Indian J. Genet., 69(2): 132-139 (2009)

Development of somaclones and their genetic diversity analysis through RAPD in Finger millet (*Eleusine coracana* L. Gaertn.)

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(Received: November 2008; Revised: April 2009; Accepted: May 2009)

Abstract

While developing a protocol for induction of variability *in vitro* in finger millet, significant differences for callus induction among genotypes, explant types, culture medium and their interactions were observed. The genotypes, IVT 10 (52.25%), IPGSM 10 (27.90%) and IVT 4 (23.60%) showed significantly higher frequencies of callus induction as compared to other genotypes. All the genotypes and explants showed highest callus induction in MS + 2 mgl^{-1} 2,4-D. MS medium induced early callus than B_5 media. Nine out of 12 genotypes showed somatic embryogenesis and morphogenesis. However, genotypes IPGSM 18, GOA 8/1 and AVT 11 failed to regenerate. Among explants, mature embryo axes showed highest (25.68%) regeneration response in nine genotypes. Half strength MS medium supplemented with 0.2 mgl^{-1} IBA + 0.1 mgl^{-1} BAP and 2 per cent sucrose showed significantly higher number of roots (10.25) in 6.55 days. The rooted plants planted in pots containing soil, sand and vermiculite in equal proportion showed 60% survivability in six genotypes. A total of 10 primers were used for amplification of genomic DNA of the 6 parents and their 45 variants for RAPD analysis. Out of 1374 amplified fragments 1268 (92.59 %) DNA fragments were monomorphic while the remaining 106 (7.41%) were polymorphic. The RAPD based dendrogram indicated subgrouping of IVT 10 variants and data showed genetic distance between the variants.

Key words: Somaclonal variation, finger millet, callus, induction, regeneration, RAPD analysis

Introduction

Finger millet has proved to be a “super cereal” in nutritional terms. It contains large proportion of carbohydrates (72%) and thus provides bulk of energy in diets (323 to 350 Kcal). Protein content in finger millet ranges from 7 to 14 per cent with a high methionine and all the essential amino acids which otherwise are lacking in grain based foods (National Research Council, 1996)

Apart from the major nutrients each 100 grams of finger millet contains 12.6 mg iron, 410 mg calcium and 290 mg phosphorus [1]. Because of its low glycemic index with high fiber it is also recommended for diabetic patients. Consumption of finger millet prevents constipation and lowers cholesterol [2].

Although this crop is nutritionally rich but its productivity is very low and genetic base of the local genotypes is very narrow. Attempt for improvement of finger millet genotypes so far has been restricted to pureline selection only due to the complicated floral biology of this crop. Finger millet is highly self-pollinated, cleistogamous plant having tiny florets, densely present on inflorescence and anthesis takes place during midnight. The only possibility to get rid of these problems is to adopt biotechnological tools for crop improvement in finger millet for yield in general and nutritional characters in particular.

High variability can be induced through *in vitro* callus cultures. Somaclonal variation is a cheaper *in vitro* method for genetic manipulation. It has been most successful in crops with limited genetic system or narrow genetic base [3]. Further morphological variation in stooling and errectness among finger millet has been reported [4, 5]. Random Amplified Polymorphic DNA (RAPD) analysis has been used to assess somaclonal variability. The present research was focused on the

development of somaclones of the finger millet genotypes and assessment of their variation through RAPD.

Material and methods

Induction and isolation of somaclones

Callus induction and *in vitro* regeneration was tried in two types of explant *viz.,* mature embryo axes and epicotyl of twelve genotypes - IPGSM 10, IPGSM 15, IPGSM 17, IPGSM 18, IVT 4, IVT 10, IVT 16, IVT 27, GOA 8/1, WARC12/4, AVTE 11 and Dapoli 1. The experiments were carried out aseptically under well defined conditions at Plant Biotechnology Unit, Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli- 415 712 (MS).

MS and B_5 medium supplemented with 2,4-D, NAA, BAP, Kinetin and IBA in different combinations and concentrations were included in the study. For preparation of explants the seeds were surface sterilized by 0.1 % Teepol for ten minutes followed by washing under running tap water. After that, seeds were quickly dipped in 70 % ethanol and further immersed in mercuric chloride (0.1%) for 10 minutes, followed by several washings with double distilled water. Treated seeds were soaked for six hours in double distilled water. Treated seeds along with mature embryo axes were inoculated on callus induction media. Seeds were also inoculated on MS basal medium without hormones for epicotyl explants. After 6-7 days epicotyl was used as aseptic explant for callus induction. Observations were recorded on - Callus induction response (%), Days to initiation of callus, Regeneration response (%), Days to shooting, Number of shoots per explant, Days to rooting, Number of roots per plantlet.

