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BIOLOGICAL EFFECT OF SODIUM AZIDE ON *PROSOPIS CINERARIA* L. DRUCE *IN VIVO* AND *IN VITRO*

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Biological effect of sodium azide, a chemical mutagen on intact plants and unorganized cultures of *Prosopis cineraria* L. Druce was studied. The effect of these treatments were evaluated by considering various aspect of growth index (GI) primary and secondary metabolic contents. Unorganised cultures were initiated from hypocotyl and maintained on MS medium supplemented with different hormones. Plants and callus treated with sodum azide caused diminutive effect on primary and secondary metabolite production, however some positive implications were also noticed during the investigation.

Keywords: Intact plants; Organized cultures; Primary and Secondary metabolites; Sodium azide.

Introduction

Sodium azide (NaN_3) a chemical mutagen, acts as a biological toxic or mutagenic agent, which greatly influences growth and differentiation by regulating given expressions. Although the variations induced by mutagens are not always useful, they are helpful in determining the effect and mechanism of action of the mutagen in question and also the sensititivy of the biological material[1].

Sodum azide is mutagenic in several plant system[2] and nodulation[3]. It can be metabolized by plants into a mutagen[4]. Nevertheless, no work, has been done on the effect of sodium azide in production of primary and secondary metabolites under in vivo and tissue culture. *Prosopis cineraria* (Khejri), one of the most, important leguminous tree of Rajasthan, which not only serve as food for man but also fodder for cattle and potential source of raw material for industrial and pharmaceutical sectors. It also has the attitude to fix atmospheric mutagen which enriches the soil. Therefore, the present study was designed to study the effect of sodium azide, a well known potent mutagen[6] on the growth of primary and secondary metabolic contents of *Prosopis cineraria* L. Druce as well as on growth *in vivo* and *in vitro* system.

Material and Methods

In vivo- Seeds of *P.cineraria* were surface sterilized with 0.1% (w/v) mercuric chloride, washed with sterilized distilled water and than soaked for 4 hours. Presoaked seeds were treated with freshly prepared solution of NaN_3 (0.001, 0.002, 0.003, 0.004M) for 24 h. The treated seeds were transferred in polypots for raising the plants. Data were recorded upto 90 days. A set of untreated seeds served as a control.

In vitro - The surface sterilized seeds were germinated under aseptic conditions on MS basal medium[6] gelled with 0.8% agar-agar. Hypocotyl explants were excised from 10-15 days old seedlings and inoculated for induction of callus on modified 2MS medium (double micronutrients); 2.4-D, 2.5 mg/l; NAA, 0.5 mg/l; BAP, 1mg/l. Growth index were calculated into different intervals of growth period after five weeks of sub-culture by taking fresh weight of callus cultures. The induced static cultures were maintained with regular intervals of subculture by transferring them on fresh MS medium.

Five weeks grown callus (~ 1g) was transferred to modified 2MS medium to study the affect of NaN_3 treatments for growth period of five weeks as short duration. Freshly prepared aqueous solution of NaN_3 (5,10, 15, 20 µm) was incorporated after 3rd, 5th and 7th day for continuous supply for NaN_3 in first week of callus growth to study the effect on static cultures.

The growth indices were calculated at different time interval of 5th and 10th week using the formula

$$GI = \frac{\text{Final fresh weight-Initial fresh weight}}{\text{Initial fresh weight}}$$

Estimation of protein, sugar and phenol and secondary metabolic contents was done in *in vivo* and cultures including control by utilizing standard protocols[7-10], respectively.

Results and Discussion

In vivo- All the concentration of NaN_3 in *P.cineraria* in general caused significant reduction in seeding length except 0.001 M (Table 1), where no change in primary metabolic content and promotory effect in β–sitosterol was observed, so it is clear from the observations that sodium azide caused a clear inhibitory effect on growth

Table 1. Effect of NaN_3 on growth, primary and secondary metabolities of *Prosopis cineraria* L. Druce *in vivo*.

