Asian J. Exp. Sci., Vol. 21, No. 2, 2007, 277-280

Isolation and Identification of Trigonelline from the Tissue Culture of *Allium sativum* Linn.

Kapil Kumar Saxena and Shikha Roy
Department of Botany,
University of Rajasthan, Jaipur, India

Abstract : Trigonelline is an important bioactive compound of plants. Tissue culture technique is being used for producing bioactive compounds. The Trigonelline was extracted the *in vitro* raised callus of *A. sativum*. The amount of Trigonelline was higher in 8 weeks old callus (0.86 mg/g d.w.). The maximum growth index of *A. sativum* (7.90) was observed in eight weeks old callus tissue. Present study deals with the isolation and identification of Trigonelline of *A. sativm.*

Key words : Trigonelline, Isolation, Tissue culture, *Allium sativum*.

Introduction

Medicinal plants are rich source of secondary metabolites like alkaloids, glycosides steroids, flavonoids, which are potential source of drugs. The interest in natural plant products is based on the fact that these are more compatible with human systems then synthetics.

Alkaloids have a long history in medicine for its tranquilizing and therapeutic effect. More than 1000 kinds of alkaloids have been isolated (Robinson, 1979). Besides being pharmacologically active, the alkaloids can be used as a model for the synthesis of thousand of new compounds in the hope of producing more effective drug for treating the human ailments.

A broad spectrum approach was employed to the biological evaluation of active principles of plants, specially alkaloids, glycosides, steroids, flavonoids and their pharmacological and chemotherapeutic activities but still there are many plants remains undiscovered in the vast backlog (Tyler, 1986).

The tissue culture technique is being used for producing primary and secondary metabolites as early as 1950's. Since then this technique has been developed for the production of various secondary metabolites which have been reviewed by Reinhard (1975), Staba (1980), Anderson *et al*. (1985) and Butenko (1985).

Secondary metabolites have been primarily used in the pharmaceutical industry as well as agents in food flavouring and perfumery. Apart from their economic importance, several secondary metabolites have been observed to have some correlation with the ecological and physiological requirements of higher plants e.g. some secondary compounds produced in plants are important either to protect these plants against microorganisms and animals or to enhance the ability of one plant species to competed with other plants in a particular habitat (Bell and Charlwood, 1980). Plant tissue culture can be used as an effective tool to regenerate such plants on a massive scale through micropropagation and attempts are even being

* **Corresponding author :** Kapil Kumar Saxena, Department of Botany,University of Rajasthan, Jaipur, India; E-mail - allium2005_06@yahoo.co.in

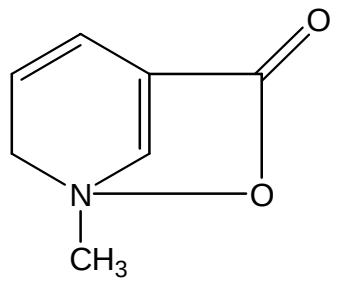
made to identify and propagate elite species so as to enhance the yield of secondary metabolites (Bajaj and Nitsch, 1975).

A large variety of plant secondary metabolites have nitrogen in their structure, included in this category are such well known antiherbivore defenses as alkaloids and cyanogenic glycosides which are of considerable interest because of their toxicity to human and their medicinal properties. Most nitrogenous secondary metabolites are biosynthesized from common aminoacids.

The alkaloids are a large family of nitrogen containing secondary metabolites found in approximately 20% of the species of vascular plants. The nitrogen atom in these substances is usually part of a heterocyclic ring, a ring that contains both nitrogen and carbon atoms. Most alkaloids are alkaline in nature. pH values commonly found in the cytosol is 7.2 (Taiz and Zeiger, 2002).

The role of alkaloids in plants has been a subject of speculation for at least 100 years. Most alkaloids are now believed to function as defenses against predators, especially mammals because of their general toxicity and deterrence capability (Hiroaoa and Tabata, 1974).

