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Micropropagation Protocol for Sugarcane (*Saccharum officinarum*) *var* 2005T50

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ABSTRACT

The *in vitro* response of sugarcane pre-release variety, 2005T50 was assessed in the present investigation for direct organogenesis, multiplication, shooting, rooting and hardening on different hormonal combinations and concentrations from shoot tip. Different combinations and concentrations of BAP, IAA, Kinetin and NAA were studied for establishment of axillary shoots and multiple shoot induction. Among the various treatments evaluated MS medium supplemented with 2 mg l^{-1} BAP, 1 mg l^{-1} IAA and 3 mg l^{-1} Kinetin was the best for establishment and MS medium supplemented with 3 mg l^{-1} BAP, 1 mg l^{-1} NAA and 3 mg l^{-1} Kinetin was efficient for multiplication. In case of root induction studies half MS medium supplemented with 6 mg l^{-1} NAA was proved to be the successful. Vermicompost : Soil : Sand (1:1:1) was found to be the most suitable medium for acclimatization and hardening.

Key words : *In vitro* regeneration, Micropropagation, Sugarcane, Shoot tip.

Sugarcane is an important commercial crop in the tropics and sub tropics. It contributes 75 per cent to total sugar pool at global level apart from sugar beet and other sources. India ranks second among the sugarcane producing countries of the world with an area of 4.396 M ha, production of 273.931 M tons and productivity of 62.3 tons per hectare. The projected sugarcane production by the year 2020 is estimated as 415 M tons. This can be achieved not only by evolving elite genotypes with high yielding potential but also supplying quality seed to the farmers. Mass multiplication of newly developed varieties through vegetative propagation is slow. Whereas, through shoot tip culture healthy seed can be produced on a large scale.

MATERIAL AND METHODS

Plant material

Sugarcane pre-release variety, 2005T50 was used in the present study. The tops of sugarcane were collected from the 7-8 months old plants at the Agicultural Research Station, Perumallapalle. Shoot tips were cultured to study micropropagation. At first the tops were sized to 10 cm with growing point in the middle. Later leaf whorls were removed one by one and upto 2-3 leaf whorls were retained on the meristem 4-5 cm explants were dissected and used.

Surface sterilization

The explants were washed thoroughly under running tap water for 20-30 minutes followed by treatment with 0.2 per cent bavistin for 10 minutes and then washed under sterile distilled water prior to transfer to laminar air flow cabinet. The young shoot tip explants were treated with 10 per cent sodium hypochlorite for 20 minutes. Finally the explants were washed thoroughly for 3-5 times with sterile distilled water and dissected to size of 1.0 - 1.5 cm length before inoculation into harmone enriched sterilized nutrient agar media pre-packed in culture bottles.

Explant inoculation

The explants were cultured on fifty four treatmants of MS medium supplemented with different concentrations and combinations of BAP (2-4 mg l^{-1}), Kn (1-3 mg l^{-1}), NAA (1-3 mg l^{-1}) and IAA (1-3 mg l^{-1}) for axillary shoot induction from shoot tip and its establishment as shown in Table1.

Shoot multiplication

The well grown axillary shoots were transferred to the fifty four treatments of MS medium with the different concentrations and combinations of BAP (2-4 mg l^{-1}), Kn (1-3 mg l^{-1}), NAA (1-3 mg l^{-1}) and IAA (1-3 mg l^{-1}) i.e. same treatment

combinations that were used for the axillary shoot induction (as shown in Table.2) were used for multiplication of shoots.

***In vitro* rooting**

Well grown multi shoots with good length were excised from 21-28 days grown axillary shoot cultures and transferred to 30 treatments of half strength and full strength MS medium supplemented with different concentrations of IBA (2-4 mg l^{-1}) and NAA (4-6 mg l^{-1}) both individually and in combinations.