Concentrations (M)	Seedling length (cm)	Protein (mg/g/dw) mean ±SE	Sugar (mg/g/dw) mean ±SE	Phenol (mg/g/dw) mean ±SE	β–Sitosterol (mg/g/dw) mean ±SE
Control	17.34±0.31	0.0198±0.02	0.0287±0.22	0.0162±0.03	0.98±0.09
0.001	17.33±0.32	0.0172±0.07	0.0261±0.01	0.0183±0.21	1.18±0.11
0.002	15.11±0.17	0.0169±0.06	0.0272±0.07	0.0157±0.01	0.86±0.15
0.003	15.01±0.42	0.0189±0.11	0.0292±0.01	0.0141±0.17	0.81±0.14
0.004	13.76±0.21	0.0172±0.13	0.0232±0.04	0.013±0.05	0.85±0.01
'r'	-0.9532	-0.7330	-0.6432	-0.5441	-0.4322
Xx	8.320	0.012	0.024	0.012	0.762

of seedling as well as metabolic contents. This reduction may be due to gross injury caused at cellular level either due to the gene controlled biochemical processes or acute chromosomal aberrations or both. Similarly, early researchers[11,12], observed NaN_3 treatments had negative effect on seed germination and seedling growth parameters of *Vigna unguiculta*. Quraing[12] openoined that physiological effect of sodium azide might be due to decline in assimilation mechanism, inhibition of catalase, peroxidase and cytochrome oxidases.

In vitro- During the experimental investigation the marked reduction in GI was observed in treated callus cultures. Five weeks grown callus cultures showed more inhibitory effect than ten weeks growth period cultures in terms of GI in treated cultures of *P. cineraria* L. Druce (Table 2).

The physiological and biochemical studies reveal that in all the concentrations of NaN_3 treated cultures, significant reduction was observed in primary and secondary metabolites of five weeks growth period. Exception to this, 5 µm treated cultures, where no change in sugar and enhanced effect in β-sitosterol control were observed (Table 2).

Deterimental effect was observed in all treated series after ten weeks and more or less complete recovery of callus growth and metabolite production.

In all the parameters studied in the present experiments comparatively, after taking the combined mean values of percentage average of occurrence (Xx), maximum damaging effect was caused by higher concentrations.

Scanty information is available, on *in vitro* effects of NaN_3 as worked out in present study also. This observation is also supported by Ahmad *et al.*[14] who proposed the same effect that sodium azide interfers with biochemical and genetic proceses. Similarly, Panda and Madhukar[15] observed that percent germination decreased with increasing concentration of mutagene and their study also indicates that the duration of exposure of leaf explants of NaN_3 influence callus induction and growth at lower concentration.

The biological influence of NaN_3 accessed in present study clearly indicated that NaN_3 plays an important role as stimulatory agent at low/high doses. Therefore, a particular dose was used for enhancing the primary and secondary metabolic contents in the desert area.

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Table 2. Effect of NaN_3 on growth, primary and secondary metabolities of *Prosopis cineraria* L. Druce *in vitro.*

Concentrations (µM)	Growth Index (GI) Callus fresh weight (g)		Primary metabolic contents						Secondary metabolic contents	
			Protein		Sugar		Phenol		β-sitosterol	
	5 weeks mean ±SE	10 weeks mean ±SE	5 weeks mean ±SE	10 weeks mean ±SE	5 weeks mean ±SE	10 weeks mean ±SE	5 weeks mean ±SE	10 weeks mean ±SE	5 weeks mean ±SE	10 weeks mean ±SE
Control	2.81±0.04	2.82±0.25	2.88±0.15	2.89±0.14	3.49±0.17	3.47±0.21	4.65±0.11	4.67±0.11	1.48±0.12	1.37±0.21
5	2.71±0.29	2.76±0.21	2.95±0.21	2.81±.11	3.48±0.23	3.41±0.26	4.73±0.13	4.60±0.10	1.87±0.21	1.81±0.13
10	2.66±0.20	2.60±0.17	2.90±0.07	2.80±0.21	3.72±0.17	3.67±0.11	4.43±0.25	4.32±0.21	1.11±0.11	1.24±0.14
15	2.52±0.24	2.63±0.16	2.89±0.13	2.79±0.27	3.17±0.13	3.13±0.17	4.17±0.21	4.07±0.17	1.01±0.14	1.21±0.21
20	2.40±0.18	2.61±0.12	2.80±0.04	2.71±0.17	2.92±0.09	2.89±0.15	3.92±0.40	3.80±0.16	0.92±0.13	1.17±0.18
'r'	-0.7431	-0.5501	-0.7501	-0.6501	-0.7321	-0.7221	-0.7431	-0.6552	-0.6441	-0.5042
Xx	3.650	3.421	1.720	2.721	2.431	2.321	2.432	2.321	1.103	1.232

Reported values are mean ± SE of 3 replicates
'r'= coefficient of correlation
Xx= Percentage average of occurence

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