

***In vitro* establishment and multiplication studies in spine gourd (*Momordica dioica* Roxb)**

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In micropropagation of *Momordica dioica* Roxb., the axillary bud was found as a best explant than shoot tip for rapid multiplication of three local genotypes comprising of two female (AKSG-5 and AKSG-35) and one male genotype (AKSGM-1). Basal MS medium containing AdSO₄ (70 mg/l or 80 mg/l) supplemented with BAP (1.0 mg/l) and NAA (1.0 mg/l) produced maximum multiple shoots (more than 5 per culture) with better growth response.

Key words: Micropropagation, *Momordica dioica*.

INTRODUCTION

KARTOLI (*Momordica dioica* Roxb) is a cucurbitaceous and perennial vegetable. It is indigenous and well distributed throughout the country (Singh, 1990). Kartoli having tuberous roots, herbaceous climber grown for nutritious immature tender green fruits. It is unexploited promising vegetable of high nutritional, medicinal and economical value.

High multiplication ratio can be achieved by micropropagation technique, which enables rapid multiplication of disease pest free elite plants within short space and time. (Morel and Martin, 1952).

Considering the importance of spine gourd and need for rapid multiplication of promising local genotypes of two female AKSG-5 and AKSG-35 and one male (AKSGM-1) was attempted for perfect investigations to standardize the technique of micropropagation of Spine gourd. Multiplication of elite genotypes through vegetative means will improve the growth rate and production of fruits in shorter span of time. All the conventionally used methods of vegetative propagation have been tried with kartoli but most of the methods have been difficult and inefficient. Thus there is vast scope for clonal selection and micropropagation of elite kartoli. Attempts were made to collect relevant literature on the *in vitro* multiplication of kartoli and other related crop species. Murashige (1974) has given the stages for *in vitro* propagation as under,

Stage I :	Explant establishment
Stage II :	Rapid multiplication of shoots through increased axillary branching
Stage III :	<i>In vitro</i> rooting
Stage IV :	Acclimatization and planting out

MATERIALS AND METHODS

The explants of spine gourd i.e., shoot tip and axillary buds were washed under tap water and surface sterilized after washing in 0.1% Teepol detergent for 2 minutes and followed by 0.1% HgCl₂ treatment for 2 minutes. The explants were then washed with sterile distilled water. The explants were then cultured *in vitro* on MS medium singly or in combination with various concentrations of BAP and NAA for establishment in test tubes (autoclaved at 115 degree C for 25 minutes in an autoclave.) cultures were maintained in a laboratory at 25 degree celsius. The cultures were sub cultured at an interval of 15 days on fresh MS with similar combination. Multiplication studies were carried out i.e. studies on number of multiple shoots in multiplication stage were carried out taking into consideration type of explant and effect of BAP and NAA. Morphological characters were also recorded during this period.

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RESULTS AND DISCUSSION

Number of *in vitro* shoot per established culture

MS media supplemented with growth hormones was used the observations were recorded in established cultures after 45 days and resulted are presented in Table 1.

From shoot tip explant in AKSG-5 the maximum number of *in vitro* shoots per culture i.e. 2 shoots per established culture were observed on EM₉ media (MS+BAP 2.0 mg/l + NAA 1.0 mg/l) followed by 1.68 shoots per established culture on EM₅ (MS+BAP 2.0 mg/l) media. Lowest number of *in vitro* shoots per established culture i.e. 1 shoot per established culture was observed on EM₁ (MS).

From shoot tip explant in AKSG-35 the maximum number of *in vitro* shoot per culture i.e. 2.02 shoots per established culture was observed on EM₉ media (MS+BAP 2.0 mg/l + NAA 1.0 mg/l) followed by 1.69 shoots per established culture on EM₁₃ (MS+BAP 2 mg/l+ NAA 2.0 mg/l). Lowest number of *in vitro* shoots per established culture i.e. 1 shoot per culture was observed on EM₁ (MS).