Trigonelline ($C_7H_7O_2N$) base is of special interest as it has been found in plants belonging to a number of botanical families and is a betaine in constitution and on that account is of importance as a possible primary material in the phytochemical synthesis of more complex alkaloids. It was isolated by Jahns from fenugreek (*Trigonella foenumgracum*), a leguminous annual cultivated in India and Egypt for the sake of its seeds, which are used in the preparation of curries, but are of principal importance as a veterinary spices (Henry, 1999).



Trigonelline

Material and Methods

Root tip of *A. sativum* var. G50 grown on MS medium was transferred to fresh MS medium, grown for 2,4,6,8 and 10 weeks after frequent subculturings of 8-10 weeks, harvested, dried and growth indices calculated separately. The various tissue samples were analysed separately for their alkaloidal contents. Five such replicates of each of the samples were examined and mean values taken.

$$GI = \frac{\text{Final dry weight of tissue} - \text{Initial dry weight of tissue}}{\text{Initial dry weitht of tissue}}$$

Extraction Procedure

Each of the dried samples were weighed, ground in a mortar, transferred to one litre Erlenmeyer flasks containing distilled water (50 mg/50 ml distilled water) to which 5 ml of 0.05 N sulfuric acid was added. Each of these mixtures was macerated for 3-4 hr and boiled gently for 20 min. Heavy magnesium oxide (2.5 gm) was added to each of the mixtures and again boiled gently for 20 min. the mixture cooled at room temperature and weighted. An equivalent amount of water was added to make up the loss of water due to boiling. Each of the mixtures was filtered through a whatman filter paper No. 12 (Kogan *et al*., 1953). The filtrate was evaporated to dryness, the

residue dissolved in water and analysed for its alkaloidal content.

TLC on silica gel in butanol : acetone: water (45:5:50) with Rf 0.06 was comparable to a standard Pre TLC was used to isolate the compound recrystallized from alcohol m.pt. 218-219°.

Results and Discussion

Maximum growth index (7.90) was observed in eighth weeks old callus tissue of *A. sativum* (Table 1, Fig. 1). The presence of trigonelline was confirmed by CO-TLC of standard (RF 0.61), Dragendroff's positive color (Brick-red) and mp (218-219°). Amount of trigonelline was found to be higher in 8 weeks old callus tissue (0.086 mg/g.d.w.) when compared with that of 10 weeks old tissue (0.029 mg/g./d.w.). (Table 1, Fig. 2)

Trigonelline has been reported from various plant species (Kogan *et al*., 1953). A. cepa (Evans *et al*., 1984), *A. sativum* (Taguchi *et al*., 1987). In the present study, *A. sativum* has been worked out for trigonelline content. It has been observed that trigonelline content increased upto the eighth week and then sharply declined in tenth week.

Table 1 : Trigonelline contents mg/g.d.w.) of static tissue culture of *Allium sativum* Linn.

Age of tissue (Weeks)	GI	Trigonelline contents (mg./g.d.w.)
2	1.70±0.19	0.015±0.003
4	2.20±0.15	0.020±0.005
6	5.40±0.32	0.027±0.007
8	7.90±0.40	0.086±0.012
10	4.30±0.20	0.029±0.005

