Applied Biological Research 19 (1): 114-116; (2017) **SHORT NOTE**
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PRODUCTION OF BIOMASS, TOTAL SAPONIN AND PROLINE UNDER VARIED NITROGEN CONCENTRATION IN SUSPENSION CULTURES OF *Gymnema sylvestre* R. Br

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Plants generally have medicinal value based on the kind and concentration of secondary metabolites they possess. One of such medicinally important plant *Gymnema sylvestre* R.Br. (Aslepiadaceae), commonly called 'gurmar', has widely been used in ayurvedic and homoepathic systems of medicine. Traditionally, this plant is used for its anti-diabetic potential attributed to the saponin named as gymnemic acid (Min *et al.*, 2007). The nitrogen concentration in medium greatly influences the amount of secondary metabolite produced (Nagella and Murthy, 2011) as it is required for the expression of specific proteins (Sugiharto and Sugiyama, 1992). The NH_4^+ to NO_3^- ratio influences the amount of biomass produced (Nagella and Murthy, 2011). Secondary metabolites are accumulated in cells in response to biotic and abiotic stress. Proline is one of the compounds accumulated in response to stress (Yaish, 2015). The present study aimed to analyze the influence of nitrogen on biomass and total saponin production in *G. sylvestre*, through a cost-effective method and relate it to associated proline content in tissues.

A 9 year old twinning shrub of *Gymnema sylvestre*, planted in the Experimental Nursery, AFRI, Jodhpur, Rajasthan (India) was selected for the collection of explants. The suspension cultures were initiated from friable callus obtained on semi-solid medium after 4-5 subcultures. Fresh callus (500 mg) was inoculated in 250 mL conical flask containing 40 mL liquid MS medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose, 2.26 µM 2,4-D and 0.46 µM kinetin as plant growth regulators. The experimentation involved 16 combinations of NH_4NO_3 and KNO_3 (Hi Media, Mumbai). The concentration of nitrogen sources in basal MS medium was 1650 mg L^{-1} for NH_4NO_3 and 1900 mg L^{-1} for KNO_3 (referred to as 1 X strength). These individual concentrations were altered with respect to NH_4^+/NO_3^- ratio to various strengths of 0X, 0.25X and 0.5X. The pH of the medium was adjusted to 5.8±0.02 with 1N NaOH or 0.1N HCl, prior to autoclaving. These were maintained on a gyratory shaker at 100 rpm under cool fluorescent light at 25±2°C, 60% RH and 16 h light/8h dark photo-period. The cells were sub-cultured after every 20 days. Batch culture was established by sub-culturing actively growing cells in fresh liquid MS medium.

The cells of suspension culture produced maximum total saponin after 15 days of incubation as per growth curve analysis (Fig. 1). Therefore, the cells were incubated for 15 days and then filtered from the medium to measure cell biomass and total saponin productivity. For the estimation of fresh biomass the cells were first washed with distilled water and then blotted dry on filter paper to remove excess water. Dry biomass was determined after drying the cells at 60°C till constant weight. This data was used for assessing growth ratio by following formula (Cui *et al.*, 2010):

$$\text{Growth ratio} = \frac{\text{Harvested dry wt.} - \text{Inoculum dry wt}}{\text{Inoculum dry wt}}$$

The total saponins were extracted from the samples using the method of Makkar *et al.* (2007). Production of total saponin along the growth curve was determined by vanillin-sulphuric acid method (Hiai *et al.*, 1976) where calibration curve was prepared using diosgenin as standard. The

suspension cultures were analyzed for the proline content accumulated. Proline was estimated using ninhydrin method (Bates *et al.*, 1973). In the above experimentation, each experiment was repeated thrice using 24 replicates for each treatment. The data so obtained was statistically analyzed by SPSS 17.0 version (SPSS Inc., Chicago, USA) using one way ANOVA and the significant difference between means was analyzed by Duncan's multiple range test (DMRT) at $p<0.05$. The result is expressed as mean ± SD.

In present study, out of the various combinations tested, the maximum biomass growth was obtained on medium with 0.5X strength NH_4NO_3 and full strength (1X) KNO_3 giving a growth ratio of 3.16. Similar observations were reported in suspension cultures of *Withania somnifera* (Nagella and Murthy, 2011) and *Panax ginseng* (Yu *et al.*, 2001) where lower concentrations of NH_4NO_3 supported highest biomass accumulation. Decrease in dry mass accumulation on lower concentrations of NH_4NO_3 was also observed in culture cells, which was concomitant with the observations made in seedling studies of *Jatropha curcas* (Wang *et al*., 2011). Hence, optimization of NH_4NO_3 andKNO_3 combination for maximum biomass production was done.