Hardening and establishment of rooted plants

While transferring rooted shoots from culture, roots were gently washed with distilled water to remove the medium attached to them. The individual rooted shoots were transferred to potting mixture containing vermiculite and covered with plastic bag with a small hole for air circulation and kept in growth chamber. For the initial 10-15 days high humidity was gradually reduced to the ambient level over a period of 2-4 weeks. The potting mixture containing soil, sand, and vermiculite (1:1:1) was used for transplantation of the plants.

DNA isolation

DNA was extracted from 1-2 g of immature leaves. DNA extraction was carried out using modified CTAB method [6]. DNA quantification was done on 1 per cent agarose gel with lambda DNA and subjected for further PCR analysis.

Completely Randomized Design (CRD) was employed for the experiment. The statistical analysis was done in MSTAT-C software programme developed by Russel D. Freed, MSTAT Director, Crop and Soil Science Department, Michigan State University, Version 2.10.

RAPD finger printing

A total of 10 decamer primers from kit OPA and OPD (Operon Technologies, USA), were used for the PCR amplification of the genome of six finger millet genotypes and their 45 variants. Amplification was carried out in 20 µl of reaction mixture containing 50 ng template DNA, 2.0 µl of 10X PCR buffer, 2.5 mM $MgCl_2$, 200 µM dNTPs, 15 ng of primer and 1 unit *Taq* polymerase (Banglore Genei). DNA amplification was performed in Applied Biosystem Cycler programmed for 40 cycles as follows: one cycle of 5 min at 94°C, 30 sec. at 37°C, 30 sec. at 72°C followed by 39 cycles each of 1 min at 94°C, 30 sec. at 37°C, 30 sec. at 72°C followed by one final extension cycle of 10 min at 72°C. The amplification products were size separated by gel electrophoresis in 1.2 per cent agarose gel (Banglore genei) with 1X TBE and stained with ethidium bromide. The gel was documented in Biovis gel doc system with Biovies 1 D software. All the reactions were repeated at least twice and reproducible bands were used in the analysis.

Results and discussion

Effect of genotypes

Significant difference was observed for callus induction amongst the genotypes (Table 1). Genotype IVT 10 recorded highest callus induction response (52.25 %) followed by IPGSM 10 (50.41 %) and IVT 4 (44.58 %). The lowest callus induction was observed in GOA 8/1 (28.74 %) when mature embryo axes was used as explant. When epicotyl was used as an explant the highest response was observed in IVT 10 (28.33 %) followed by IVT 4 (26.66 %). The lowest callus induction was observed in GOA 8/1 (15.41 %).

Effect of growth regulators

The mean values for callus induction response in mature embryo axes are presented in Table 1. It ranged from 27.08 to 54.66 per cent. The maximum response for callusing was recorded in 2 mgl^{-1} 2,4-D (64.31%) followed by 1.00 and 0.5 mgl^{-1} 2,4-D concentrations. The highest callus induction (95.00 %) was observed in 2.00 mgl^{-1} 2,4-D whereas, lowest (8.33 %) was recorded

Table 1. Genotypic variability in relation to auxin levels (mg/l) for callus induction response in mature embryo axes on MS medium.