Culture conditions

The explants were inoculated on to sterilized solid basal MS medium (Murashige and Skoog's, 1962) supplemented with different concentrations and combinations of different plant growth regulators. All the cultures of establishment, multiplication and rooting were incubated in a growth room with 16 hours photo period (2500 lux) 8 hours dark and the temperature was maintained at 26±1^{0}C with 70-75 per cent relative humidity in the culture room. Each treatment consisted of eight replicates with three repeats for establishment. Whereas six replicates were repeated thrice for multiplication and rooting.

Acclimatization and hardening of plantlets

Plantlets with well developed roots were removed from the culture medium. Roots gently washed under running tap water were transferred to plastic cups containing six different hardening mixture combinations of cocopeat, vermi compost, press mud, soil and sand as mentioned in Table.4. All these plants were irrigated when ever required and they are provided with quarter strength MS medium twice a day for two weeks and after that water is provided twice in a day.

Observations

Experiment were set up in a completely randomized design and observations were recorded on different parameters

- Frequency of explants induced axillary shoots (%)
- Mean number of days taken for axillary shoot initiation from explant
- Mean number of axillary shoots per explant
- Mean length of axillary shoots (cm)
- Mean number of days taken for multishoot initiation from axillary shoots
- Frequency of axillary shoots producing multishoots (%)
- Mean number of multishoots per axillary shoot
- Mean length of multi shoots (cm)
- Mean number of days taken for root initiation
- Frequency of shoots producing roots (%)
- Mean number of roots per shoot
- Mean length of roots (cm)
- Mean number of days taken for acclimatization
- Survival percentage (%)

Statistical analysis

The data recorded on various parameters during the course of investigation was statistically analyzed by following the analysis of variance for completely randomized design on basis of the model proposed by Panse and Sukhatme (1985).

The analysis of variance (ANOVA) for each character was carried out, statistical significance was tested with 'F'-test at five percent level of probability and compared the treatmental means with critical difference. Due to larger variation in percentage data for various parameters, the corresponding data was transformed by arc sine transformation before subjecting to stastical analysis as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Studies were conducted to determine suitable media for establishment, development of multiple shoots, shoot and root induction, acclimatization from shoot tip explants of 2005T50. By this study we can confirm that shoot tip explants responded well for micropropagation of sugar cane. Similarly Khan *et al.,* (2008) reported that shoot tips with apical meristem were found to be the excellent starting material for micropropagartion of sugarcane. Many other workers used shoot tip explants for micropropagation due to less contamination, systemic pathogen elimination and more multiplication rate than other explants (Chen *et al.,* (1988), Dhumale *et al.,* (1994), Sood *et al.* (2000), Singh *et al.,* (2001), Khan *et al.,* (2006), Ali *et al.,* (2008), Khan *et al.,* (2009)).

Axillary shoot induction

Various concentrations of cytokinins (BAP, 2-4 mg l^{-1} and Kinetin, 1-3 mg l^{-1}) and auxins (IAA, 1-3 mg l^{-1} and NAA, 1-3 mg l^{-1}) were used in fifty four different treatment combinations for shoot regeneration. Shoot initiation was highly influenced by concentrations and type of growth regulators

used in the experiment. Among fifty four different combinations of hormones for shoot induction, MS medium supplemented with 2 mg l^{-1} BAP + 1 mg l^{-1} IAA + 3 mg l^{-1} Kinetin (T_3) recorded the maximum of 87.5 per cent of explants inducing axillary shoots within 6.3 days, with 5.2 shoots per explant and with a mean height of 4.8 cm followed by MS medium with 3 mg l^{-1} BAP + 1 mg l^{-1} NAA + 3 mg l^{-1} Kinetin (T_{39}) with 82.5 per cent of explants induced axillary shoots within 6.8 days, with 5 shoots per explant and mean height of 4.8 cm (Table 1, Fig. A & B). However, there were no significant differences between the treatments.

