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In Vitro PLANT REGENERATION FROM MESTA (*Hibiscus sabdariffa* L.)

T. K. Roy[1], A. Khatun[2], M. S. Hossain[3] and R. Mondal[4]

ABSTRACT

An experiment was conducted in the Genetic Engineering Laboratory of the Cytogenetics Department, Bangladesh Jute Research Institute (BJRI), Dhaka. Cotyledons (with attached petioles) of mesta (*Hibiscus sabdariffa* L.) were used as explant to investigate its *in vitro* regeneration potentiality. Seed germination percentage was found to be better on clinical cotton supported MS liquid medium (90%) compared to agar-supported MS solidified medium (81.33%). Among the combinations, MS medium supplemented by BAP (2.0 mg/l) and IAA (0.5 mg/l) showed the highest (58.33%) shoot regeneration. Shoot regeneration was not observed in MS media without hormone (MSO) and also when more than 4mg/L BAP with 0.5 mg/l IAA combination was used. MS medium without hormone (MSO) showed good response for root formation from regenerated shoots of mesta and root development occurred very nicely. The regenerated plantlets of mesta subsequently survived in soil.

Key words: Regeneration, MS medium, phytohormone, cotyledons, protocol, tissue culture

INTRODUCTION

Mesta (*Hibiscus sabdariffa* L.) is the best fibre crop next to jute and kenaf in importance. It is a world wide adapted potential fibre and biomass producing crop. Mesta is a crop of tropical African origin and was domesticated in ancient times for its edible leaves, young shoots and flower parts as well (Maiti, 1997). In the recent years, there has been an increasing trend in both area and production of mesta in Bangladesh. The average yield of mesta in Bangladesh is 1.9 t/ha, which is much lower than that of many other mesta growing countries (Ahmed and Vossen, 2003). Mesta is usually propagated by seed, but can also be grown from stem cutting. Mesta has a narrow genetic base and low adaptability to our agro-ecological conditions and susceptibility to several diseases and insects. One of the major constraints to increase mesta productivity is the non-availability of modern varieties and susceptibility to diseases, nematodes and many other environmental factors. For improving the agronomic characters of mesta, usually conventional breeding methods were practiced. In order to produce desirable lines of mesta with high fibre yield and higher biomass production, an alternate technique is necessary. Biotechnology is a recently developed novel approach, which includes a range of techniques including plant tissue culture. Plant regeneration of a crop through tissue culture of a crop is also a prerequisite for the improvement of any crop. In the present study, attempts were made to establish a suitable regeneration protocol for mesta. This plant regeneration protocol from the explants of mesta may be used for genetic transformation work by which transgenic mesta can be produced for disease or any pest resistance. Healthy seedling is one of the prerequisite for plant regeneration from *Hibiscus sabdariffa*. Seeds of *H. sabdariffa* were collected from gene bank of BJRI, Dhaka. Seeds were surface sterilized by immersing in absolute alcohol for 1 minute. and then in 0.1% Mercuric Chloride for 20 minutes. Seeds were thoroughly washed with autoclaved water for 4-5 times.

[1]MS. student, [3] Professor, Dept. of Genetics and Plant Breeding, [4]M.S. student, Dept. of Soil Science, Sher-e-Bangla Agricultural University, [2]Chief Scientific Officer, Genetic Resource and Seed Division, Bangladesh Jute Research Institute, Dhaka, Bangladesh.

The sterilized seeds were transferred on clinical cotton supported MS (Murashige and Skoog, 1962) liquid medium and agar (Sigma, UK, 0.8%, w/v) supported MS solidified medium in 500 ml conical flasks. Each flask contained 15 seeds and was placed in a growth room with 27±2°C temperature under 1500 lux fluorescent illumination with 12 h photoperiod.

MATERIALS AND METHODS

In vitro seed germination

Healthy seedling is one of the prerequisite for plant regeneration from Hibiscus sabdariffa. Seeds of H. sabdariffa were collected from gene bank of BJRI, Dhaka. Seeds were surface sterilized by immersing in absolute alcohol for 1 minute and then in 0.1% Mercuric Chlorid for 20 minutes. Seeds were thoroughly washed with autoclaved water for 4-5 times. The sterilized seeds were transferred on clinical cotton supported MS (Murashige and Skoog, 1962) liquid medium and agar (Sigma, UK, 0.8%, w/v) supported MS solidified medium in 500 ml conical flasks. Each flask contained 15 seeds and was placed in a growth room with 27.2°C temperature under 1500 lux fluorescent illumination with 12 h photoperiod.