S.No.	Genotype	2,4-D (mg/l)					NAA (mg/l)				
		0.1	0.5	1.00	2.00	Mean	1.00	2.00	3.00	4.00	Mean
1.	IPGSM 10	31.67 (34.23)	38.33 (38.24)	61.67 (51.78)	93.33 (75.24)	56.25 (49.87)	28.33 (38.24)	43.33 (41.16)	53.33 (46.91)	76.67 (61.14)	50.41 (42.10)
2.	IPGSM 15	16.67 (24.05)	28.33 (32.14)	43.33 (41.13)	56.67 (48.87)	36.25 (36.54)	28.33 (32.14)	38.33 (38.24)	51.67 (45.97)	46.67 43.09)	41.25 (38.78)
3.	IPGSM 17	16.67 (24.05)	21.67 (27.71)	51.67 (45.96)	55.00 (47.88)	36.25 (36.54)	21.67 (27.71)	33.33 (35.25)	55.00 (47.88)	53.33 (46.93)	40.83 (39.44)
4.	IPGSM 18	16.67 (24.05)	23.33 (28.86	43.33 (41.07)	71.67 (57.86)	38.75 (40.99)	18.33 (25.31)	28.33 (32.14)	51.67 (45.96)	50.00 (45.00)	37.08 (37.10)
5.	IVT 4	13.33 (21.34)	18.33 (25.31)	46.67 (43.05)	73.33 (59.05)	37.91 (37.18)	21.67 (27.71)	38.33 (38.22)	65.00 (53.76)	53.33 (46.92)	44.58 (41.65)
6.	IVT 10	32.67 (27.71)	37.33 (32.14)	63.33 (52.91)	95.00 (79.55)	57.08 (37.58)	29.33 (21.34)	51.67 (45.96)	56.33 (44.03)	71.67 (57.91)	52.25 (42.31)
7.	IVT 16	16.67 (24.05)	21.67 (27.71)	38.33 (38.22)	58.33 (49.80)	33.75 (34.94)	16.67 (24.05)	31.67 (34.18)	43.33 (41.16)	48.33 (44.04)	35.00 (35.85)
8.	IVT 27	23.33 (16.60)	28.33 (23.74)	45.00 (35.17)	43.33 (41.16)	34.99 (29.16)	13.33 (21.34)	18.33 (25.31)	53.33 (46.91)	30.00 (33.16)	28.74 (31.68)
9.	GOA 8/1	8.33 (21.34)	16.67 (25.31)	33.33 (42.12)	43.33 (52.80)	25.41 (35.39)	13.33 (24.05)	18.33 (41.16)	53.33 (51.78)	30.00 (37.26)	28.74 (31.68)
10.	WARC 12/4	13.33 (21.34)	18.33 (25.31)	45.00 (42.12)	63.33 (52.80)	34.99 (35.39)	16.67 (24.05)	43.33 (41.16)	36.67 (37.26)	61.67 (51.78)	39.58 (38.56)
11.	AVTE 11	21.67 (27.52)	28.33 (32.14)	48.33 (44.04)	61.67 (51.76)	40.00 (38.86)	18.33 (25.31)	43.33 (41.15)	51.67 (45.96)	56.67 (48.87)	42.50 (40.32)
12.	DAPOLI 1	18.33 (25.31)	28.33 (32.14)	40.00 (39.21)	56.67 (48.84)	35.83 (36.37)	21.67 (27.71)	43.33 (41.16)	40.00 (39.21)	48.33 (44.04)	38.33 (38.03)
Mean		18.19 (25.31)	25.00 (32.14)	46.67 (39.21)	64.31 (53.31)	38.95 (37.39)	20.14 (27.71)	37.08 (41.16)	49.44 (39.21)	53.89 (44.04)	39.94 (38.70)

	Genotype	Auxin	Genotype x Auxin
$SE_m \pm$	6.20	5.32	4.33
C. D. at 1 %	19.30	17.20	13.23

(Figures in parenthesis indicate transformed values).

in 0.1 mgl^{-1} 2,4-D. All the genotypes recorded friable embryogenic callus in these media (Fig. 1a). Eapen and George [1] recorded somatic embryogenesis and plant differentiation in media supplemented with 2,4-D. Characteristics of callus in terms of its colour and appearance plays an important role in determining regeneration [4].

Similarly, maximum response for callusing was recorded in 4.00 mgl^{-1} NAA (53.89%) followed by 3 mgl^{-1} NAA (49.44%) and 2 mgl^{-1} NAA (37.08%). The lowest (20.14 %) response for callusing was recorded by 1 mgl^{-1} NAA. The per cent callus induction response ranged from 13.33 to 29.33%, 18.33 to 51.67%, 36.67 to 65.00% and 30.00 to 76.67 per cent with NAA concentration of 1.00, 2.00, 3.00 and 4.00 mgl^{-1}, respectively. The callus induced in NAA was compact and green in colour [7, 8]. Thus, the deciding factor in callusing in tissue culture of fingermillet is the concentration of 2, 4-D and NAA in the medium. In this way large genotypic variations in callus induction might have attributed to the differences in the levels of 2, 4-D and NAA [8].

Epicotyl explant in MS media when enriched with different concentration of 2,4-D and NAA recorded

variable response for callus induction (Table 2). Highest (48.33 %) response for callusing was observed in 1 mgl^{-1} 2,4-D concentrations followed by 2.00 mgl^{-1} (21.66 %) and 0.5 mgl^{-1} (24.02 %). No callusing was recorded in media supplemented with 0.1 mgl^{-1} 2,4-D. Per cent callus induction ranged from 0.00 to 58.33 in different concentrations of 2,4-D.