Sharma (2005) also observed the similar results with the BAP + IAA + KM hormonal combinations. Shukla *et al.,* (1994) reported the positive effects of BAP + IAA + Kin combination on shoot formation in sugarcane and stated that 'The development of axillary shoots dependent ultimately on the supply and favourable balance of cytokinin'. Inclusion of cytokinin in nutrient media promotes the growth of axillaries in cultured buds (Phillips, 1975).

Shoot multiplication

The effect of the presence of two cytokinins (BAP and KM) along with two auxins (IAA and NAA) was examined during multiplication phase of propagation. Among fifty four combinations of different harmonal concentrations tested the best response of multi shoot initiation was observed on MS medium supplemented with 3 mg l^{-1} BAP + 1 mg l^{-1} NAA + 3 mg l^{-1} Kinetin (T_{39}) with 88.89 per cent of axillary shoots inducing multi shoots within 13.6 days with 11.8 multi shoots per shoot and mean length of 4.6 cm followed by MS medium supplemented with 3 mg l^{-1} BAP + 2 mg l^{-1} IAA + 2 mg l^{-1} Kinetin (T_{14}) with 77.78 per cent of axillary shoots inducing multi shoots within 15.2 days with 11.0 mean number of multi shoots per shoot and mean length of 4.5 cm (Table 2, Fig. C & D). However, there were no significant differences between the treatments.

Results of the present investigation suggested that auxins and cytokinins are essential for shoot regeneration which was also supported by Baksha *et al.,* (2002). The higher and lower concentrations did not show better shoot multiplication. Chen *et al.,* (1988) also opined that at lower concentrations of cytokinins, there was loss of proliferation and vigour. Nagai (1988) also reported that high concentration of cytokinins suppressed the shoot proliferation in sugarcane. The degree of shoot multiplication similar to the present result was shown by Khan *et al.,* (2009) who observed 6-11 shoots per shoot on three varieties studied.

***In vitro* rooting of shootlets**

NAA and IBA were used at thirty different concentrations and combinations with full strength and half strength MS media to regenerate adventitious roots in the present study and the results were presented in Table 3. In general NAA was found to be relatively better responsive than IBA. Whereas, half strength MS media supplemented with 6 mg l^{-1} NAA (R_{12}) which is a higher concentration was found to be the best for inducing roots with 94.4 per cent of shoots inducing roots within 9.6 days. The highest number of roots of 13.4 per shoot with a mean length of 2.87 cm was obtained in the next 20 days (Table 3, Fig. E & F). Significant differences were observed between the treatments.

Shenk and Hildebrandt (1972) also reported the requirement of high concentration of auxin for rooting in sugarcane. These results were also supported by Singh *et al.,* (2001) who observed the best rooting response of 75 per cent on MS medium with 5 mg l^{-1} NAA in 7-15 days and profuse rooting within 30-40 days. Whereas, Ali *et al.,* (2008) observed root induction in 8-10 days on full strength MS medium supplemented with 1.0 mg l^{-1} NAA + 2.0 mg l^{-1} IBA. Karim *et al*., (2002) and Sood *et al.* (2000) reported rooting in 10-15 days using MS medium with 3 mg l^{-1} IBA and 7 mg l^{-1} NAA, respectively.

Combination R_8 which contained half strength MS medium supplemented with 3 mg l^{-1} IBA was the next best treatment. Dhumale *et al.* (1994) induced roots on half strength MS medium containing 2 mg l^{-1} IBA and 1 mg l^{-1} IAA. Whereas, Naritoom *et al.,* (1993), Mamun *et al.,* (2004) and Khan *et al.,* (2006) initiated rooting with 1.0 mg l^{-1} IBA. However, no significant root induction was observed in combination of NAA and IBA than either NAA or IBA alone in the present study.

Acclimatization of plantlets

The ultimate success of in vitro propagation lies in successful establishment of plants in soil. The most difficult during micropropagation is the recovery of plants up on their transfer from lab conditions to the soil.