Culture of cotyledons with attached petioles

Cotyledons with attached petioles were taken from *in vitro* raised mesta (var. HS-24) seedlings. Fifteen days old seedlings were used for cotyledon culture. It was made sure before the culture that the emerging shoots were not remained attached with the petioles. Four cotyledons with attached petioles were cultured on MS medium in 250 ml conical flasks and gently pressed on the surface of the sterilized culture medium, so that the cut end of the petioles were immersed into the medium to a depth of 2 mm. Various combinations and concentrations of hormone i.e., IAA (0.5 mg/l) and BAP (0.0, 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/l) were used. The cultures were maintained in a growth room with 27.2°C temperature under1500 lux fluorescent illumination of 12 h photoperiod. The conical flasks were checked daily to note the response and the development of contamination. Number of cotyledons responded and number of shoots regenerated from each cotyledon in the culture medium were recorded after 42 days of culture.

Shoot culture for root induction

When the regenerated shoots were 2-3 cm in length, they were rescued aseptically from the culture flasks and cultured individually on 250 ml conical flask with freshly prepared MS medium without hormone (MSO) for root induction.

Transfer of plantlets into soil

Mesta plantlets were transferred into pots containing mixed soil. Saver Dairy soil (70%) was mixed with 30% commercial sand. The mixture was sterilized before use. The earthen pots (6 cm dia. and 7 cm height) with a small hole at the bottom were used for transfer of plantlets. Pots were placed on 9 cm petridishes each containing 20 ml of water. The root of plants were washed with sterilized tap water to remove agar and then transferred into the pots. The plantlets were then covered with a cellophane paper bag (Plate 3) and placed in a well ventilated room. After one week, two holes were made in each bag. During the third week, the bags were removed. Survival rate of the plants was recorded.

Statistical analysis

The data for the parameters under present experiments were statistically analyzed. The analyses of variances for different parameters were performed and means were compared by the Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Germination of seeds from mesta in different media

Seeds of mesta (var. HS-24) were germinated on both clinical cotton-supported MS liquid medium and agar-supported MS solidified medium. Percent seed germination from mesta variety was found to be higher on cotton-supported MS liquid medium (90.00%) compared to agar-supported MS solidified medium (81.33%) (Table1). Growth of the seedlings in cotton-supported MS liquid medium was

Table 1. Germination of mesta seeds on clinical cotton-supported MS liquid medium and agar-supported MS solidified medium.

Name of medium	Number of seeds germinated	Total no. seeds for germination	Percent seed germination
Cotton supported MS liquid medium	45.00a	50	90.00a
Agar supported MS solidified medium	40.67b	50	81.33b
Level of significance	*		*
LSD 0.05	3.33		6.67

Figure in column, having same letter(s) do not differ significantly at 5% level of significance

found to be comparatively better than agar-supported MS solidified medium (Plate 1). This finding agreed with the finding of Khatun (2001), who reported about similar result.