Similarly, NAA concentration of 4 mgl^{-1} gave maximum (60.00 %) response for callus induction while no response was recorded in 1 mgl^{-1} NAA concentration. Per cent callus induction ranged from 0.00 to 60.00 in varying concentrations of NAA.

Effect of genotype on days to callus induction

Genotype IVT 10 took 12.98 days for callusing while genotype IVT 4 took 16.99 days for callusing when mature embryo axes was used as explants. Similarly, when epicotyl was used as an explant the minimum days (16.67) required for callusing were by genotype IVT 10 and WARC 12/4. Mean response for days to callus initiation ranged from 13.52 to 16.17 in both the media tried. When MS media was used for recording the observation for number of days required for callusing it was observed that genotype IVT 10 took minimum 10.77 days and maximum 15.07 days were required for

Table 2. Genotypic variability in relation to auxin levels (mg/l) for callus induction response in epicotyl explant on MS media

S.No.	Genotype	2,4-D (mg/l)					NAA (mg/l)				
		0.1	0.5	1.00	2.00	Mean	1.00	2.00	3.00	4.00	Mean
1.	IPGSM 10	0.00 (0.00)	26.66 (31.07)	21.66 (27.71)	55.00 (47.88)	25.83 (26.66)	0.00 (0.00)	8.33 (16.60)	28.33 (32.14)	41.66 (40.20	19.58 (16.24)
2.	IPGSM 15	0.00 (0.00)	16.66 (24.05)	18.33 (25.31)	38.33 (38.22)	18.33 (21.89)	0.00 (0.00)	8.33 (16.60)	23.33 (28.86)	31.66 (34.23)	15.83 (19.92)
3.	IPGSM 17	0.00 (0.00)	23.33 (28.86)	36.66 (37.26)	55.00 (47.88)	28.74 (28.50)	0.00 (0.00)	13.33 (21.34)	33.33 (35.22)	46.66 (43.09)	23.33 (24.91)
4.	IPGSM 18	0.00 (0.00)	21.66 (27.71)	13.33 (21.34)	51.66 (45.96)	21.66 (23..75)	0.00 (0.00)	6.66 (14.76)	21.66 (27.71)	36.66 (37.26)	16.24 (19.93)
5.	IVT 4	0.00 (0.00)	25.00 (29.80)	41.66 (40.20)	55.00 (47.88)	30.41 (29.47)	0.00 (0.00)	16.66 (24.05)	35.00 (36.24	55.00 (47.88)	26.66 (23.97)
6.	IVT 10	0.00 (0.00)	36.66 (37.26)	35.00 (36.24)	58.33 (49.82)	32.49 (30.83)	0.00 (0.00)	11.66 (19.89)	41.66 (40.20)	60.00 (50.79)	28.33 (27.72)
7.	IVT 16	0.00 (0.00)	21.66 (27.71)	23.33 (28.86)	43.33 (41.16)	22.08 (24.43)	0.00 (0.00)	18.33 (25.31)	18.33 (25.31)	46.66 (43.09)	20.83 (16.87)
8.	IVT 27	0.00 (0.00)	16.66 (24.05)	21.66 (27.71)	31.66 (34.18)	17.49 (21.48)	0.00 (0.00)	6.66 (14.76)	21.66 (27.71)	36.66 (37.26)	16.24 (19.93)
9.	GOA 8/1	0.00 (0.00)	23.33 (28.86)	18.33 (25.31)	53.33 (46.91)	23.74 (25.27)	0.00 (0.00)	8.33 (16.60)	26.66 (31.07)	26.66 (31.07)	15.41 (19.63)
10.	WARC 12/4	0.00 (0.00)	20.00 (26.57)	23.33 28.86)	50.00 (45.00)	23.33 (23.85)	0.00 (0.00)	5.00 (12.92)	21.66 (27.71)	53.33 (46.91)	19.99 (22.20)
11.	AVTE 11	0.00 (0.00)	16.66 (24.05)	16.66 (24.05)	46.66 (43.08)	19.99 (22.79)	0.00 (0.00)	8.33 (16.60)	26.66 (31.07)	43.33 (41.16)	19.58 (22.20)
12.	DAPOLI 1	0.00 (0.00)	11.66 (19.89)	18.33 (25.31)	41.66 (40.17)	17.91 (21.34)	0.00 (0.00)	11.66 (19.89)	23.33 (28.86)	38.33 (38.24)	18.33 (21.74)
Mean		0.00 (0.00)	24.02 (29.02)	48.33 (44.01)	21.66 (27.49)	24.02 (29.02)	0.00 (0.00)	10.27 (18.27)	26.80 (30.53)	26.80 (30.53)	20.02 (26.57)