Among six different combinations evaluated as mentioned in Table 4, the treatment combination H5 (vermicompost + soil + sand (1:1:1)) recorded the highest survival percentage of 86 per cent within

Table 1. Effect of different combinations of BAP, IAA, Kinetin and NAA on axillary bud induction.

Treatments	Hormonal combinations BAP+IAA+Kn (mg l^{-1})	Frequency of explants induced axillary shoots	Mean no. of days for axillary shoot initiation	Mean no. of axillary shoots per explant	Mean length of axillary shoots(cm)
T1	2+1+1	57.50 (49.31)	9.0	2.1	3.0
T2	2+1+2	65.00 (53.73)	8.3	3.0	3.3
T3	2+1+3	87.50 (69.30)	6.3	5.2	4.8
T4	2+2+1	38.75 (38.50)	14.3	1.2	1.6
T5	2+2+2	53.75 (47.15)	13.1	2.3	2.3
T6	2+2+3	54.13 (47.37)	10.6	2.3	3.4
T7	2+3+1	37.50 (37.76)	9.6	1.3	3.6
T8	2+3+2	66.25 (54.48)	7.6	3.7	4.6
T9	2+3+3	63.75 (52.98)	8.6	2.8	3.2
T10	3+1+1	35.00 (36.27)	16.5	0.8	2.8
T11	3+1+2	47.50 (43.57)	13.2	1.2	3.0
T12	3+1+3	66.25 (54.48)	11.7	2.1	3.2
T13	3+2+1	76.25 (60.83)	11.1	3.1	4.2
T14	3+2+2	78.75 (62.55)	6.8	3.6	4.5
T15	3+2+3	70.00 (56.79)	7.0	2.3	4.1
T16	3+3+1	42.50 (40.69)	8.1	1.7	3.8
T17	3+3+2	51.25 (45.72)	9.4	1.4	3.3
T18	3+3+3	62.50 (52.24)	7.1	3.5	3.6
T19	4+1+1	57.50 (49.31)	9.6	1.0	3.3
T20	4+1+2	65.00 (53.73)	9.4	2.1	2.8
T21	4+1+3	62.50 (52.24)	7.8	3.2	2.4
T22	4+2+1	70.00 (56.79)	9.0	3.5	3.9
T23	4+2+2	53.75 (47.15)	10.5	3.4	3.6
T24	4+2+3	58.75 (50.04)	11.1	4.6	4.1
T25	4+3+1	35.00 (36.27)	12.3	1.3	2.6
T26	4+3+2	56.25 (48.59)	9.4	1.2	3.2
T27	4+3+3	40.00 (39.23)	10.2	1.6	3.0
Treatments	BAP+NAA+Kn				
T28	2+1+1	45.00 (42.13)	12.0	2.1	3.6
T29	2+1+2	41.25 (39.96)	11.5	2.7	3.3
T30	2+1+3	45.00 (42.13)	10.3	3.7	4.5
T31	2+2+1	53.75 (47.15)	18.2	1.2	1.2
T32	2+2+2	61.25 (51.50)	15.6	1.8	1.8
T33	2+2+3	57.50 (49.31)	13.0	2.0	3.5
T34	2+3+1	53.75 (47.15)	10.3	2.3	2.3
T35	2+3+2	32.50 (34.76)	9.1	1.3	2.9
T36	2+3+3	65.00 (53.73)	9.4	2.1	2.8
T37	3+1+1	56.25 (48.59)	10.4	2.5	2.8
T38	3+1+2	72.50 (58.37)	9.2	3.2	3.7
T39	3+1+3	82.50 (65.27)	6.8	5.0	4.8
T40	3+2+1	67.50 (55.24)	11.5	3.8	4.3
T41	3+2+2	78.75 (62.55)	7.8	4.2	4.6
T42	3+2+3	62.50 (52.24)	9.5	2.3	3.6
T43	3+3+1	58.25 (49.75)	11.1	2.8	3.9
T44	3+3+2	65.00 (53.73)	11.5	3.1	3.3
T45	3+3+3	70.00 (56.79)	8.3	3.0	3.7
T46	4+1+1	63.75 (52.98)	8.6	3.2	3.8
T47	4+1+2	78.75 (62.55)	7.2	4.4	4.3
T48	4+1+3	75.00 (60.00)	9.2	2.5	3.8
T49	4+2+1	40.00 (39.23)	9.5	1.5	2.8
T50	4+2+2	45.00 (42.13)	11.0	2.6	3.4
T51	4+2+3	47.50 (43.57)	11.3	3.4	3.9
T52	4+3+1	53.25 (46.54)	10.0	3.2	4.2
T53	4+3+2	70.00 (56.79)	9.0	3.8	4.0
T54	4+3+3	50.00 (45.00)	10.2	3.2	3.2
(±) S.Em		4.070	0.802	0.203	0.226
C.D @ 5%		11.424	2.252	0.569	0.634

