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MICROPROPAGATION STUDIES IN ASHWAGANDHA (*WITHANIA SOMNIFERA*)

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Key words : *In vitro, Micropropagation, Withania somnifera, Ashwgandha, Plant growth regulator.*

Abstract – The present investigation on micropropagation studied in ashwagandha *(Withania somnifera*) were to study the response of various genotypes on different media. The various genotypes were collected from Medicinal and Aromatic Plant Project, MPKV, Rahuri, Dist, Ahmednagar. Among the different media concentration tried for the establishment of the culture. Least period for the establishment for all genotypes responded on MS media supplemented with 2 mg/l BAP and 0.4 mg/l KIN. However embryogenic callus was formed on MS medium containing 2,4-D (0.5 mg/l) after 2 weeks of subculture. Regeneration was noticed within 3.66 to 12.33 days. The genotype RAS-2 took minimum days 3.66 for emergence of first shoots on media MS + 2 mg/l BAP+2 mg/l KIN. Maximum number of multiple shoots (7.00) were produced by RAS-2 genotype on media MS+3mg/l BAP+1mg/l IAA). The optimums rooting to the shoot were recorded on MS supplemented with 1 mg/l IBA. Maximum percentage of survival 80% observed in genotype RAS-2 plantlets was established after hardening.

INTRODUCTION

India has a long history of the use of a large number of medicinal and aromatic plants for various purposes. India is also a primary and secondary centre of origin and centre of diversity of several medicinal and aromatic plants. *Withania somnifera* have been used in herbal formulations (Kamboj, 2000). Ashwgandha or Asgandh *(W. Somnifera)* is an important medicinal plant cultivated only in North Western region of Madhya Pradesh on about 4000 ha in India (Nigam, 1995). The pharmacological activity of the roots is attributed to the presence of several alkaloid content of the Indian roots is reported between 0.13 to 0.31 per cent.Roots are prescribed in medicines for hiccup, several female disorders, bronchitis, rheumatism, dropsy, stomach,lung inflammation and skin diseases (Chopra *et al.,* 1958).

Propagation of ashwagandha is mainly by seeds, but seed viability is limited to one year. Therefore, it is important to develop an efficient micropropagation technique rapidly disseminate superior clones. Once they are identified the genotype with alkaloid content and higher yield will give higher returns to farmers (Madhvilata and Sing, 2008).

MATERIALS AND METHODS

Murashige and Skoog (1962) nutrient media was used for the present study. The stock solutions of growth regulators of concentrations is used as, NAA (1N NaOH), IBA (1N NaOH), IAA(1N NaOH) and BAP(1NNaOH) (Razdan, 2000). The specific media (pH 5.7-5.8) poured hot at the rate of 25-30 mL per sterilized bottle and 15-18 ml per sterilized test tube (150mm x 25mm). The bottles were plugged with caps or non absorbent cotton and autoclaved at 15 lbs (1.06 kg/$m^{2)}$ pressure and 121 ^{o}C for 15-20 minutes (Bhojwani and Razdan, 1983). The inoculated cultures were incubated at 25$\pm$2 ^{o}C in an air conditioned culture room. Photoperiod was maintained 16 hrs (3000-3500 lux) supplied by cool white florescent tube lights daily followed by 8 hrs of darkness as suggested by Conger (1981).

The explants of all genotypes under study of ashwagandha collected from Medicinal and Aromatic Plant project, M.P.K.V. Rahuri served as source material. The explants of optimum size were

washed thoroughly under running tap water for 25 minutes followed by distilled water containing detergent (Tween-20) solution for 10 min. with constant shaking. The shoot tip and nodal segments of 0.5-1 cm were taken for inoculation pre sterilize with $HgCl_2$. The culture bottles/ test tubes after inoculation were kept in culture room at 25±2 ^{0}C temperature and photoperiod of 16 hours light and 8 hours dark in culture room.