	Genotype	Auxin	Genotype x Auxin
$SE_m \pm$	5.30	3.40	12.00
C. D. at 1 %	16.30	12.30	25.23

(Figures in parenthesis indicate transformed values).

genotype IVT 16. However, when B_5 media was used IPGSM 18 showed quickest response 13.86 days and Dapoli 1 was least in responding taking 19.30 days for callusing.

Effect of growth regulators on plantlet regeneration

Different growth regulators at varying concentrations resulted in causing variability in plantlet regeneration. The levels of cytokinin were significant for plantlet regeneration. Highest response (26.18 %) was observed in media containing BAP, while lowest response was observed (23.05 %) in media enriched with kinetin. The range for regeneration response in BAP was 0.00 to 44.72 per cent and that of media supplemented with kinetin was 0.00 to 40.00 % suggesting that the presence of required level of hormones is a pre-requiste for the initiation of organogenesis *in vitro* [9].

Media containing 2 mgl^{-1} BAP with mature embryo axes showed highest (40.13 %) plantlet regeneration (Figs. 1b and 1c) followed by 1.00 mgl^{-1} BAP (28.88%) and 3.00 mgl^{-1} (26.38%). Mean values ranged from 0.00 to 54.99 per cent. Similarly 2.00 mgl^{-1} Kinetin proved to be effective showing highest regeneration response (36.10%) followed by 1.00 mgl^{-1} (27.91 %) and 3.00 mgl^{-1} (26.52 %). Mean values for regeneration response ranged from 0.00 to 53.33 per cent.

Significant differences between the two cytokinins with epicotyl explant were observed. BAP 2 mg/l^{-1} concentration was found to be effective with highest (26.10%) regeneration response followed by 1.00 mg/l^{-1} (19.58%) and 3.00 mg/l^{-1} (15.96%) with values ranging from 0.00 to 38.32 per cent.

Genotype x explant x cytokinin interaction in regeneration response

The observations recorded in Table 3 indicate that the mature embryo axes as explants of genotype IVT 10 recorded maximum number of plantlet in BAP (8.53) as well as kinetin (7.91) media combinations. However, IPGSM 18, GOA 8/1 and AVTE 11 failed to regenerate in BAP as well as kinetin. Maximum plantlet regeneration was observed in IVT 10 genotype (13.30) when media was supplemented with 2.0 mgl^{-1} BAP.

When epicotyl were used as explants the genotypes IPGSM 10 recorded maximum plantlet in BAP (3.46) which was significantly superior over kinetin (2.83).

Table 3. Genotypic variability in relation to cytokinin levels for number of plantlets per explant in mature embryo axes and epicotyl explant.

S.No.	Genotype	Mature Embryo Axes								Epicotyl explant							
		BAP				Kinetin				BAP				Kinetin			
		1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
1.	IPGSM 10	3.99	11.29	6.44	7.24	4.09	9.47	6.14	6.56	2.59	5.50	2.30	3.46	2.30	4.27	1.94	2.83
2.	IPGSM 15	1.59	4.32	3.19	3.03	2.4	4.19	2.61	3.06	1.17	3.84	2.11	2.37	1.09	2.53	1.92	1.84
3.	IPGSM 17	1.86	5.29	3.26	3.47	1.99	5.69	4.12	3.93	1.00	3.61	2.42	2.34	1.15	3.40	1.94	2.16
4.	IPGSM 18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.	IVT 4	2.88	6.32	5.51	4.90	2.21	6.01	5.25	4.49	1.18	4.24	2.15	2.52	1.28	3.54	1.87	2.23
6.	IVT 10	4.12	13.30	8.19	8.53	4.06	11.60	8.07	7.91	2.15	5.09	2.58	3.27	2.13	3.16	1.87	2.38
7.	IVT 16	1.49	4.71	3.70	3.3	1.54	5.19	4.07	3.60	1.07	4.53	3.11	2.90	1.07	3.72	2.52	2.43
8.	IVT 27	2.26	6.96	4.45	4.55	2.01	5.99	5.36	4.45	1.04	4.25	2.54	2.61	1.16	3.57	2.45	2.39
9.	GOA 8/1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10.	WARC 12/4	3.99	5.53	5.54	5.02	3.91	6.00	4.47	4.79	1.23	4.16	2.38	2.59	1.12	2.72	1.91	1.91
11.	AVTE 11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.	DAPOLI 1	3.40	5.40	5.42	4.74	2.25	4.73	4.39	3.79	1.23	4.16	2.38	2.59	1.12	2.72	1.91	1.91
	Mean	2.13	5.26	3.81	3.73	2.04	4.90	3.70	3.54	1.06	3.26	1.86	2.06	1.04	2.51	1.53	1.69

