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Study of callus induction with domestic sugar and sucrose in different concentration of PGR in Ashwagandha (*Withania somnifera*) Dunal

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Abstract
The Experiment work was conducted at High Altitude Plant Physiology Research Centre (HAPPRC), HNBGU, Srinagar Garhwal, and Uttarakhand, India. Ten months old plant parts were used as explant. The tissue culture work was done with the plant of Ashwagandha (*Withnia sominfera*) which is one of the major herbal components of geriatric tonics mentioned in Indian systems of medicine. It is greatly use in Ayurveda. The work was started with the media preparation, domestic sugar and sucrose were used with the different concentrations of PGR. Inoculated all the explants in the prepared media with different hormonal concentrations under the aseptic condition. Swelling of the explants was observed one week after inoculation. However, callus initiation was observed in the second week after inoculation. Four weeks later, callus initiated at the cut edge of the explants and developed into a full grown callus. The callus was morphologically found to be yellowish-white, compact, dry and nodular. The result was observed that the domestic sugar and sucrose with the concentration of 3µM BAP gave better response respectively. The callus (3µM BAP) was sub cultured again with the different concentrations of BAP and observed high rate of callus mass increased in 3µM and 5µM of BAP.

Keywords: Tissue culture, domestic sugar, sucrose, explant, Callus, contamination, inoculation, sub culture

Introduction
Withania somnifera Dunal popularly known as 'Ashwagandha' is one of the major herbal components of geriatric tonics mentioned in Indian systems of medicine. It is a erect branching under shrub reaching about 150 cm in height, usually clothed with minutely stellate tomentum; leaves ovate up to 10 cm long, densely hairy beneath and sparsely above, flowers greenish or lurid yellow in axillary fascicles, bisexual, pedicel long, fruits globose berries which are orange colored when mature, enclosed in a persistent calyx. The fleshy roots when dry are cylindrical, gradually tapering down with a brownish white surface and pure white inside when broken.

Ashwagandha is effective for insomnia but does not act as a sedative. It rejuvenates and nervine properties produce energy which in turn help the body to settle and sleep. Thus it helps the body to address a stress related condition rather than masking it with sedatives. An herb that rejuvenates the nervous system erases insomnia and eases stress. It has also been shown to lower blood pressure and is highly effective in stopping the formation of stress induced ulcers. In arthritis, which involves joints that are painful, dry, swollen and inflamed, Ashwagandha would be the herb of choice. It increases hemoglobin (red blood count) and hair melanin. It stabilizes blood sugar and lowers cholesterol (Naveen Gaurav *et al*.) [6].

In the traditional system of medicine Ayurveda, this plant is claimed to have potent aphrodisiac rejuvenates life prolonging properties. It has general animating and regenerative qualities and is used among others for the treatment of nervous exhaustion, memory related conditions, insomnia, tiredness, potency issues, skin problems and coughing. It improves learning ability and memory capacity. The traditional use of 'Ashwagandha' was to increase energy, youthful vigour, endurance, strength, health, nurture the time elements of the body, increase vital fluids, muscle fat, blood, lymph, and semen and cell production. It helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature aging emaciation, debility, and convalescence and muscle tension. It helps invigorate the body by rejuvenating the reproductive organs, just as a tree is invigorated by feeding the roots.

In recent years market of plant products expand rapidly and this trend will continue in the 21st century because more and more people prefer natural products. (Singh N, *et al*.1982).
In present work during the research *in vitro* protocol for rapid regeneration of *Withania somnifera* practiced. It deals with *in vitro* plant growth of *Withania somnifera* through tissue culture for propagation. Control use of fertilizers in cultivated crops at the time of germination may be effect the quality of plant and seeds. The rapid multiplication of *W. somnifera* by tissue culture techniques can help to solve these problems and the benefits are extensive in the agricultural.

Materials and Methods
The Experiment was conducted at High Altitude Plant Physiology Research Centre (HAPPRC), HNBGU, Srinagar Garhwal, and Uttarakhand, India. Ten months old plant parts were used as explant.

Media Preparation
Glass wares were washed with tween 20. After that distilled water was used to clean glass wares. A small amount of distilled water was taken in sterilized flask. Added macronutrients 25ml + $CaCl_2$ 25ml, micronutrient 1ml and vitamins 1ml from stock solution accordingly. The volume of the medium was made up by adding1000 ml of distilled water. Divided the media stock into equal amount. Added 15gm sucrose into 500ml of media and 15gm domestic sugar to rest of 500 ml of media. Required amount of growth regulators were added. After that added BAP in 500 ml (250 ml with sucrose and 250ml with domestic sugar) of media and 2, 4-D in another 500ml (250ml with sucrose and 250 ml with domestic sugar) stock solution. Poured the media stock solution in 20 jam jars of different concentration (2µM, 3µM, 5µM, 10µM) of BAP and 2, 4-D and we kept 4 jars without any concentration. The pH of media was adjusted to 5.6-5.8 by adding 1N HCL or 1N NaOH. After maintaining the pH, 400mg Agar was added in each vials. The vials with medium were kept in microwave to dissolve the agar. Finally the culture vials with medium were capped and autoclaved at 15 1bs, 121c for 20 to 30 minutes.
For sterilization of media the minimum time required depends upon the volume of the media in the vessel. Prolonged autoclaving may result in breaking and denaturation of media ingredients.