Treatment combinations for establishment is used as ES_1 – MS+0.5 mg/l BAP+ 0.1 mg /l KIN, ES_2 – MS+ 1 mg/l BAP+ 0.2 mg /l KIN , ES_3 – MS+ 1.5 mg/l BAP+ 0.3 mg /l KIN, ES_4 – MS+ 2 mg/l BAP+ 0.4 mg /l KIN. Treatment combinations for shoot multiplication is used as MS_1 – MS+0.5 mg/l BAP+ 0.5 mg /l NAA, MS_2 – MS+1 mg/l BAP+ 1 mg /l NAA, MS_3 – MS+ 2 mg/l BAP+ 0.5 mg /l IAA, MS_4 – MS+3 mg/l BAP+ 0.5 mg /l IAA.

RESULTS AND DISCUSSION

Days required for establishment of explants for different genotype

The days required for establishment of explants for different genotype on various concentration of media in Table 1. Significant results were found when concentration of BAP was increased up to a certain level (*viz*., 2.0 mg/l) after that it reduced with further increase. Thus the minimum 5 days were required by RAS-2 genotype for establishment on treatment ES_4 – MS+ 2 mg/l BAP+ 0.4 mg /l KIN. The observations on number of regenerated shoots for all genotypes recorded.

Table 1. Days required for establishment of explants

Genotype	Media				Mean
	ES_1	ES_2	ES_3	ES_4	
Askand	7.333	9.333	6.667	5.667	7.250
Nagori	7.667	6.667	6.000	5.333	6.417
RAS-2	8.667	6.333	6.000	5.000	6.500
RAS-3	8.000	9.000	7.000	6.000	7.500
RAS-4	8.333	7.333	6.333	6.333	7.083
		S.E.±		C.D. at 5 %	
Genotype		0.16245		0.46406	
Media		0.14530		0.41507	
Interaction		0.324890		0.92813	

ES_1 – MS+0.5 mg/l BAP+ 0.1 mg /l KIN
ES_2 – MS+ 1 mg/l BAP+ 0.2 mg /l KIN
ES_3 – MS+ 1.5 mg/l BAP+ 0.3 mg/l KIN
ES_4 – MS+ 2 mg/l BAP+ 0.4 mg /l KIN

Effect of Plant Growth Regulators combination on multiple shoot formation and percent shoot multiplication from shoot tip: The effect of different concentration of BAP and NAA on multiple shoots formation furnished in (Table 2)**.**

Number of multiple shoots per explants : The number of shoots formed per genotype was ranged from 1 to 7 (Table .2)

Table 2. Number of shoots produced per explant

Genotype	Media				Mean
	MS_1	MS_2	MS_3	MS_4	
Askand	—	1.333	3.333	5.333	2.500
Nagori	—	2.000	5.000	6.667	3.417
RAS-2	—	2.667	5.333	7.000	3.750
RAS-3	—	2.333	5.000	6.333	3.417
RAS-4	—	2.333	4.333	6.000	3.167
Mean	—	2.133	4.600	6.267	
		S.E.±		C.D. at 5 %	
Genotype		0.16245		0.46406	
Media		0.14530		0.41507	
Interaction		0.32489		0.92813	

MS_1 – MS + 0.5 mg/l BAP + 0.5 mg /l NAA
MS_2 – MS + 1 mg/l BAP + 1 mg /l NAA
MS_3 – MS + 2 mg/l BAP + 0.5 mg/l IAA
MS_4 – MS + 3 mg/l BAP + 1 mg /l IAA

The treatment MS_4 – MS+3 mg/l BAP+ 0.5 mg /l IAA recorded maximum number of multiple shoots (7.00) were produced by RAS-2 (Fig. 2). However, shoots did not survive on MS_1 – MS+0.5 mg/l BAP+ 0.5 mg /l NAA and resulted into death of cells.