From shoot tip explant in AKSGM-1 the maximum number of *in vitro* shoot per culture i.e. 2 shoots per established culture was observed on EM₁₃ media (MS+BAP 2.0 mg/l+ NAA 2.0 mg/l). followed by 1.68 shoots per established culture on EM₈ (MS+BAP 1.5 mg/l+ NAA 1.0 mg/l) media. Lowest number of *in vitro* shoots per established culture was 1 shoot per culture was observed on EM₄ (MS+BAP 1.5 mg/l).

From axillary bud explant in AKSG-5 the maximum number of *in vitro* shoot per culture i.e. 2.35 shoots per established culture was observed on EM₉ media (MS+BAP 2.0 mg/l + NAA 1.0 mg/l) followed by 2.33 shoots per established culture on EM₁₁ (MS+BAP 1.0 mg/l + NAA 2.0 mg/l). Lowest number i.e. 1.31 *in vitro* shoots per established culture was observed on EM₅ (MS+BAP 2 mg/l).

From axillary bud explant in AKSGM-35 the maximum number of *in vitro* shoot per culture i.e. 2.68 *in vitro* shoots per established culture was observed on EM₈ media (MS+BAP 1.5 mg/l + NAA 1.0 mg/l) followed by 2.34 *in vitro* shoots per established culture on EM₁₃ (MS+BAP 2.0 mg/l+ NAA 2.0 mg/l) and EM₉ (MS + BAP 2.0 mg/l + NAA 1.0 mg/l). Lowest number of *in vitro* shoots i.e. 1 shoot per established culture was observed on EM₁ (MS).

From axillary bud explant in AKSGM-1 the maximum number of *in vitro* shoot per culture i.e. 2.70 *in vitro* shoots per established culture was observed on EM₈ media (MS+BAP 1.5 mg/l + NAA 1.0 mg/l) followed by 2.67 *in vitro* shoots per established culture on EM₁₃ (MS+BAP 2.0 mg/l+ NAA 2.0 mg/l). Lowest number of *in vitro* shoots i.e. 1.64 shoot per established culture was observed on EM₂ (MS+BAP 0.5 mg/l). None of the media combination gave significant multiple shoots per culture on establishment medium.

Table 1 : Number of *in vitro* shoot per established culture

Medium	AKSG-5		AKSG-35		AKSGM-1		Mean	
	ST	AB	ST	AB	ST	AB	ST	AB
EM1	1.00	1.33	1.00	1.00	1.34	1.67	1.11	1.33
EM2	1.02	1.68	1.04	1.64	1.01	1.64	1.02	1.65
EM3	1.67	2.01	1.67	2.02	1.67	2.00	1.67	2.01
EM4	1.31	1.67	1.33	1.68	1.00	1.69	1.21	1.68
EM5	1.68	1.31	1.35	2.00	1.04	1.68	1.36	1.66
EM6	1.02	1.67	1.04	2.03	1.03	2.02	1.03	1.91
EM7	1.33	2.00	1.38	2.33	1.34	2.34	1.35	2.23
EM8	1.65	2.33	1.68	2.68	1.68	2.70	1.67	2.57
EM9	2.00	2.35	2.02	2.34	1.33	2.35	1.78	2.34
EM10	1.35	2.00	1.34	2.31	1.00	2.37	1.21	2.23
EM11	1.67	2.33	1.68	2.32	1.67	2.30	1.67	2.32
EM12	1.33	1.67	1.34	2.33	1.38	2.31	1.35	2.10
EM13	1.31	1.65	1.69	2.34	2.00	2.67	1.67	2.22
Mean	1.41	1.85	1.43	2.08	1.35	2.13	1.39	2.01

	Variety		Medium		Variety x Medium	
	ST	AB	ST	AB	ST	AB
SE±	0.69	0.72	1.45	1.49	2.51	2.58
CD at 5%	1.96	2.01	4.07	4.19	7.05	7.26

Effect of growth hormones on multiple shoot formation in Spine gourd

A single shoot from well established culture were transferred to shoot proliferation media to study their response for induction and development of multiple shoots. After 30 days, multiple shoots were observed and data was recorded on number of multiple shoots produced per culture has been presented in Table 2.

Significant differences were observed for number of

multiple shoots per culture. The highest multiple shoots (5.42) were produced on SM₁₄ (MS + ADS 70 mg/L + BAP 10 mg/L + NAA 1.0 mg/L) and SM₁₇ (MS + ADS 80 mg/L + BAP 10 mg/L + NAA 1.0 mg/L) medium.