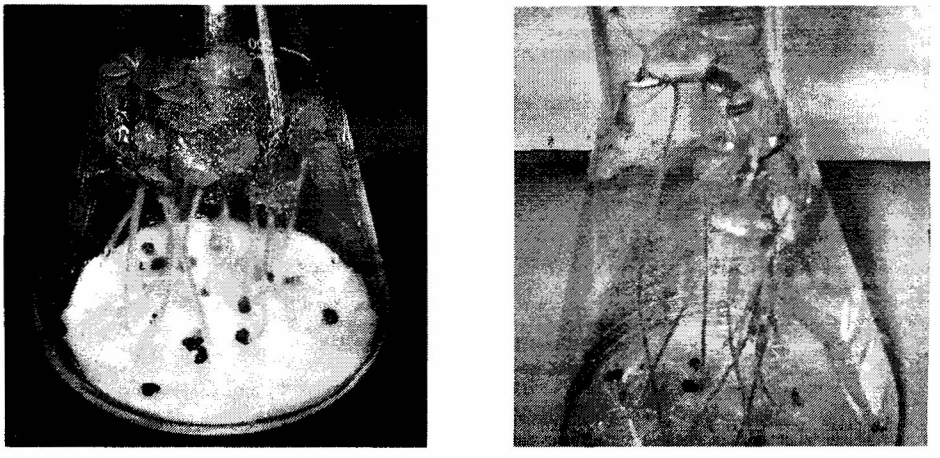

Plate 1. Seed germination on clinical cotton-supported MS liquid medium (left) and agar-supported MS solidified medium (right)

Effect of different concentration of hormones on shoot regeneration

Percent shoot regeneration of mesta in IAA (0.5 mg/l) with different concentrations of BAP (0.25, 0.50, 1.0, 2.0, 3.0 and 4.0 mg/l) is presented in Table 2. The highest regeneration potentiality (58.33%) was observed on MS medium supplemented by IAA (0.5 mg/l) with BAP (2.0 mg/l) (Plate 2)

Plate 2. Shoot regeneration from cotyledons with attached petioles on MS medium supplemented by IAA (0.5mg/l) and BAP (2.0mg/l)

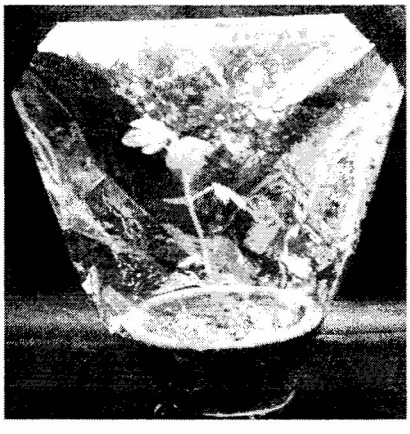

Plate 3. Regenerated plantlets of mesta were covered with cellophane bag after transferred into soil

and the lowest shoot regeneration percent (44.44%) was observed on MS medium supplemented by IAA (0.5 mg/l) with BAP (0.5 mg/l). However, no shoot regeneration was observed without hormone and also when higher doses of hormones (4 mg/l) were applied. This observation is similar to the finding of Khatun (2001), who reported that the highest number of shoots regenerated from the cotyledons with attached petioles of *C. capsularis* on MS medium supplemented by IAA (0.5 mg /l) and BAP (2.0 mg/l). Shoot regeneration was restricted to the proximal cut end of the petioles. This finding was in consistence with the results found in *Brassica juncea* (Sharma *et al.*, 1991), jute (Khatun *et al.*, 1993) and kenaf (Khatun *et al.*, 2003).

Root induction from the regenerated shoots of mesta

Regenerated shoots were transferred on MS medium without hormone (MSO) for root induction. Root induction was observed from the regenerated shoots on hormone free MS medium. This finding also agreed with the finding of Khatun (2001), who reported that the shoot of *C. olitorius* and *C. capsularis* were rooted on MS medium without hormone.

Table 2. Effect of different hormone concentrations on shoot regeneration in mesta

IAA (mg/l)	BAP (mg/l)	Average number of cotyledons producing shoots	Number of shoots per cotyledon	Percent shoot regeneration
	0.00	0.00b	0.00c	0.00b
	0.25	5.67a	2.17b	47.22a
	0.50	5.33a	2.33b	44.44a
0.50	1.00	6.00a	1.67b	50.00a
	2.00	7.00a	4.00a	58.33a
	3.00	6.00a	3.67a	50.00a
	4.00	0.00b	0.00c	0.00b
Level of significance		**	**	**
LSD 0.01		1.576	1.064	13.13

Figure in column, having same letter(s) do not differ significantly at 1% level of significance

Survivability of plantlets into soil

Root production was observed on hormone free MS medium. Plantlets were successfully transferred to mixed soil (dairy soil and sand). Most of the mesta plantlets survived after transfer to soil and grew up to maturity (Plate 4).

Plate 4. Successfully transferred plantlets 15 days after transferred into soil

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