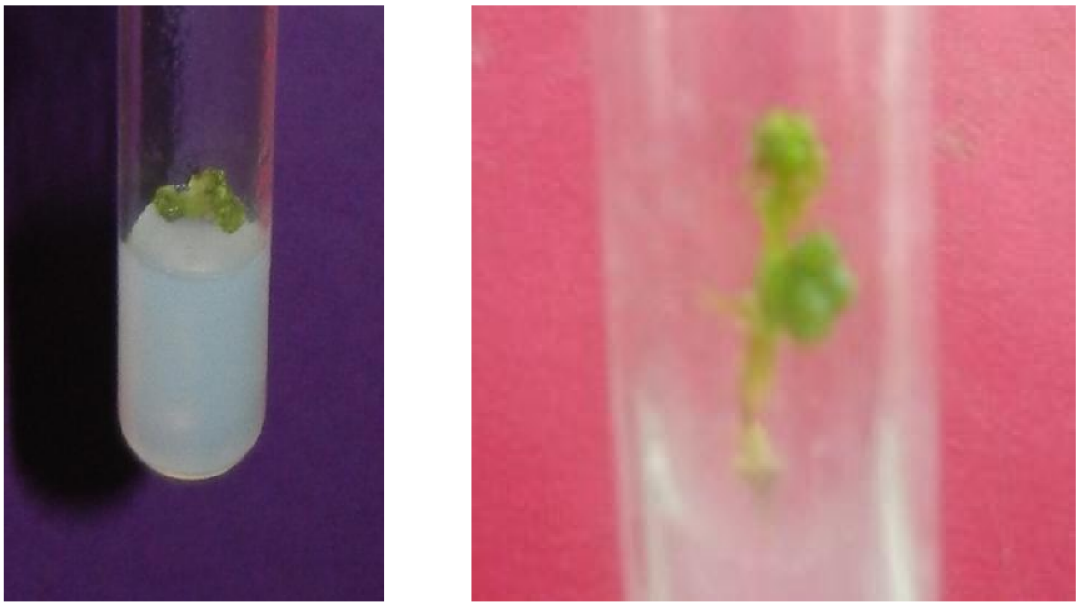
Callogenesis 80% and 40% regeneration were observed with leaf explants NAA 3.5mg/l-2.5mg/l in MS basal medium respectively (Fig.2, Table.2) whereas in a study carried out by Venkatachalam [15] the best callogenesis was found with a combination of NAA and kinetin 0.1mgl^{-1} each. Similarly 40% callogenesis and 20% regeneration were obtained using leaf explant on MS basal medium supplemented with BAP (2mg/l) (Fig.3, Table.2). Whereas Sheeja and Mandal, in 2003 reported the *in vitro* induction of fruiting and flowering from calli of regenerated plants of Pant 11variety of *Lycopersicon esculentum* L. (tomato) using leaf explants. BAP (2mgl^{-1}) showed the best results [16].

Fig.2. (a) Showing maximum Callogenesis and; (b) Showing maximum Regeneration using leaf explant and MS basal media fortified with NAA



(**a**) (**b**)

Fig.3. (a) Showing maximum Callogenesis and; (b) Showing maximum Regeneration using leaf explant and MS basal media fortified with BAP



(**a**) (**b**)

An association of bioactive compounds was detected between *in vitro* and *in vivo* grown plant samples of tomato. Furthermore, based on GC-MS results, the compounds detected were also found different than compared to reported by other authors [17]. Out of all the bioactive compounds detected by both *in vitro* and *in vivo* samples three compounds *i.e.* Propanoyl fluoride; p-pentylacetophenone, and Butanoic acid, detected from *in vivo* sample were found correlated with bioactive compounds detected using three cultivars of tomato and these were (*E*)-2-heptenal,

hexanal, 6-methyl-5-hepten-2-one, (*E*,*E*)-2,4-decadienal, 2-isobutylthiazole, geranylacetone, geranial and 1-nitro-2-phenylethane while one volatile *i.e.* methyl salicylate was seen to be present in all the three cultivars. One volatile compound was found to be decreased in *Lycopersicon esculentum* cv. Mickey *i.e.* (*Z*)-3-hexenal whereas another volatile compound *i.e.* (*E*)-2-hexenal also decreased in *Lycopersicon esculentum* cv. Vanessa. No uniform tendency was found for 1-nitro-3-methylbutane, 1-penten-3-one and 3-methyl-butanal.

In vitro and *in vivo* grown tomato plant samples were analyzed by GC-MS. Overall, a number (>63) of bioactive compounds were detected by GC-MS from both, the *in vitro* and *in vivo* grown tomato. Importantly, nine bioactive compounds were found in *in vivo* samples whereas fifty-four were detected in *in vitro* samples.

CONCLUSION

The current study indicated that *in vitro* cultures of *Lycopersicon esculentum* L. (Tomato) gave fifty-four metabolites whereas *in vivo* tomato plants gave only nine metabolites. These results suggest that different PGRs take part effectively in the manufacturing of bioactive compounds during the *in vitro* growth of the plants and one can increase/decrease or alter the quantity/quality of these compounds by changing the PGRs composition.

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Abbreviations

PGRs	Plant Growth Regulators
NAA	α-Naphthalene acetic acid
BAP	6-Benzylaminopurine
2,4-D	2,4-Dichlorophenoxyacetic acid
GC-MS	Gas Chromatography Mass Spectrometry

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