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In-vitro sterilization and shoot induction in spine gourd

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Abstract

The present investigation entitled “*In-vitro* sterilization and shoot induction in spine gourd” was conducted at Department of Vegetable Science, Dr. PDKV, Akola and Centre of Excellence, Plant Biotechnology, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, (M.S.) during the academic year 2022-23 and 2023-2024 with the aim to standardized the protocol for *in-vitro* propagation of spine gourd with (CRD) Completely Randomized Design having 5 treatments and 4 replications of surface sterilization of spine gourd explant, *in-vitro* shoot induction with 10 treatments and 3 replications.

In this study treatment of standardization of surface sterilization of explant, the maximum survival percentage and minimum contamination percentage was found in treatment T₃ i.e. Carbendazim 0.1% for 5 min + HgCl₂ 0.1% for 5 min + NaOCl 1.0% for 5 min.

In this experiment, standardization of *in-vitro* shoot induction protocol for spine gourd micropropagation, the maximum number of shoots initiated per explant, maximum number of leaves per shoot, maximum length of shoot, maximum number of nodes per explant and maximum shoot induction percentage whereas, minimum days to shoot initiation were recorded in treatment T₆ i.e. MS media + 2.0 mg/l BAP + 2.0 mg/l LGA.

In the present study above treatments were found suitable for the successful propagation of spine gourd with the help of tissue culture technique.

Keywords: Spine gourd, Plant tissue culture, Propagation, Media, *in-vitro*, etc.

Introduction

Spine gourd (*Momordica dioica* Roxb.) is an underutilized cucurbitaceous fruit vegetable with diploid chromosome number of 2n=28. It is dioecious perennial vine and native of tropical Asia and it belongs to family *Cucurbitaceae* mainly grows during rainy season as a wild crop (Bharathi *et al.* 2007)^[1].

Spine gourd is commercially grown in tropical and sub-tropical regions of India. It can be grown successfully in areas where annual rainfall of 1000-1500 mm with temperature range of about 25-40°C. It can be grown on wide range of soils ranging from sandy loam to clay soils with pH range of about 5.5 to 7. Spine gourd is largely propagated through tuberous roots followed by stem cutting and seeds. Planting is usually done during the summer months at the onset of monsoon. It thrives well under warm humid climate. In most of the areas flowering usually starts in June to July and fruiting are available from August to September. During severe winter underground tubers undergo dormancy (Ram *et al.* 2002)^[2]. Rapid multiplication would greatly depend on the micropropagation technique. Thus, the goal of the current study was to validate the quick and effective methodology for spine gourd surface sterilization, shoot induction and proliferation, root induction and hardening of *in-vitro* grown plantlets utilizing various growth regulator doses.

Materials and Methods

Present study was conducted at Instructional farm, Department of Vegetable Science, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola along with the help of Plant Tissue Culture Laboratory, Biotechnology Centre, Department of Agril. Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, (M.S.) during the academic year 2022-23 and 2023-24.

Materials

- **Source of explant used and variety:** Healthy and disease-free nodal explant of spine gourd local cultivar used in this study were cultivated at Instructional farm, Department of Vegetable Science, Dr. PDKV, Akola.
- **Chemicals:** The salts for supplying major, minor elements and other ingredients required for the preparation of media were obtained from Himedia Laboratories Limited, Mumbai, India. The amino acids, vitamins and plant growth regulators used were of plant tissue culture grade from Himedia.
- **Glassware and Laboratory Equipment:** Thermostable, autoclavable and high grade borosil make glassware, various equipment and instruments were used as available at plant tissue culture laboratory of Dr. PDKV, Akola to carry out present work.
- **Culture Medium:** In the present study, Murashige and Skoog (MS) (Murashige and Skoog, 1962) ^[3] media was used in combination with different plant hormones for *in-vitro* micro-propagation.

