Plant Archives Vol. 26, Supplement 1, 2026 pp. 2163-2167 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.290

IN-VITRO SHOOT AND ROOT INDUCTION IN SPINE GOURD

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(Date of Receiving : 19-10-2025; Date of Acceptance : 27-12-2025)

ABSTRACT

The present investigation entitled "*In-vitro* shoot and root induction in spine gourd" was conducted at Centre of Excellence, Plant Biotechnology, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, (M.S.) during the academic year 2022-23 with the aim to standardize the protocol for *in-vitro* shoot and root induction in spine gourd with (CRD) Completely Randomized Design having 10 treatments and 3 replications of *in-vitro* shoot induction and 5 treatments and 4 replications of *in-vitro* root induction. In this study the surface sterilized nodal part of spine gourd were cultured on MS medium supplemented with different combinations of BAP and LGA, the highest number of shoots per explant, number of leaves per shoot, length of shoot, number of nodes per explant and shoot induction percentage was recorded in treatment MS media + 2mg/l BAP + 2mg/l LGA, whereas, minimum number of days required for shoot initiation were also recorded in the same treatment combination. In *in-vitro* root induction half strength of MS medium was used with different combinations of IBA, the highest number of roots per plantlet, number of primary roots per plantlet, root length, shoot length, number of leaves per shoot, number of tendrils per plant and root induction percentage and lowest number of days for root initiation, days to root proliferation after root initiation and number of days for initiation of tendrils were observed in treatment combination ½ MS media + 1mg/l IBA. In the present study above combinations of growth regulators were found suitable for the successful propagation of spine gourd with the help of tissue culture technique.
Keywords **:** Spine gourd, plant tissue culture, propagation, induction, 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). It is an indigenous and distributed throughout India, China, Nepal, Bangladesh, Myanmar, Pakistan and Sri Lanka. It is widely cultivated in Indian states such as Odisha, Maharashtra, Bihar, Jharkhand, West Bengal and Chhattisgarh. In some of the Indian language it is named English: Teasle gourd, Spine gourd, Small bitter gourd; Marathi: Katval, Kartule; Bengali: Kartoli, Kakrol; Assam: Batkarila; Hindi: Kantola, Parora, Kakora; Tamil: Paluppakkay; Telugu: Agakara, Karkotaki; Malayalam: Venpaval; Kannada: Madahagala-Kaya; Sanskrit: Vahisi; Gujarati: Katwal (Das *et al.,* 2022).

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 25-40ºC. 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).

Spine gourd is quite palatable, sweet and entirely free from bitterness, good source of antioxidants, and other nutrients viz. β-carotene, vitamin C, folic acid, magnesium, phosphorous and potassium. Fresh fruit has about 84.1-87% moisture content. The fruit is a rich source of protein, carbohydrates and vitamin C. Nutritional composition showed that per 100 g edible fruit on dry weight basis is crude protein (19.38 g), crude lipid (4.70 g), carbohydrates (47.92 g), crude fibre (21.30 g), ash (6.70 g), calcium (33.00 mg), phosphorous (42.00 mg), iron (4.60 mg), riboflavin (0.18 mg), Thiamine (0.05 mg), calorific value (311.50 kcal) (Tiwari *et al.,* 2022).

Commercial multiplication using the tuberous roots and stem cuttings are critically limited due to inadequate availability of tuberous roots and late availability of stem cuttings in fruiting season. As the conventional methods of spine gourd propagation impose several limitations for large-scale propagation of sex specific plants an efficient clonal propagation method is must. An *in-vitro* propagation system offers unlimited availability of planting material early in the planting season (Hoque *et al.,* 2007). To overcome all these drawbacks plant tissue culture is extremely desirable for commercial production of spine gourd with predetermined sex (Patel and Ishnava, 2015). As an alternative to traditional methods tissue culture offers an efficient method for propagation of spine gourd as it required only small amount of propagating material and has the potential to provide the large number of cloned plants thus tissue culture offers a viable tool for the *in-vitro* propagation of the spine gourd species (Arekar *et al.,* 2012). Thus, the goal of the current study was to validate the quick and effective methodology for spine gourd shoot proliferation utilizing various growth regulator doses.