Collection of Explants
10 months old plant was selected and parts were separated with the help of surgical blade and forceps, which were collected from HAPPRC (High Altitude Plant Physiology Research Canter) Srinagar Garhwal (Uttarakhand).

Surface sterilization process of explant
The collected explants were washed thoroughly (10 times) with tap water in order to remove large dust particles and then with distilled water. Later the explants were washed with detergent (tween-20) to avoid contamination and again washed with distilled water Washed explants were treated with fungicide Endokil (200mg/200ml of water) and kept it for 30 min. Prepared 7ml/100ml $NaClO_3$ and 10mg/100ml $Hgcl_2$ of water. Place the treated explants, $NaClO_{3zs}$ & $Hgcl_2$ in the laminar flow for 1-2min and then washed with autoclaved water.

Micro propagation of *Withania somnifera Dunal*
Cut the leaves and nodule parts with the help of forceps and surgical blade.
Selected leaves and nodule parts were inoculated in the prepared media with the help of forceps and then jars were covered with caps tightly under laminar airflow. After the process of inoculation all the jars were kept into the laboratory (temperature 23-25 C).

Result and Discussion
Swelling of the explants was observed one week after inoculation. However, callus initiation was observed in the second week after inoculation. Four weeks later, callus initiated at the cut edge of the explants and developed into a full grown callus. The callus was morphologically found to be yellowish-white, compact, dry and nodular. The time when the callus appears is influenced by several factors, including the source of plants used as the ex-plants and growth regulators used. It is known that 2,4-D and BAP concentrations can play a role in the acceleration when callus appears.
Table 1. shows the number of callus appears after inoculation. In line with the results, it showed that the domestic sugar with the concentration of 3µM BAP and sucrose with the concentration of 3µM BAP was the concentration that induced the highest number of callus initation Whereas concentration of Domestic sugar and sucrose, 2,4-D of concentration 10 µM and BAP of 10 µM did not give any signs of callus initation. The time when the callus appears is influenced by several factors, including the source of plants used as the ex-plants and growth regulators used.It is known that 2,4-D and BAP concentrations can play a role in the acceleration when callus appears.
The production of a yellowish, compact and nodular callus at cut edge of explant may be due to the wound caused during the process of cutting which resulted in a synchronous cell division. This is considered as a process of de-differentiation of organized tissue and is similar to the work of Hamish and Sue (1989) Pellegrinechi *et al*. (2004); Qin *et al*. (2005) [8] and Xing *et al*. (2010) [12]. The development of callus from immature leaf explants is directly related to the presence of BAP which is a suitable growth hormone responsible for callus induction in most plant Species in plant tissue culture work. This is similar to the findings of Mamun *et al*. (2004) [4] and Baskaran *et al*. (2005) [2].

Table 1: Response of Domestic sugar in different concentrations of BAP and 2, 4-D

S. No	Conc. Of PGR	No. of explants	Response
BAP (Benzyl Aminopurin)			
1	Control	3	3
2	2 µM	3	2
3	3 µM	4	4
4	5 µM	4	3
5	10 µM	3	1

2,4-D (2,4 Dichloro phenoxy Acetic Acid)			
6	Control	3	3
7	2 µM	2	0
8	3 µM	3	2
9	5 µM	3	0
10	10µM	2	2

Table 2: Response of sucrose in different concentrations of BAP and 2, 4-D

S. No.	Conc. of PGR	No. of explants	Response
BAP (Benzyl Aminopurine)			
1	Control	3	2
2	2µM	2	1
3	3µM	3	3
4	5µM	4	2
5	10 µM BAP	4	3
2,4-D (2,4 Dichloro phenoxy Acetic Acid)			
6	Control	2	1
7	2 µM	3	2
8	3 µM	3	2
9	5 µM	3	0
10	10 µM	3	0

Table 3: Subculture of 3µM BAP with different concentrations

S. No	Treatment concentration	No of callus part	Response
1	Control	2	2
2	3µM	4	4, Callus mass increased at very good rate
3	5µM	4	4, Callus mass increased at very good rate
4	10µM	4	2

Fig 1: Initiation of callus

Fig 2: Growth of callus

Conclusion

It has been concluded that the domestic sugar and sucrose with the concentration of 3µM BAP gave better response respectively. The callus (3µM BAP) was sub cultured again with the different concentrations of BAP and observed high rate of callus mass increased in 3µM and 5µM of BAP.

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