Methodology

Healthy and disease-free nodal explants were selected from spine gourd vine and washed in running tap water to remove the adhering dirt and soil. Surface sterilized using liquid detergent labolene 5% and disinfected by Carbendazim, HgCl₂ and NaOCl solutions at specific concentrations followed by several washings by double distilled water to remove the traces of sterilant under the hood of laminar air flow cabinet. The sterilised nodal part was then inoculated on MS media fortified with different concentrations of growth regulators as per the treatment combinations. For every treatment, 20 nodal explants were used per replication i.e. 60 explants used per treatment. The airtight sealed culture bottles were then maintained in a culture room at 25±2 °C under 1500 to 2000 lux was provided by white fluorescent light with 14 hours of light and 10 hours of dark period.

The shoots formed *in-vitro* were isolated and subculture on a same medium for multiplication and rooting. After the 5th

subculture cycle, individual shoots of length 4- 5 cm were excised and transferred to rooting media containing different combinations of auxin (IBA) on half strength MS medium. The rooted plantlets of spine gourd then removed from the glass bottles and then transferred to the black polythene bags (growing bags) with different medias for hardening under greenhouse conditions. The experiment was laid out in CRD experimental design having 5 treatments 4 replications of surface sterilization of spine gourd explant, *in-vitro* shoot induction with 10 treatments and 3 replications.

Result and Discussion

Surface sterilization of explants

The data regarding the establishment of spine gourd explant was found significantly influenced by different surface sterilization treatments having different concentrations of carbendazim, HgCl₂ and NaOCl with different time periods forming 5 treatments. The data presented in Table 1 revealed that maximum survival percentage (86.25%) was found in treatment T₃ (Carbendazim 0.1% for 5min+HgCl₂ 0.1 % for 5 min + NaOCl 1.0% for 5 min) which was found at par (83.52%) with treatment T₂ (Carbendazim 0.1% for 5min+HgCl₂ 0.1 % for 3 min + NaOCl 1.0% for 3 min). whereas, the minimum survival percentage was observed in the treatment T₁ (1.02%) i.e. Control (DDW). Also, the data revealed that minimum contamination percentage (13.75%) was found in treatment T₃ (Carbendazim 0.1% for 5 min + HgCl₂ 0.1% for 5 min + NaOCl 1.0% for 5 min) which was found at par (16.48%) with treatment T₂ (Carbendazim 0.1% for 5min+HgCl₂ 0.1 % for 3 min + NaOCl 1.0% for 3 min). whereas, the maximum contamination percentage was observed in the treatment T₁ (98.98%) i.e. Control (DDW). The increase in the survival percentage of the healthy nodal explants of spine gourd is due to the low concentration of carbendazim with optimum time, standard concentration of HgCl₂ and NaOCl with standard time. Similar results were found by Nabi *et al.* (2002) ^[4], Ghive *et al.* (2006) ^[5], Devendra *et al.* (2009) ^[6].

Table 1: Surface sterilization of explant

Tr. No.	Treatment details	Survival percentage (%)	Contamination Percentage (%)
T ₁	Control (DDW)	1.02	98.98
T ₂	Carbendazim 0.1% for 5 min+ HgCl ₂ 0.1% for 3 min+ NaOCl 1.0% for 3 min	83.52	16.48
T ₃	Carbendazim 0.1% for 5 min+ HgCl ₂ 0.1% for 5 min+ NaOCl 1.0% for 5 min	86.25	13.75
T ₄	Carbendazim 0.1% for 7 min+ HgCl ₂ 0.2% for 3 min+ NaOCl 1.0% for 3 min	59.35	40.65
T ₅	Carbendazim 0.1% for 7 min+ HgCl ₂ 0.2% for 5 min+ NaOCl 1.0% for 5 min	41.12	58.88
	'F' Test	Sig	Sig
	SE (m)±	0.95	0.95
	CD at 1%	3.97	3.97





Fig 1: (a) Inoculation of spine gourd (b) Initiation of shoot

***In-vitro* shoot induction**

In present study, the surface sterilized nodal explant of spine gourd was inoculated in different media combinations containing growth regulators such as BAP & LGA of varied concentrations forming ten different combinations.