Material 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, and Department of Agril. Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, (M.S.) during the academic year 2022-23.

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) 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, $HgCl_2$ 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 5^{th} 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 experiment was laid out in CRD experimental design.

Result and Discussion

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 and 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 1. 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 T_6 (MS media + 2mg/l BAP + 2mg/l LGA) which was found at par with treatment combination T_5 (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 T_6 (MS media + 2mg/l BAP + 2mg/l LGA) which was found at par with treatment combination T_5 (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 founds more in treatment where BAP is supplemented 2.0 mg/l rather than other doses.

Plate 1: *In-vitro* shoot induction

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), Devendra *et al.* (2009), Shekhawat *et al.* (2011), Thiruvengadam and Chung (2011), Arekar *et al.* (2012), Mustafa *et al.* (2012), Mustafa *et al.* (2013), Rai *et al.* (2012), Debnath *et al.* (2013), Patel *et al.* (2015) and Raju *et al.* (2015) in spine gourd.

Table 1: *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**

In-vitro root induction

In present study, the *in-vitro* grown plantlets of spine gourd were transferred to the rooting media of different combinations containing growth regulator IBA in five different concentrations. Effective rooting treatments result in a highly rooted shoots and a well-developed root system. The data regarding the number of roots per plantlet was found significantly influenced by different concentrations of growth regulator IBA. The data presented in table 2. shows that significantly maximum number of roots per plant (7.70), number of primary roots per plantlet (12.57) were found in treatment combination T_3 (½ MS media + 1 mg/l IBA). Similarly, significantly minimum number of number of days for root initiation (14.35), minimum days to root proliferation after root initiation (19.40) were also found in same treatment combination.

To improve root development *in-vitro,* auxin supplementation is necessary due to cytokinin inhibition. Auxin like IBA that is a rooting hormone when applied in proper quantity gives better results. It is a myth that high concentrations of IBA reduces the days for root initiation rater it causes some reverse effects. Excessive IBA can have an inhibitory effect, sometimes causing callus at the base instead of proper roots, which delays root initiation. Optimal concentration of IBA reduces the number of days required for root initiation by accelerating root primordial formation. Adding activated charcoal to rooting media is beneficial and it darkens and absorb inhibitory chemicals, which promotes *in-vitro* rooting. Such kind of similar findings were also reported by Hoque *et al.* (1998), Arekar *et al.* (2012) and Rekha *et al.* (2013) in spine gourd.

Plate 2: *In-vitro* root induction

Table 2: *In-vitro* root induction

Treatment number	Treatment details	No. of days for root initiation	No. of roots per plantlet	Days to root proliferation after root initiation	No. of primary roots per plantlet
T_1	Control (½ MS media)	22.77	1.45	32.75	1.40
T_2	½ MS+0.5 mg/l IBA	15.02	6.45	21.57	4.20
T_3	½ MS+1 mg/l IBA	14.35	7.70	19.40	4.75
T_4	½ MS+ 2 mg/l IBA	17.72	4.52	25.55	3.27
T_5	½ MS+ 3 mg/l IBA	18.72	3.60	27.45	2.50
	'F Test'	**Sig**	**Sig**	**Sig**	**Sig**
	SE(m)±	**0.18**	**0.09**	**0.11**	**0.15**
	CD (1%)	**0.74**	**0.37**	**0.48**	**0.66**

Conclusion

Based on the results mentioned above, it is concluded that in *in-vitro* shooting of the spine gourd, the parameters *viz*., number of shoots initiated per explant, number of leaves per shoot, length of shoot, number of nodes per explant and shoot induction percentage were found significantly maximum in treatment (MS media + 2.0 mg/l BAP + 2.0 mg/l LGA) while in the same treatment combination number of days required for shoot initiation were found significantly minimum.

In *in-vitro* rooting of the spine gourd, the parameters *viz.,* number of roots per plantlet and number of primary roots per plantlet were found significantly maximum at treatment (½ MS media + 1mg/l IBA) whereas in the same treatment number of days required for root initiation and days required to root proliferation after root initiation were found significantly minimum.

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