Note: Figures in parentheses represent arc sine transformed values

Table 2. Effect of different combinations of BAP, IAA, Kinetin and NAA on shoot multiplication.

Treatments	Hormonal combinations BAP+IAA+Kn (mg l^{-1})	Mean no. of days taken for multi shoot initiation	Frequency of shoots producing multi shoots	Mean no. of multi shoots per shoot	Mean length multi shoots (cm)
T1	2+1+1	19.6	44.44 (41.75)	4.2	1.6
T2	2+1+2	16.2	27.78 (31.54)	5.3	2.3
T3	2+1+3	17.3	55.56 (48.25)	8.3	3.6
T4	2+2+1	26.2	44.44 (41.75)	2.6	1.5
T5	2+2+2	22.4	38.89 (38.51)	3.4	1.8
T6	2+2+3	19.3	33.33 (34.79)	7.5	3.2
T7	2+3+1	16.3	11.11 (16.06)	7.6	3.4
T8	2+3+2	15.4	50.00 (45.00)	9.7	3.7
T9	2+3+3	18.7	27.78 (31.54)	8.3	3.6
T10	3+1+1	25.8	38.89 (38.51)	3.5	1.5
T11	3+1+2	24.6	22.22 (27.82)	3.8	1.8
T12	3+1+3	27.3	16.67 (24.09)	5.0	2.5
T13	3+2+1	17.9	55.56 (48.25)	9.4	3.6
T14	3+2+2	15.2	77.78 (62.18)	11.0	4.5
T15	3+2+3	15.7	61.11 (51.49)	9.3	4.1
T16	3+3+1	23.4	38.89 (38.51)	7.2	3.0
T17	3+3+2	18.3	27.78 (31.54)	8.1	3.2
T18	3+3+3	15.3	72.22 (58.46)	9.2	3.8
T19	4+1+1	24.3	11.11 (16.06)	6.6	3.1
T20	4+1+2	21.6	44.44 (41.75)	8.3	2.9
T21	4+1+3	15.7	33.33 (34.79)	7.5	3.4
T22	4+2+1	16.6	72.22 (58.46)	8.7	4.0
T23	4+2+2	16.2	22.22 (27.82)	7.3	3.8
T24	4+2+3	14.7	77.78 (62.18)	10.2	4.2
T25	4+3+1	20.3	44.44 (41.75)	7.1	3.1
T26	4+3+2	18.6	50.00 (45.00)	8.2	3.8
T27	4+3+3	22.5	38.89 (38.51)	6.8	2.6
Treatments	BAP+NAA+Kn				
T28	2+1+1	19.6	5.56 (8.03)	5.8	2.8
T29	2+1+2	18.5	22.22 (27.82)	6.2	2.4
T30	2+1+3	17.5	27.78 (31.54)	7.4	3.6
T31	2+2+1	24.3	5.56 (8.03)	3.3	2.0
T32	2+2+2	19.2	16.67 (19.79)	5.4	2.6
T33	2+2+3	18.5	55.56 (48.25)	8.1	3.2
T34	2+3+1	19.2	38.89 (38.51)	5.1	2.8
T35	2+3+2	20.8	50.00 (45.00)	6.5	3.6
T36	2+3+3	18.2	44.44 (41.75)	6.3	2.8
T37	3+1+1	15.8	72.22 (58.46)	8.6	3.5
T38	3+1+2	15.2	61.11 (51.97)	9.2	4.0
T39	3+1+3	13.6	88.89 (73.94)	11.8	4.6
T40	3+2+1	21.5	72.22 (58.46)	4.9	2.2
T41	3+2+2	14.3	77.78 (62.18)	9.8	4.3
T42	3+2+3	16.3	38.89 (38.51)	7.2	3.2
T43	3+3+1	27.6	16.67 (19.79)	3.0	1.3
T44	3+3+2	24.0	22.22 (27.82)	3.4	1.6
T45	3+3+3	15.7	50.00 (45.00)	4.5	2.6
T46	4+1+1	16.1	22.22 (27.82)	7.6	3.2
T47	4+1+2	14.8	72.22 (58.46)	9.3	4.0
T48	4+1+3	16.2	22.22 (27.82)	8.1	3.6
T49	4+2+1	22.4	27.78 (31.54)	6.3	2.6
T50	4+2+2	20.6	44.44 (41.75)	6.8	3.5
T51	4+2+3	15.4	38.89 (38.51)	7.1	4.0
T52	4+3+1	16.7	44.44 (41.75)	8.5	3.5
T53	4+3+2	14.4	61.11 (51.49)	8.3	4.1
T54	4+3+3	17.3	55.56 (48.25)	7.3	3.4
(±) S.Em		1.068	4.952	0.278	0.266
C.D @ 5%		2.999	13.901	0.781	0.746