	Genotype	Cytokinin	Genotype x Cytokinin		Genotype	Cytokinin	GenotypexCytokinin
SEm±	0.15	0.11	0.37	SEm±	0.06	0.04	0.14
CD at 1%	0.56	0.40	1.37	CD at 1%	0.21	0.15	0.52

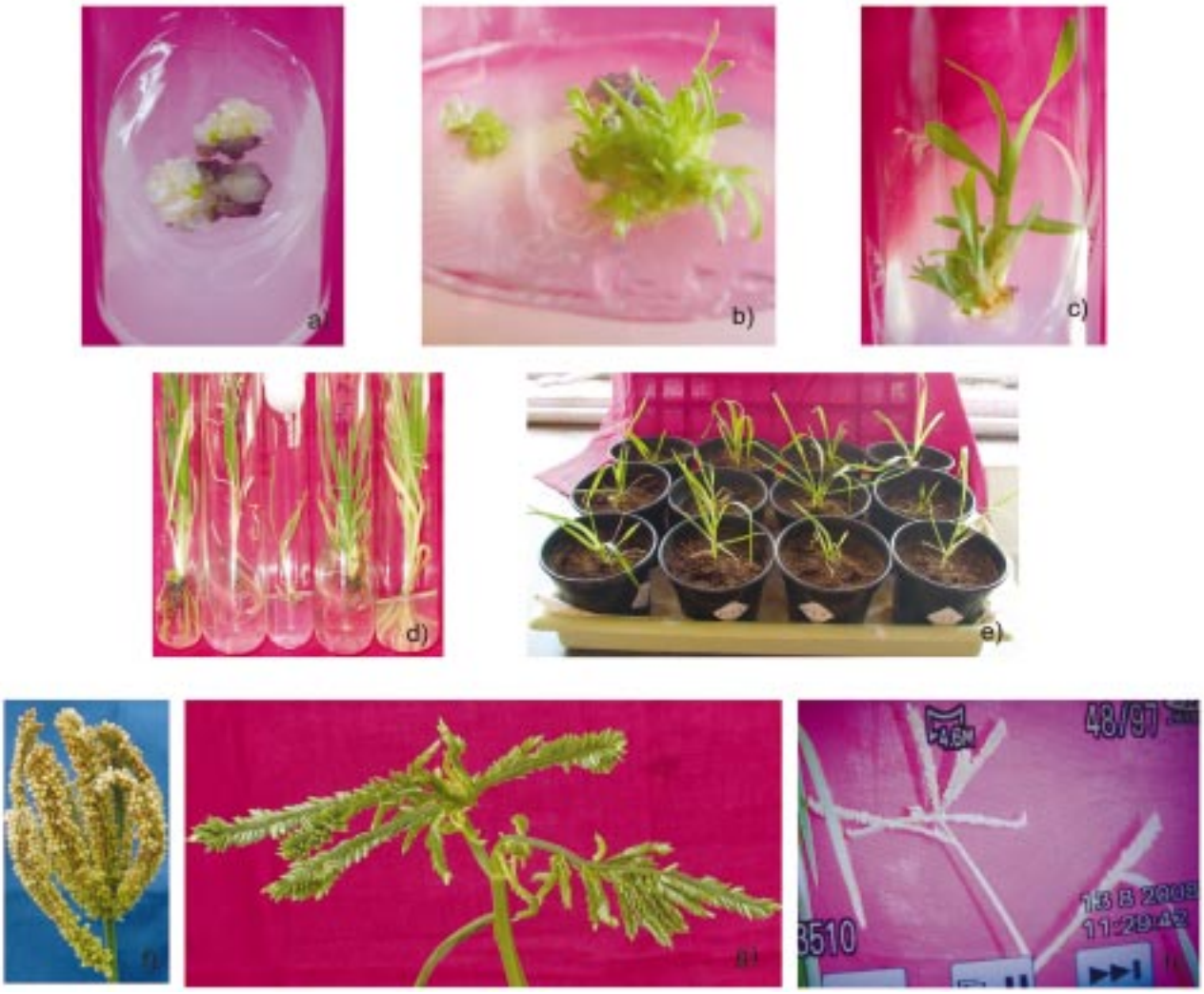

Fig. 1. a) Friable and embryogenic callus of finger millet developed in MS + 2mg/l, 2, 4-D; b & c) Germination of proembroys and multiple shooting in MS + 2 mg/l BAP; d) Variable response of rooting in different rooting media; e) Establishment of somaclones of finger millet in potting mixture containing soil, sand and vermiculture (1:1:1); f, g & h) Variants of IVT 10 genotypes of developed through somatic embryogenesis

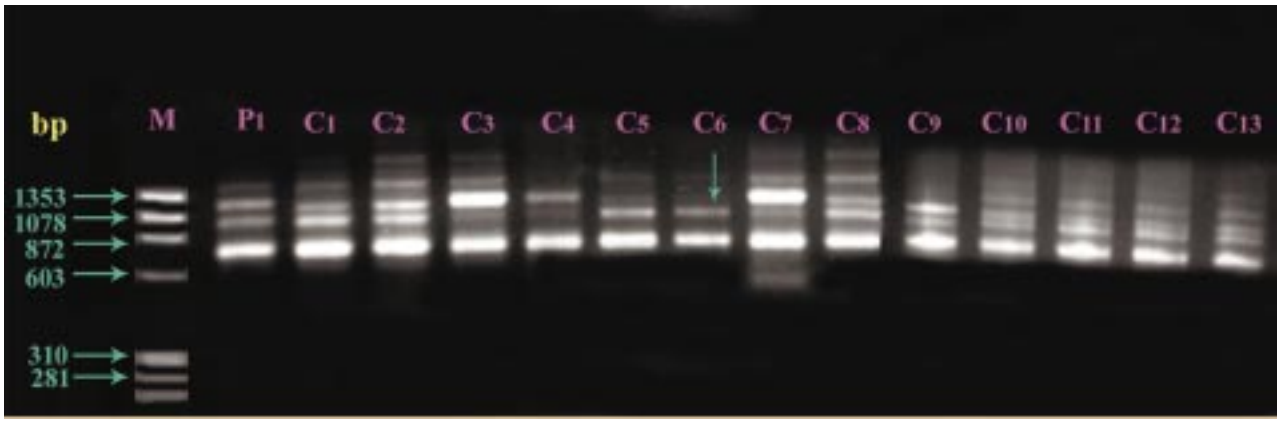

Fig. 3. Agarose gel electrophoresis pattern of PCR amplification products obtained with Finger millet cultivar IVT 10 and its clones using OPA08 primer, M: x174 marker; Lane 1: Parent; C1-C13: Clones, respectively