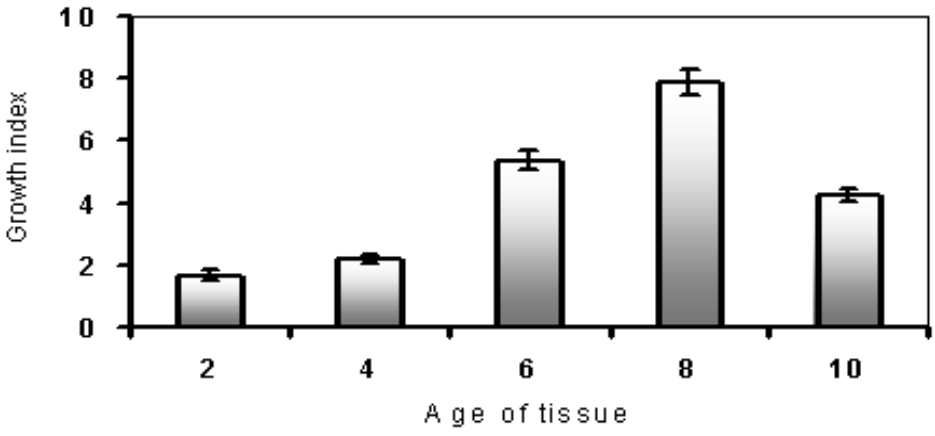


Fig. 1 : Growth index in relation to incubation period of culture

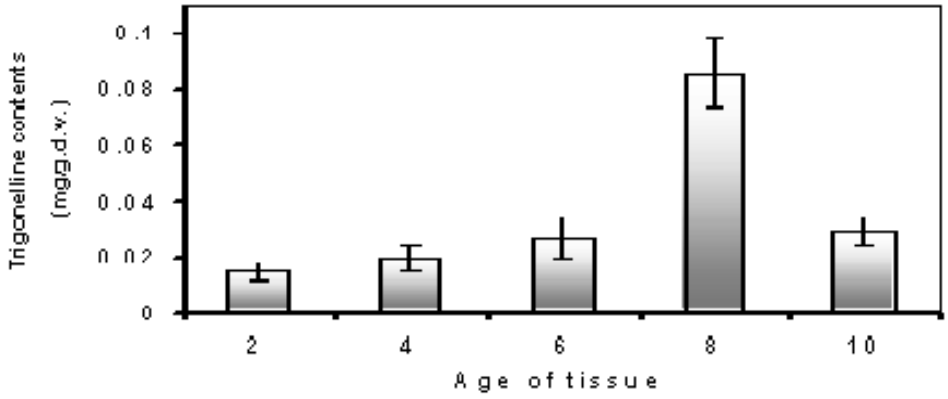


Fig. 2 : Total trigonelline content at different age of *A. sativum* tissue culture

References

Anderson L.A., Phillipson J.D. and Roberts M. (1985) : Biosynthesis of secondary products by cell cultures of higher plants. Adv. Biochem. Eng./Biotechnol. In : Fiechter, A (Ed) *Plant Cell Culture* **31** :1-36.

Bajaj Y.P.S and Nitsch P. (1975) : In vitro propagation of red cabbage, *J. Exp. Biol*. **26** : 883-890.

Bell E.A. and B.V. Charlwood (1980) : Encyclopedia of plant physiology vol. secondary plant products. Springer-Verlag Berlin. Heidelberg. New York.

Butenko R.G. (1985) : Plant cell culture (Biological series). MIR publisher, MOSCOW USSR.

Evans L. and Tramontana W. (1984) : Trigonelline and promotion of cell arrest in G2 of varitious legumes ACW *Symp. Ser*. **257** : 75-82.

Henry T.A. (1999) : The plant alkaloids anmol publication Pvt. Ltd. New Delhi pp. 16-17.

Hiroaoa N. and Tabata M. (1974) : Alkloid production by plants regenerated from cultured cells of Datura innoxia. Phytochemistry. **13** : 1671.

Kogan L., Dicarb J. and Maynard W.E. (1953) : Determination of coffee by paper chromatography. *Anal. Chem*. **25** : 1118-1120.

Reinhard E. (1975) : Possible use of plant tissue culture in medicinal plant research. Planta Medica (Suppl.) p. 1.

Robinson T. (1979) : The evolution ecology of alkaloids. In : G.A. Rosenthal and D.H. Tazen (Eds.) The alkaloids p. 413-448.

Staba E.J. (1980) : Plant tissue culture as a source of Biochemicals. CRC press, Boca Raton, Florida. p. 59-67.

Taiz L. and Zeiger E. (2002) : Plantd physiology II Edition sinquer Associates, Inc., Publishers, Sunderland, Massachusetts pp. 1-792.

Toguchi H., Sakaguchi M. and Shimabayashi Y. (1985) : Trigonelline content on coffee beans and the thermal conversion of trigonelline and nicotinic acid during the roasting of coffee beans. Agric. *Biol. Chem*. **49** : 3467-3471.

Tyler V.E. (1986) : Plant drugs in twenty first century. *Economic Botany* **40(3)** : 279-288.