Note: Figures in parentheses represent arc sine transformed values

Table 3. Effect of different concentrations of IBA and NAA with full strength and half strength MS medium on initiation of roots from shootlets

Treatments	MS medium	Hormonal combination BA+NAA (mg l^{-1})	Mean no. of days taken for root initiation	Frequency of shoots producing roots	Mean no. of roots per shoot	Mean length of roots (cm)
R1	Full Strength	2+0	23.3	22.22 (27.82)	1.2	1.15
R2	Full Strength	3+0	21.6	27.78 (31.54)	1.8	1.20
R3	Full Strength	4+0	19.3	33.33 (34.79)	3.4	1.67
R4	Full Strength	0+4	16.6	50.00 (45.00)	4.3	1.32
R5	Full Strength	0+5	12.3	61.11 (51.49)	8.6	2.26
R6	Full Strength	0+6	18.6	44.44 (41.75)	5.8	1.63
R7	Half strength	2+0	17.3	50.00 (45.00)	4.5	1.83
R8	Half strength	3+0	8.3	72.22 (58.46)	6.3	3.25
R9	Half strength	4+0	16.6	61.11 (51.49)	5.7	2.14
R10	Half strength	0+4	10.6	88.89 (73.94)	10.3	2.67
R11	Half strength	0+5	10.3	77.78 (62.18)	9.6	2.42
R12	Half strength	0+6	9.6	94.44 (81.97)	13.4	2.87
R13	Full Strength	2+4	15.6	38.89 (38.51)	5.4	1.52
R14	Full Strength	2+5	13.6	55.56 (48.25)	6.8	1.76
R15	Full Strength	2+6	10.3	66.67 (55.21)	9.4	2.47
R16	Full Strength	3+4	20.3	16.67 (19.79)	2.2	1.20
R17	Full Strength	3+5	18.6	22.22 (27.82)	2.6	1.45
R18	Full Strength	3+6	24.6	11.11 (16.06)	1.6	0.58
R19	Full Strength	4+4	14.3	50.00 (45.00)	6.3	1.73
R20	Full Strength	4+5	11.6	61.11 (51.49)	7.6	2.23
R21	Full Strength	4+6	16.3	44.44 (41.75)	5.4	1.48
R22	Half strength	2+4	22.3	16.67 (24.09)	1.3	0.53
R23	Half strength	2+5	21.6	22.22 (27.82)	1.8	1.24
R24	Half strength	2+6	20.3	38.89 (38.51)	2.5	1.64
R25	Half strength	3+4	13.6	50.00 (45.00)	5.6	1.87
R26	Half strength	3+5	11.3	55.56 (48.72)	7.3	2.43
R27	Half strength	3+6	13.3	44.44 (41.75)	6.4	2.00
R28	Half strength	4+4	18.6	22.22 (27.82)	4.2	1.96
R29	Half strength	4+5	17.3	33.33 (34.79)	5.4	2.15
R30	Half strength	4+6	16.3	27.78 (31.54)	5.8	2.34
(±) S.Em			1.121	2.709	0.169	
C.D @ 5%			3.180	7.683	0.479	

