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Short Communication

Comparison of Sweet taste Suppression by Callus with different Leaf Explants of *Gymnema sylvestre*

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Abstract

To explore the possibility of in vitro production of gymnemic acid in cell & callus cultures at commercial level, callus was regenerated from leaf and nodal explants of Gymnema on MS medium supplemented with different combinations of auxins and cytokinins. Best response (100%) was observed with leaves incubated on MS + 0.5mgl^{-1}Kn+ 1.5 mgl^{-1} 2,4-D.For estimating GA content, recovery time of sweet taste after chewing the sample was taken as parameter. Callii obtained from leaves from juvenile and mature plates were compared for GA activity. Samples were chewed separately by volunteers of different age groups (10-40years) before and after taking meal. Maximum time (4 hours) in sweet taste recovery was observed by 10-20 years old volunteers by chewing mature leaves before taking meal, while with callii this time was very short (15-20min).Callus obtained on different PGR combinations responded differently. Hence by altering media composition production of gymnemic acid can be enhanced and such oral test can be used as preliminary test for estimation of GA in sample.

Keywords: *Gymnema sylvestre,* gymnemic acid, MS medium, PGR.

Introduction

In India, almost 45000 plant species are growing naturally or being cultivated. There are many popular Indian herbs used in traditional practices to cure diabetes[1,2]. *Gymnema sylvestre* R.Br. has an important place among such antidiabetic medicinal herbs. It belongs to the family Asclepiadaceae. It is known as "Gurmar" (destroyer of sugar) or Periploca of the woods" and is distributed over most parts of India, tropical Africa, Vietnam, Malaysia, Srilanka, Japan, Germany and USA. Its leaves contain triterpene saponins[3]. As a result of chewing the leaves**,** the sensation of sweet taste buds issuppressed. Gymnemic acid present in the leaves is responsible for this property. Component molecules of gymnemic acid are similar in arrangement to that of glucose molecules. These molecules occupy the receptors present on the taste buds for one to two hours, hence glucose molecules present in food cannot bind to these receptors, thus suppressing the sweet taste. The same phenomenon occurs in the absorptive external layers of the intestine, thereby preventing the intestine from absorbing the sugar molecules. However, it has no effect on pungent, salty, astringent or acidic tastes[4]. In present study a reproducible callus regeneration protocol was developed in view to produce GA in *vitro*. Then GA effect of various plant parts was compared with that of callii obtained on different PGR combinations.

Material and Methods

Leaves and nodal stem segments from 5-6 years old *G. sylvestre* were collected from NATP garden of College of Agriculture, S.K.R.A.U, Bikaner. Explants were first treated with a detergent (Clextron 0.5%, CDH) for 10 – 15 minutes and washed with tap water. The explants were dipped for 1-2 seconds in 70 percent ethanol and then immediately immersed in sterilized water. This pre-treated explant material was surface sterilized with 0.1 percent (w/v) aqueous solution of $HgCl_2$ (E. Merck -India) for 3 – 4 minutes and washed several times with sterilized distilled water. The process of pre-treatment and surface sterilization was carried out under aseptic condition in laminar air flow bench. The surface sterilized explants were inoculated on MS media[5] supplemented with cytokinins and auxins in combination for callus culture. Cultures were incubated in 16 hour photoperiod at 1000 lux light intensity and 25 ± 2^0C temperature.

Leaves segments from juvenile and mature (5-6yrs.) *G. sylvestre* were tested orally by various volunteers group each of 5 members of different age group (10-40 years old) of male and female. Juvenile and young leaf derived fresh callii of MS medium supplemented with combinations of Kn (0.5 mgl^{-1}) +2,4-D (0.5,1.0,1.5,2.0, 5.0 mgl^{-1}) were also tested orally on the same group. Sample weighing (juvenile leaf; 20-30 mg and young leaves/nodal segment; 80-100 mg) was chewed by individuals for 2 minutes after and before taking the meal. Individuals were given 5gm of sugar at an interval of 15 minute and sweet taste recovery time was recorded.

Results and Discussion

Callus induction: When both leaf (young/juvenile) and stem (inter node, young node) explants were incubated on MS

medium supplemented with combination of Kn (0.5 mgl^{-1}) and 2,4-D (0.5,1.0,1.5, 2.0,5.0 mgl^{-1}) , low concentration of Kn (0.5 mgl^{-1}) and higher concentration of 2,4-D (1.5,2.0,5.0 mgl^{-1}) induced light green callus (80-100%) within 30 days of incubation from leaf explants. Young nodal explants induced white, light green friable callus (60-70%) on 0.5 Kn+ 1.5, 2.0 mgl^{-1} 2,4-D (table-1).

Effect of leaf explants on volunteer's sweet taste recovery time: After chewing sample sweet taste was disappeared immediately. When food was not taken by person, before chewing sample, it was found that recovery of sweet taste took longer time (1 -4 hrs.) by all the volunteers. In 10-20 years old group recovery of sweet taste took 3-4 hours after chewing mature leaves before taking meal. When volunteer's chewed juvenile /young leaves after meal, it was found that recovery of sweet taste took shorter time (1-3 hrs.) by volunteers of different age group. Sweet taste recovery was slowest (3hrs.) in 10-20 yrs. age group volunteers after taking mature leaves (table-2).

