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Bacoside - A production in embryogenic callus of *Bacopa monniera* (L.) Pennell.

Parale A. P.*and Sangle S. M.**

*Department of Botany, Rajaram College, Kolhapur-416008 (MS) India.

** Department of Botany, Government Institute of Science, Aurangabad-431004 (MS) India.

Abstract:

Bacopa monniera (L.) Pennell, (Scrophulariaceae) is an important plant in the Ayurvedic system of medicine, mainly used for treating age-related brain disorders, and for improving cognitive processes. Triterpenoid saponin, Bacoside -A is an active ingredient of *Bacopa* monniera responsible for its memory enhancing property. Due to improper harvesting from natural resources, seedling death at the 2-leaf stage and specific habitat requirement has lead to rapid depletion of *B. monniera* from its natural habitat. So there is a need to apply *in-vitro* techniques for increasing production of bacoside. Although somatic embryogenesis have focused on rapid propagation or fundamental work examining the differentiation and development in plants, this techniques was also reported for secondary metabolite production in few plants as secondary metabolite production is associated with tissue differentiation and development. The present study revealed that induction of somatic embryos associated with enhancement of bacoside A content in *Bacopa monniera* (L.) Pennell. Maximum bacoside A content recorded in embryogenic calli was significant to the bacoside A content of natural seeds.

Key words: *Bacopa monniera*, somatic embryogenesis, bacoside- A.

Introduction

One of the major research interests of today's world is to improve the cerebral ability of humans. Several plants have been selected based on their use in traditional system of medicine and identified some natural compounds in them that could act as nootropic agents. *Bacopa monniera* (L.) Pennell, (Scrophulariaceae) is an important plant in the Ayurvedic system of medicine, mainly used for treating age-

related brain disorders, and for improving cognitive processes. The triterpenoid saponin, baoside – A is considered as the active ingredient of *Bacopa* responsible for its cognitive effect (Deepak and Amit, 2004).

Somatic embryogenesis is defined as a non-sexual developmental process, which produces bipolar embryos from somatic tissues. Under natural conditions somatic cells did not showed the ability to induce embryogenesis. But we can induce embryos from somatic cells using in-vitro techniques. It can be an alternative to organogenesis for regeneration of whole plants, production of mutants, artificial seeds and materials for use plant genetic engineering (Tanaka *et al*., 2000). Also, somatic embryogenesis is also associated with the increase in secondary metabolite production (Profumo *et al*., 1991). Most studies on somatic embryo culture have focused on rapid propagation or fundamental work examining the differentiation and development of a wide variety of plants. In rare cases, this technique has also been applied to the production of useful secondary metabolites such as furanochrome in *Ammi visnaga* (El-Fiky *et al*., 1989), saponin in *Panax ginseng* (Asaka *et al*., 1994) and naphthoquinone in *Plumbago rosea* (Komaraiah *et al*., 2004). In the present investigation we observed the production of bacoside –A in embryogenic callus of *Bacopa monniera*. The present study also reports the influence of sucrose percentage, MS media strength and plant growth regulators on bacoside - A production.

Materials and Methods:

Induction and Maintenance of cultures:

Cultures were established and maintained as Parale and Sangle (2020).

Bacoside A extraction and estimation:

For quantification of bacoside, the samples were extracted by following the modified method of Watoo *et al.* 2007. In brief, the dried biomass was soaked in distilled water; the residue was extracted with 95% ethanol and dried under vacuum. The dried residue was redissolved in 1 ml methanol. The bacoside-A content in *in-vitro* samples were determined by HPTLC according to the procedure of Prakash *et al.* 2008. 10 µl of each sample extract was spotted on aluminium sheets of silica gel 60F254 (Merck), in the form of a band using CAMAG sample applicator. After the complete evaporation of applied sample the chromatogram was developed in a CAMAG twin trough chamber using solvent system toluene/ethanol /methanol/glacial acetic acid (4:3:3:1, v/v). The plates were completely dried at room temperature and derivatized by spraying 5% methanolic sulphuric acid followed by heating the plate at 100^{o}C for 10 min. Densitometric scanning was performed with a CAMAG TLC scanner-III at 500 nm. The spot of bacoside-A in all the samples was confirmed by comparing the R_f value and spectra of the spot with that of the standard. For quantitative analysis, peak areas were used to calculate the amount of the compound present in the cultured tissues as compared to standard.

Statistical analysis:

All the experiments were setup in completely randomized design. Experiments were conducted at least thrice with minimum 14 replicates per treatment. Observations were recorded at the intervals of eight days and wherever necessary daily observations were recorded. Means and standard deviation for the data were calculated and were analyzed using Microsoft Excel package. ANOVA was carried out to detect significant differences between means. Means differing significantly were compared using Duncan's (1955) Multiple Range Test (DMRT) at 5% probability level. Variability in data has been expressed as mean ± standard error.

Results

Bacoside A production in embryogenic callus:

As depicted in Table 1, all the cultures producing embryogenic calli showed presence of detectable amount of bacoside A.