Influence of plant growth regulators combination

Effect of BAP on explants establishment from nodal segment: The effects of different

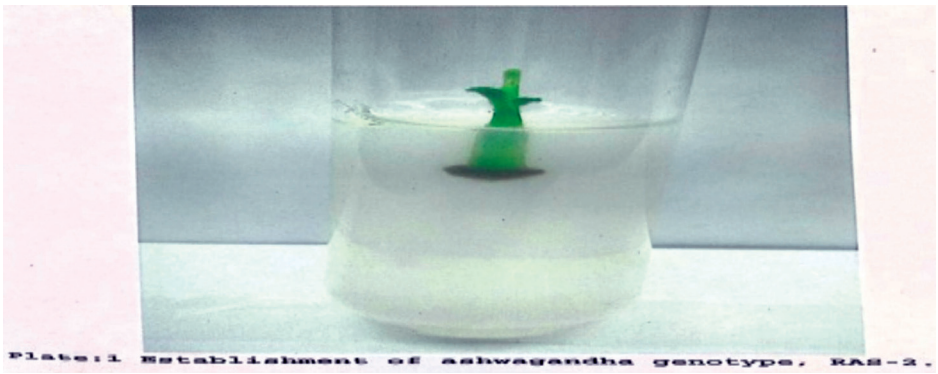

Fig. 1. Establishment of shoot tip in ashwagandha

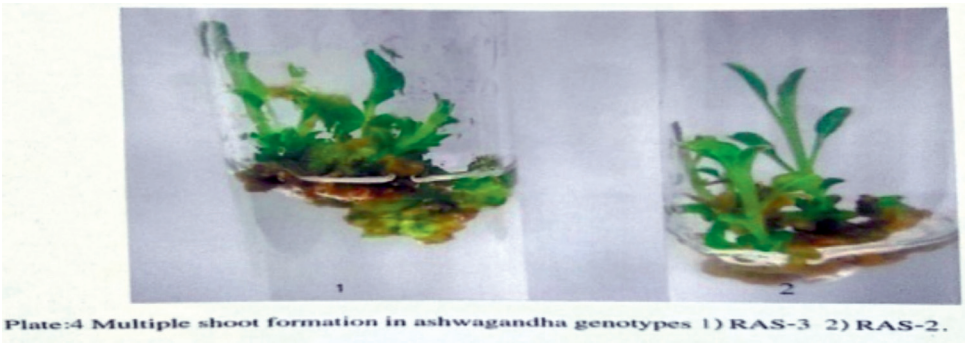

Fig. 2. Establishment of multiple shoot in ashwagandha genotypes

concentration of BAP for explants establishment from nodal segment furnished in Table 1. Optimal explants establishment was obtained when concentration of the BAP was increased up to a certain level (*viz.,* 2.0 mg/l) after that it reduced with further increase (Fig. 1).

Days required for establishment

The number of days required for explants establishment ranged from 5 to 9.33 days (Table 1). The treatment ES_4 (MS+ 2 mg/l BAP+ 0.4 mg/l KIN) recorded significantly earliest in RAS-2 genotype (5 days) for explants establishment, followed by Nagori (5.33 days) on same media. Treatment ES_1 – MS+0.5 mg/l BAP+ 0.1 mg/l KIN, ES_2 – MS+ 1 mg/l BAP+ 0.2 mg /l KIN recorded maximum number of days (8.33 to 9.33 days) for explants establishment.

Effect of Plant Growth Regulators combination on multiple shoot formation : The number of shoots formed per genotype was ranged from 2.00 to 7.00 (Table 2). The treatment MS_4 – MS+3 mg/l BAP+ 0.5 mg/l IAA recorded maximum number of multiple shoots (7.00) were produced by RAS-2 (Plate 2). The minimum number of shoots per genotype was recorded by Askand on MS_2 – MS+1 mg/l BAP + 1 mg /L NAA.

CONCLUSION

The results from present investigation clearly indicated that mass multiplication of *Withania Somnifera* through micro propagation is reliable and promising for conservation of this endangered species. From the present investigation *in vitro* response of *W. somnifera,* clearly indicated that the RAS-2, Nagori, RAS-3 are most suitable for rapid multiplication.

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