Table 4. Sequence of RAPD primers used for amplification of genomic DNA of Finger millet cultivars

Sr. No.	Primer	Sequence	Total Bands	Polymorphic bands	Per cent polymorphism (%)
1..	OPA 03	AGTCAGCCAC	-	-	-
2.	OPA 04	AATCGGGCTG	271	32	11.80
3.	OPA 08	CTGACGTAGG	188	09	4.78
4.	OPA 10	CTGATCGCAG	229	16	6.9
5.	OPA 13	CAGCACCCAC	203	08	3.9
6.	OPD O2	CGACCCAACC	238	32	13.44
7.	OPD 04	TCTGGTGAGG	245	09	3.67
8.	OPD06	ACCTGAACGG	-	-	-
9.	OPD 11	AGCGCCATTG	-	-	-
10.	OPD 12	CACCGTATCC	-	-	-
	Total		1374	106	-

Effect of explant and growth regulators on plantlet production per callus

The effect of explant and growth regulators on plantlet production reveals that maximum mean number of plantlets (5.26) were produced when BAP concentration was 2 mgl^{-1} followed by 3.0 mgl^{-1} (3.81) and 1 mgl^{-1} (2.13) in mature embryo axes.

Similar trend was observed when epicotyls were used as explant. Media supplemented with 2.0 mgl^{-1} BAP produced maximum plantlet (3.26).