In AKSG-5 the maximum number multiple shoots i.e. 5.67 shoots per culture on SM₁₄ media (MS + ADS 70 mg/l + BAP 10 mg/l + NAA 1.0 mg/l) were obtained followed by 5.33 shoot per culture on SM₁₇ media (MS+AdSO₄ 80 mg/l + NAA 1.0 mg/l).

Table 2 : *In vitro* multiple shoots per culture

Medium	Genotype			Mean
	AKSG-5	AKSG-35	AKSGM-1	
SM1	1.43	1.50	1.00	1.31
SM2	1.77	1.50	1.27	1.51
SM3	1.67	1.87	1.43	1.65
SM4	1.67	1.60	1.40	1.56
SM5	1.70	1.43	1.10	1.41
SM6	1.40	1.13	1.07	1.20
SM7	1.47	1.33	1.33	1.38
SM8	1.67	1.67	1.67	1.67
SM9	1.93	2.13	2.00	2.02
SM10	4.27	4.33	3.27	3.96
SM11	4.87	4.93	4.53	4.78
SM12	2.67	3.13	2.47	2.76
SM13	4.20	4.33	3.33	3.96
SM14	5.67	5.73	4.87	5.42
SM15	2.20	2.27	1.73	2.07
SM16	3.93	4.00	3.20	3.71
SM17	5.33	5.67	5.27	5.42
Mean	2.81	2.86	2.41	2.69

	Variety		Medium		Variety x Medium	
	ST	AB	ST	AB	ST	AB
SE+	0.06	0.06	0.16	0.16	0.28	0.28
CD at 5%	0.19	0.19	0.46	0.46	0.80	0.80

lowest number of shoots 1.40 per culture SM₆ media (MS+ADS 50 mg/l + BAP 5 mg/l + NAA 1.0 mg/l) were obtained.

In AKSG 35 the maximum number of multiple shoots i.e. 5.73 shoots per culture on SM₁₄ media MS+ ADS (70 mg/l) + BAP (10 mg/l) + NAA (1.0 mg/l) were obtained followed by 5.67 shoots per culture on SM₁₇ media (MS+AdSO₄ 80 mg/l + BAP 10 mg/L + NAA 1.0 mg/L). Lowest number of shoots 1.13 per culture were obtained on SM₆ media (MS+ADS 50 mg/l + BAP 5.0 mg/l + NAA 1.0 mg/l) were obtained.

In AKSGM-1 the maximum number of multiple shoots i.e. 5.27 shoots per culture on SM₁₇ media MS+ ADS (80mg/l) + BAP (10 mg/l) + NAA (1mg/l) were obtained followed by 4.87 shoots per culture on SM₁₄ media (MS+ADS 70 mg/l + BAP 10 mg/l + NAA 1mg/l) were obtained. Lowest number of shoots 1.0 per culture were obtained on SM₁ (MS).

REFERENCES

- Haberlandt, G.** (1902). Kulturyersuche mit isolierten pflanzhellen S.B.Sber. Akad. Wiss. Wien. **111**: 69-92.
- Morel, G. and C. Martin** (1952). Guerson de dahlias atteiner's dune maladie a virus. C.R. Acad. Sci. Paris. **235**: 1324-1325.

Murashige, T. (1974). Plant propagation through tissue culture. *A Rev. Pl. Physiol.* **25**: 135-136.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Pl.* **15**: 473-484.

Singh, A.K. (1990). Cytogenetics evolution in cucurbitaceae. In: Bates, D.M.; R.W. Rofinson and E. Joffery (Eds.). Comstock publishing Associates, Cornell University Press, Ithaca, London. PP.10-28.

Wehner, T.C. and R.P. Locy (1981). In vitro adventitious shoot and root formation of cultivars and lines of Cucumis sativus L. *Hort. Sci.* **16(6)**: 759-760.

White, P.R. (1939). Potentially unlimited growth of excised plant callus in an artificial medium. *Am. J. Bot.*, **26**: 263-269.