The results showed that nutrient strength of MS media did not produce any remarkable effect on bacoside A production in embryogenic calli. At similar concentration of sucrose, bacoside A content recorded from calli regenerated on ½ strength MS media was significantly similar to the bacoside A content of calli regenerated on full strength MS medium. On the other hand, concentration of sucrose in culture media influenced the bacoside A accumulation in embryogenic calli. Although, there was no significant difference in bacoside A production in cultured media containing 1.5 - 3% sucrose, addition of still higher (4%) sucrose in culture media strongly affects the bacoside A production. Inclusion of 4% sucrose in ½ strength MS media was least effective for bacoside A production.

Bacoside A accumulation in embryogenic calli was influenced by the type and concentration of cytokinin present in culture media. Inclusion of Kin in 2,4-D containing MS medium was more effective than BA for somatic embryo formation as well as bacoside A production. Also higher concentration of Kin (20 µM) was superior to its low concentration (10 µM) for bacoside A production. However, concentration of 2,4-D in culture medium did not influence the bacoside A production. The embryogenic calli developed on MS medium supplemented with 20 µM 2,4-D in combination with 20 µM Kin produced maximum number of embryo per 100 g for fresh callus; which also produced maximum amount of bacoside A per gram callus. The calli developed on the said media produced 7.8 ± 0.2 mg/g D.W. bacoside A. Similar to this, the calli developed on MS medium fortified with 10 µM 2,4-D together with 20 µM Kin produced 7.5 ± 0.3 mg/g bacoside A on the dry weight basis. The bacoside A content detected in the calli produced on these two culture media was not significantly different then the bacoside A content of natural seeds (8.0 ± 0.2 mg/g D.W.). However, maximum bacoside A content recorded in embryogenic cultures (7.8 ± 0.2 mg/g D.W.) and bacoside A content of natural seeds (8.0 ± 0.2 mg/g D.W.) was significantly less compared to bacoside A content of natural stem (8.5 ± 0.2 mg/g D.W.) and leaves (28.7 ± 1.1 mg/g D.W.) of *B. monniera*.

Discussion:

It is well known that the synthesis and accumulation of many secondary plant metabolites is in some way associated with tissue differentiation and development. However the interrelation between somatic embryo formation and secondary metabolite synthesis is obscure. In the present study, it was observed that synthesis of bacoside A was related to the embryo formation. The culture treatment supporting embryo formation, also support maximum bacoside A production.

Depending upon plant species, more or less production of secondary metabolites compare to intact plant has been reported. The corydaline content of *Corydalis ambigua somatic* embryos cultured in the standard medium lower than that in the tuber of intact clonal plants (Hiraoka *et al*., 2004). Zhang *et al*. (1991) reported that *Corydalis yanhusuo* callus tissues possess the ability to redifferentiate tubers and roots and accumulated secondary metabolites comparable to the the intact tuber. Somatic embryos of *Ammi visnaga* produced 0.57% khellin and 0.72% visnagin similar to those of mature fruits (El-Fiky *et al*., 1989). In the present study, bacoside A content of embryogenic calli obtained on MS medium fortified with 20 µM 2,4-D and 20 µM Kin was significant to that of seeds collected from natural *in vivo* plants. Inclusion of 20 µM 2,4-D in combination with 20 µM Kin in MS medium supports maximum embryo formation as well as maximum bacoside A production. Similar phenomenon was observed in somatic embryos of *Salvia officinalis* and *Salvia fruticosa* somatic embryo cultures (Kintzios *et al*., 1999). The results from present study and earlier published literature indicate that, the biosynthesis of bacoside A in *B. monniera* might be associated with organ formation. This can be supported by the higher bacoside A content detected in intact plant parts.

Conclusion

The present study revealed that induction of somatic embryos associated with enhancement of bacoside A content in *Bacopa monniera* (L.) Pennell. The bacoside A content of embryogenic calli was more than the bacoside A content of normal callus. Maximum bacoside A content recorded in embryogenic calli was significant to the bacoside A content of natural seeds.

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Table: Bacoside A production in embryogenic callus of *B. monniera*

Growth regulators (µM)			Callus response parameter	Bacoside A mg/g D.W
2,4-D	Kin	BA		
10	10	-	GC	3.5 ± 0.1 hi
	-	10	GC	3.3 ± 0.1 i
	20	-	EC	7.5 ± 0.3 bcde
	-	20	EC	3.8 ± 0.1 hi
20	10	-	EC	6.7 ± 0.2 efg
	-	10	EC	4.1 ± 0.1 hi
	20	-	EC	7.8 ± 0.2 bcd
	-	20	EC	5.8 ± 0.2 g
MS basal + 1.5% sucrose			EC	6.9 ± 0.1 def
MS basal + 3% sucrose			EC	6.6 ± 0.2 efg
MS basal + 4% sucrose			GC	3.2 ± 0.1 i
½ MS basal + 1.5% sucrose			EC	7.0 ± 0.2 cdef

½ MS basal + 3% sucrose	EC	6.1 ± 0.2 fg
½ MS basal + 4% sucrose	GC	3.4 ± 0.1 i
Seeds		8.0 ± 0.2 bc
Roots		4.5 ± 0.1 h
Stem		8.5 ± 0.2 b
Leaves		22.7 ± 1.1 a

Data scored after 4 weeks of culture incubation; Mean values within a column followed by the same letters did not differ at 5% level of probability by DM