Effect of fresh callus on volunteer's sweet taste recovery time: When volunteers chewed 50 mg fresh friable juvenile and young leaf derived callus obtained on MS medium supplemented with combinations of Kn (0.5 mgl^{-1}) +2,4-D (0.5,1.0,1.5,2.0,5.0 mgl^{-1}), sweet taste disappeared due to presence of gymnemic acid after chewing juvenile and young leaf derived callus of Kn (0.5 mgl^{-1}) +2,4-D(1.5 mgl^{-1}), but for a shorter duration (2-20 minutes.).Here also slowest recovery was shown by 10-20 years group before or after taking meal (table-2). When volunteer's chewed juvenile /young leaf derived callus before and after meal, it was found that mature leaf derived callus have more gymnemic acid, so it took longer time (5-20 min.) in recovery of sweet taste by all the volunteers before or after taking meal.

Table-1
Combined effect of Kn and 2,4-D on callus proliferation from both stem node and leaf explant of *G. sylvestre*

Kn (mgl^{-1})	2,4-D (mgl^{-1})	Callus morphology	Amount of callus	Response (%)
0.5	0.5	Light pale yellow, compact callus (leaf)	++	60
	1.0	Light pale yellow, compact callus(leaf)	+++	75
	1.5	light green ,yellow compact callus (leaf) white ,friable callus (stem)	++++ +++	100 70
	2.0	light green ,yellow compact callus (leaf) white ,friable callus (stem)	+++ ++	80 60
	5.0	Light green, yellow, compact callus(leaf)	+++	80

Days of observations - after 30 days of incubation, ++ Slight/ poor, +++ medium callusing, ++++ profuse callusing

Table-2
Chewing effect of *in vitro* leaf explants and *in vitro* raised callus of *G sylvestre* on recovery time of sweet taste buds

Age group (years)	Juvenile Leaf		Juvenile leaf derived callus (0.5Kn+1.5mgl^{-1}2,4-D)		Mature leaf		Mature leaf derived callus (0.5Kn+1.5mgl^{-1}2,4-D)	
	Recovery time (hours)		Recovery time (minute)		Recovery time (hours)		Recovery time (minute)	
	Pre food	Post food	Pre food	Post food	Pre food	Post food	Pre food	Post food
10-20	2	2	15	7-8	3-4	3	20	10
20-30	1	1	5	2	2	1	10	5
30-40	2	1	10	5	3	1	10	5

Pre food- Sweet taste recovery time before taking meal, Post food- Sweet taste recovery time after taking meal



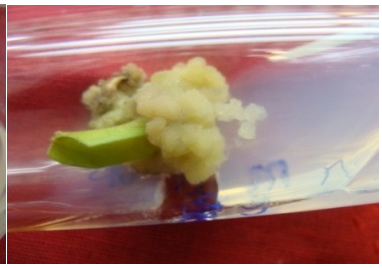

Figure-1 **Figure-2** **Figure-3**

Fig-1-3:1. *Gymnema sylvestre* plant; 2. Callus on 0.5 mgl^{-1}Kn+1.5 mgl^{-1}2, 4-D; 3. Callus on 0.5 mgl^{-1}Kn+2.0 mgl^{-1}2,4-D

Conclusion

In the present investigation, it was found that young leaf explant of *Gymnema* proved to be best in term of callus induction. Similar kind of results was observed in *G. sylvestre* by Gopi and Vatsala[6]. Callii of different morphological features has been formed on various auxins and cytokinins.[7]When both explants were incubated on MS medium supplemented with combinations of Kn (0.5mgl^{-1}) and 2,4-D(0.5-5.0 mgl^{-1}), it was found that that low concentration of Kn (0.5 mgl^{-1}) and higher concentrations of 2,4-D (1.5,2.0,5.0 mgl^{-1}) induced light green callus (80-100%) from leaf explants and white, light green, friable callus (60-70%) was induced on concentration of 0.5 Kn+ 1.5, 2.0 mgl^{-1}2,4-D from young nodal explants.

For estimation of GA, leaves of juvenile and mature *G. sylvestre* and both leaf explant derived fresh callii were tested by various volunteers of different age group (10-40 years old). It was found that oral taste buds of volunteer's (20-30 years) took very short time in recovery of sweet taste by chewing young leaf and leaf derived callus (0.5 mgl^{-1}Kn +1.5 mgl^{-1}2,4-D),when the food was taken or not. Leaf explants took longer time in sweet taste recovery than callus, hence it can be concluded that leaves have more gymnemic acid than callus. In different callii also, callus raised on Kn (0.5 mgl^{-1}) +2, 4-D(1.5 mgl^{-1}) had more gymnemic acid because it took longer time in recovery of sweet taste. According to the sweet taste recovery time recorded the amount of GA in callus cultures was very minute and it was found that sweet taste recovery time was more when samples were fed before meal and recovery was slowest in 10-20 yrs. age volunteers. It can be improved by altering media composition, hormonal combinations and physical conditions or by giving elicitation[8].

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