Days to rooting

Supply and regulation of endogenous auxin may play a crucial role in root initiation; similarly exogenous auxins are essential for rooting [10]. In some species it is reported that reduced sugar levels (10-20 gl^{-1}) have a role in root formation [4]. Data regarding total number of days required for rooting in different growth regulators is depicted Fig. 2. Minimum mean days (8.95) were required by media supplemented with IBA as compared to NAA (10.73 days). Mean value for days required to rooting ranges from 8.86 days to 11.16 days. The most suitable combination which required only 6.55 days to rooting was seen when IBA 2 mgl^{-1} +0.1 mgl^{-1} BAP+ 2% sucrose was supplemented whereas 9.88 days to rooting were required when ½ MS was supplemented with 1 mgl^{-1} IBA, 10.42 days when 0.3 mgl^{-1} IBA. The media combination of ½ MS with 1 mgl^{-1} IBA and 1% sucrose failed for root induction (Fig. 1d).

NAA concentration of 2.00 mgl^{-1} required minimum days to rooting (8.87 days) followed by 1.0 mgl^{-1} NAA (11.01 days).

Hardening and establishment

Rooted plants were transferred to the potting mixture of soil, sand and vermiculite (1:1:1) which showed maximum (60%) survivability.

RAPD analysis

A total of 10 primers were used to amplify the genomic DNA of the 6 parents and 45 variants (Table 4). Out of these, 6 primers (OPA04, OPA08, OPA10, OPA13, OPD02 and OPD04) gave amplification, whereas remaining 4 primers failed to show the amplification.

A total of 1374 DNA fragments were amplified with an average of 5.08 bands per primer per variants. Out of 1374 amplified fragments, 1268 (92.59 %) DNA fragments were found to be monomorphic, while the remaining 106 (7.41%) were polymorphic. The random decamer primer OPD 02 was most informative as it yielded 13.44% of polymorphism with a total of 238 DNA fragments, followed by OPA 04 which yielded 11.80% polymorphism and 271 DNA fragments. The approximate size of longest DNA fragment amplified was 1353 bp with primer OPA 08 (Fig. 3). When cluster analysis using RAPD data was performed, clone No. 6 of IVT 10 made a distinct node where as all clones made one separate cluster which joined mostly to IVT 10 (Fig. 4). It indicated that there was genetic distance between variants and genotype.

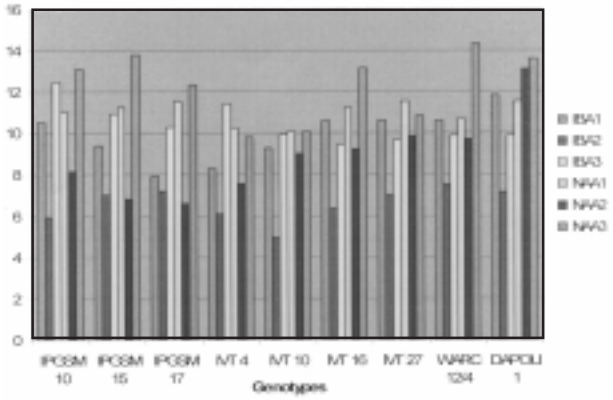

Fig. 2. Genotypic variability in relation to auxin levels for days to rooting

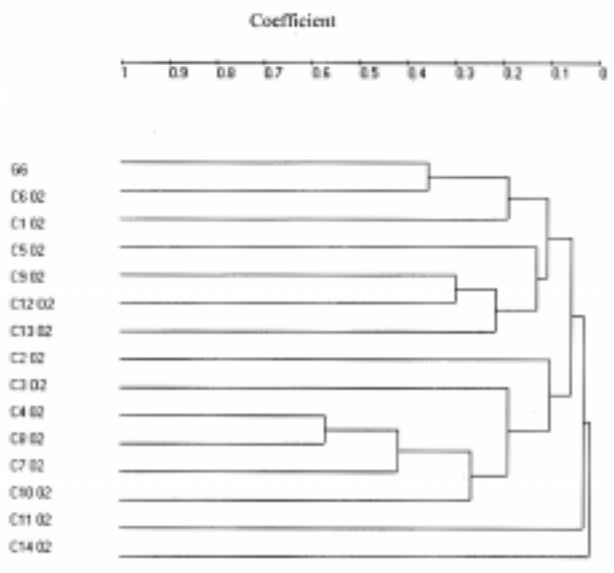

Fig. 4. Dendrogram of fingermillet parent IVT 10 and its somaclonal variants generated by RAPD data

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