The data regarding the number of shoots initiated per explant was found significantly influenced by different concentrations of growth regulators such as BAP and LGA in MS media. The data presented in Table 2. Shows that significantly maximum number of shoots initiated per explant (14.16), number of leaves per shoot (19.80), length of shoot (9.93 cm), number of nodes per explant (5.56), shoot induction percentage (95.76%) were found in treatment T₆ (MS media + 2mg/l BAP + 2mg/l LGA) which was found at par with treatment combination T₅ (MS media + 2mg/l BAP + 1.5mg/l LGA), similarly in the same treatment combination significantly minimum number of days required for shoot initiation (5.53) i.e. in T₆ (MS media + 2mg/l BAP + 2mg/l LGA) which was found at par with treatment combination T₅ (MS media + 2mg/l BAP + 1.5mg/l LGA).

For improving the culture response, synthetic auxins were often combined with cytokinin. BAP is a recognized cytokinin that promotes cell division, shoot induction and plant regeneration and LGA acts as an organic nitrogen source and a growth enhancer in plant tissue culture. The number of shoots initiated per explant was found more in treatment where BAP is supplemented 2.0 mg/l rather than other doses. The rise in the number of multiple shoots did not correspond with the rise in BAP concentration. The number of multiple shoots produced decreases proportionately as BAP rises above 2.00 mg/l. Optimal BAP concentration enhances shoot bud differentiation and reduces apical dominance leading to multiple shoots. BAP induces multiple shoot buds while LGA supports elongation and healthy development of shoots. Similar results were also reported by Agrawal and Kamal (2004), Sultana *et al.* (2005) [7], Devendra *et al.* (2009) [6], Shekhawat *et al.* (2011) [8], Thiruvengadam and Chung (2011) [9], Arekar *et al.* (2012) [10], Mustafa *et al.* (2012) [11], Mustafa *et al.* (2013) [12], Rai *et al.* (2012) [13], Debnath *et al.* (2013) [14], Patel *et al.* (2015) and Raju *et al.* (2015) [15] in spine gourd.

Table 2: *In-vitro* shoot induction

Tr. no.	Treatment details	No. of shoots initiated per explant	Days for shoot initiation	No. of leaves per shoot	Length of shoot	No. of nodes per explant	Shoot induction percentage (%)
T ₁	Control (MS media)	3.60	9.50	6.50	3.46	1.80	68.73
T ₂	MS media+1.5 mg/l BAP+1.5mg/l LGA	7.80	7.80	8.66	4.10	3.46	74.63
T ₃	MS media+1.5 mg/l BAP+2.0mg/l LGA	8.30	7.50	10.53	5.23	3.60	75.70
T ₄	MS media+1.5 mg/l BAP+2.5mg/l LGA	7.80	7.73	9.80	5.96	3.83	73.36
T ₅	MS media+2.0 mg/l BAP+1.5mg/l LGA	13.86	5.80	19.60	9.50	5.33	93.43
T ₆	MS media+2.0 mg/l BAP+2.0mg/l LGA	14.16	5.53	19.80	9.93	5.56	95.76
T ₇	MS media+2.0 mg/l BAP+2.5mg/l LGA	7.66	6.70	10.63	4.63	4.13	73.80
T ₈	MS media+2.5 mg/l BAP+1.5mg/l LGA	8.06	7.33	10.63	4.86	3.70	78.40
T ₉	MS media+2.5 mg/l BAP+2.0mg/l LGA	7.70	6.80	10.93	5.73	3.80	77.43
T ₁₀	MS media+2.5 mg/l BAP+2.5mg/l LGA	4.20	9.30	6.93	3.96	2.33	73.06
	'F Test'	Sig	Sig	Sig	Sig	Sig	Sig
	SE (m)±	0.08	0.10	0.12	0.14	0.11	0.61
	CD (1%)	0.34	0.41	0.48	0.56	0.44	2.45



Fig 2: Shooting of spine gourd plantlets

Conclusion

Based on the results mentioned above, it is concluded that in case of surface sterilization of spine gourd the treatment of Carbendazim 0.1% for 5 min + HgCl₂ 0.1% for 5 min + NaOCl 1.0% for 5 min had been found best. The full MS media with 2.0 mg/l BAP + 2.0 mg/l LGA was found to be most effective for *in-vitro* shooting of spine gourd.

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