In present study, the maximum total saponin was produced (4.94 mg g^{-1} dry wt callus) on NH_4NO_3 free medium (0X) with full strength (1X) KNO_3 (Table 1). The maximum total saponin was produced in NH_4NO_3 free medium with varied strengths of KNO_3. These results were in concordance with the studies done in *Withania somnifera* where highest withanolide A production was observed in NH_4NO_3 free medium (Nagella and Murthy, 2011). Decrease of total saponin with increase in ratio of NH_4^+/NO_3^- was in agreement with the decreased level of alkanin production in *Arnebia hispidissima* (Shekhawat and Shekhawat, 2011). Reports reveal that a good relation exists between the

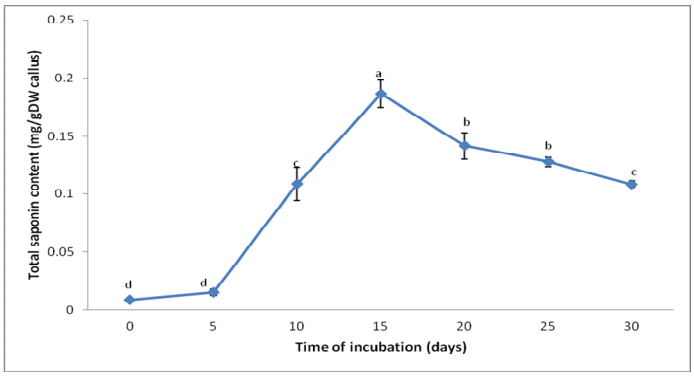

Fig. 1: Temporal effect on total saponin production in suspension culture of *G. sylvestre*

Table 1: Effect of various combinations of NH_4NO_3 and KNO_3 on growth ratio, total saponin production and proline yield

		Strength of NH_4NO_3 used			
		0X	0.25X	0.50X	1.00X
			Growth ratio		
Strength of KNO_3 used	0X	0.16	0.56	0.48	0.32
	0.25X	0.66	1.76	1.92	0.56
	0.50X	0.88	2.12	1.26	1.36
	1.00X	2.48	2.46	3.16	1.80
			Total saponin (mg g^{-1}DW callus)		
Strength of KNO_3 used	0X	0.71 ± 0.030d	1.00 ± 0.009d	1.01 ± 0.009a	1.01 ± 0.005a
	0.25X	3.73 ± 0.045c	1.97 ± 0.040c	1.18 ± 0.056c	1.11 ± 0.052b
	0.50X	4.66 ± 0.058b	2.27 ± 0.050b	2.34 ± 0.095d	1.11 ± 0.003b
	1.00X	4.94 ± 0.075a	2.33 ± 0.040a	1.16 ± 0.025b	1.12 ± 0.004c
			Proline yield (mg g^{-1} fresh wt callus)		
Strength of KNO_3 used	0X	0.12 ± 0.01g	0.21 ± 0.03f	0.22 ± 0.09f	0.18 ± 0.02f
	0.25X	0.59 ± 0.03c	0.52 ± 0.06d	0.60 ± 0.04c	0.37 ± 0.04e
	0.50X	0.66 ± 0.05b	0.61 ± 0.06c	0.61 ± 0.05c	0.41 ± 0.05e
	1.00X	0.74 ± 0.04a	0.62 ± 0.04b,c	0.49 ± 0.06d	0.40 ± 0.01e

Data points represent mean ± SD of three replicates. Mean values with common letters are not significantly different at $P < 0.05$ according to DMRT.

amount of N added and the amount of proline accumulated in cells of *Trifolium repens* (Kim *et al*., 2004). Highest proline content of 0.74 mg g^{-1} FW callus was recorded in combination of NH_4NO_3 free (0X) with full strength (1X) KNO_3 medium. It was observed that the amount of proline accumulated in the cells was higher in NH_4^+ free medium in combination with various strengths of KNO_3. The present study can be utilized for cost effective analysis and large scale production of total saponin. Effects of various concentrations can also be utilized for optimum production of total saponin under bioreactor system.

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