Note: Figures in parentheses represent arc sine transformed values

Table 4. Effect of different hardening mixtures on acclimatization of regenerated plantlets.

Treatments	Hardening mixture combination	Mean no. of days taken for acclimatization	Survival percentage
H_1	Coconut peat	-	-
H_2	Vermicompost	45	50 (45.00)
H_3	Pressmud	-	-
H_4	Coconut peat:Soil:Sand (1:1:1)	42	50 (45.00)
H_5	Vermicompost:Soil:Sand (1:1:1)	35	86 (68.04)
H_6	Pressmud:Soil:Sand (1:1:1)	52	25 (29.98)
(±) S.Em		1.012	0.836
C.D @ 5%		3.790	2.603

Note: Figures in parentheses represent arc sine transformed values

Fig. A Induced axillary shoots on MS medium + 2 mg l^{-1} BAP + 1 mg l^{-1} IAA + 3 mg l^{-1} Kn

Fig. B Established axillary shoots on MS medium + 2 mg l^{-1} BAP + 1 mg l^{-1} IAA + 3 mg l^{-1} Kn

Fig. C Multiple shoot induction on MS medium + 3 mg l^{-1} BAP + 1 mg l^{-1} NAA + 3 mg l^{-1} Kn

Fig. D Elongated shoots on MS medium + 3 mg l^{-1} BAP + 1 mg l^{-1} NAA + 3 mg l^{-1} Kn

Fig. E Root initiation on Half MS medium + 6 mg l^{-1} NAA

Fig. F Well developed roots on Half MS medium + 6 mg l^{-1} NAA

Fig. G Hardened Plantlets on vermicompost + soil + sand (1:1:1)

35 days of minimum period. The better survival of the plantlets in the treatment combination may be attributed to the presence of all nutrients including minor nutrients in vermi compost. Significant differences were observed between the treatments.

In contrast Sreenivasan and Sreenivasan (1992) and Purushothaman *et al.,* (2000) observed 90 percent of survival with sand, silt and pressmud in a ratio of 1:1:1. Whereas Singh *et al.,* (2001) and Ali *et al.,* (2008) carried out hardening with sieved mixture of soil, sand and cocopeat (1:1:1) and observed the best survival rates.

By and large, the present studies reveals that the shoot tip explant of 2005T50 could be well established with full strength solid MS medium supplemented with 2.0 mg l-1 BAP, 1.0 mg l-1 IAA and 3.0 mg l-1 Kinetin. The established explants can also be efficiently subcultured with full strength solid MS medium supplemented with 3.0 mg l-1 BAP, 1.0 mg l-1 NAA and 3.0 mg l-1 Kinetin. Similarly, the rooting can be best achieved in half strength solid MS medium supplemented with 6.0 mg l-1 NAA alone. The plantlets with well developed shoot and roots were acclimatized on hardening mixture of vermicompost + soil + sand (1:1:1), that showed 86 per cent survivability (Fig.G).

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