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Regeneration of plantlets from leaf explants of spine gourd (*Momordica dioica* Roxb.)

RK Yadav**Abstract**

The present investigation was carried out to develop a protocol for rapid callus induction and plant regeneration of spine gourd (*Momordica dioica* Roxb.). Leaf explants of three spine gourd genotypes viz. RMD-2, RMD-3 and Indira Kankoda-1 (released variety) were cultured. The explants were cultured on to MS media supplemented with different combinations of BAP, Kinetin and NAA. Combinations of MS + BAP (1 mg/lit) was best for callus induction and MS + BAP (0.5 mg/lit) + Kinetin (0.5 mg/lit) was best for multiple shooting induction for all genotypes i.e. RMD-2, RMD-3 and Indira Kankoda-1. On the other hand hormone free MS media with charcoal (2gm/lit) was found best for rooting in only one genotype named Indira Kankoda-1. The regenerated shoots were rooted in MS₀ medium and successfully transplanted to field during Kharif 2018.

Keywords: callus, genotypes, media, growth regulators, explants, spine gourd

Introduction

Spine gourd (*Momordica dioica* Roxb.) also known as teasel gourd, Kankoda, kheksi, meetha karela, kantola and kakrola is an underutilized cucurbitaceous fruit vegetable. It is widely cultivated in Odisha, Maharashtra, Bihar, Jharkhand, West Bengal and Chhattisgarh. It possesses several medicinal and curative properties. As a crop, spine gourd has number of problems including dormancy of seeds, large number of hard seeds, low germination rate and unpredictable sex ratio in seedling progeny. Now a days there is no protocol developed in this crop. So it is important to standardize the protocol for plant regeneration through callus of spine gourd genotypes grown in Chhattisgarh. Because of plants regenerated through callus culture are genetical identical to the parent, huge number of plantlets in a short period of time, predictable sex progeny. But the response of explants for callus induction and regeneration are not same in different media concentrations. Therefore, the present study was taken to establish the effective protocol for rapid regeneration of spine gourd.

Materials and Methods

Three genotypes including one standard check variety (Indira Kankoda-1) were carried out during the period from July 2017 to Feb.2018 at Dept. of Plant Molecular & Plant Biotechnology Laboratory, Indira Gandhi agricultural University, and Raipur (C.G.). Auxiliary buds were used for callus induction and plant regeneration. MS^[2] semi solid media with different concentration and combination of BAP, Kinetin and NAA were used for this purpose. In each treatment 10 explants were inoculated. Explants were cultured in culture tubes containing MS medium (30g L⁻¹ sucrose, 7 g L⁻¹ agar). The pH of medium was adjusted to 5.8 by using 1 N NaOH. The media were autoclaved at 121⁰ for 20 min after adjusting the pH. The explants were inoculated on callus induction medium at 25 ± 2 °C for 306 weeks. The calli were transferred to the fresh callus inducing medium about 21 days interval for further proliferation and maintenance. After 40-60days of incubation in the dark, the callus induction frequency was determined and well developed calli were selected and sub-cultured on regeneration media. After 4-6 weeks differentiated shoot formation was observed the shoots were excised and transferred to MS₀ (without growth regulators) medium with charcoal (2g/lit) for root induction. Days required for shoot initiation, days required for root initiation and number of multiple shooted plants were recorded to investigate the effect of different treatments and response of different genotypes of spine gourd.

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Results and Discussion

The results revealed that a high variation among three genotypes for induction ability was demonstrated by explants and treatments under present investigation. Among varieties explants auxiliary bud performed best for callus induction. Auxiliary buds had highest frequency of callus and required minimum number of days (12 days) Table-1. MS and BAP (1mg/lit) combinations had significant effect on callus induction in a variety of Indira Kankoda-1. However, for the leaf explants the rate of callus formation was very poor in two other promising genotypes i.e. V 1 (RMD 15-2) and V2 (RMD 15-3). Different concentrations of BAP with kinetin and NAA were used to investigate the shoot induction capability of different explants (Fig. 1 & 2). Among the explants used, shoot tip explants showed best performance in the concentration of MS + BAP (0.5 mg/lit) + Kinetin (0.5 mg/ lit) in all the three genotypes. Similar findings were also reported by Akter et al., 2007 [1]. The least number of days

(12) and more number in genotype of the V2 (RMD 15-2) and V1 (RMD 15-1) for shoot initiation. More number of days (45days) required for root initiation was seen in only one genotype i.e. Indira Kankoda-1. Other two genotypes did not showed root initiation. Other combinations of hormones i.e. (MS + BAP+ NAA) did not showed root and shoot initiations. Best media i.e. MS0 + charcoal (2gm./lit) was used for rooting purpose. The number of multiple shooted plants (4) was exhibited in the genotype of V 3 (Indira Kankoda-1).

Results obtained from the present investigation, it may be concluded that combinations of MS + BAP (0.5 mg/lit) + Kinetin (0.5 mg/lit) was best for callus induction and MS + BAP (0.5 mg/lit) + Kinetin (0.5mg/lit) was best for multiple shooting induction for all the three genotypes. On the other hand hormone free MS media with charcoal (2gm/lit) was found best for rooting for only one genotype i.e. Indira Kankoda-1 than the others in Spine gourd (Fig.).

Table 1: Main effect of hormone concentrations on shoots and root induction of spine gourd

Treatments	Hormone concentration (mg/lit.)	Genotypes	Days required for shoot initiation	Days required for root initiation	No. of multiple shooted plant
MS+ BAP (T 1- T 4)	0.5	V1	25	-	-
	1.0	V2	13	-	2
	0.5	V3	12.5	-	-
	2.0				
½ MS + BAP (T 5 –T 8)	0.5	V1	-	-	-
	1.0	V2	-	-	3
	1.5	V3	-	4	-
	2.0				
MS + BAP+ Kinetin T 9-T 10	0.5	V1	24	-	1
	+	V2	12	-	4
	0.5	V3	13	45	1
MS + BAP+ NAA T 11-T12	0.5	V1	-	-	-
	+	V2	-	-	-
	0.1	V3	-	-	-
MS + BAP+NAA T 13-T 14	1.0	V1	-	-	-
	+	V2	-	-	-
	0.1	V3	-	-	-
MS + BAP+ NAA	1.5	V1	-	-	-
	+	V2	-	-	-
	0.1	V3	-	-	-



Callus and regeneration in Spine guard



Hardening of Spine guard (Indira Kankoda-1)

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