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Original Article

OPTIMIZATION OF *IN VITRO* REGENERATION PROTOCOL FOR TOMATO (*SOLANUM LYCOPERSICON* MILL.) CV. JUNAGADH TOMATO-3

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Abstract: ***The study was undertaken during the year 2018-2019 in Department of Biotechnology Junagadh Agricultural University. The objective of study was to standardize the in vitro regeneration protocols of tomato using cotyledons as source of explant material. This study was carried out as a pre-requisite for the genetic transformation studies for herbicide resistance in tomatocv. Junagadh Tomato-3 (JT-3), which is extensively grown in Saurashtra region of Gujarat. In vitro regeneration is genotype specific hence efforts being taken to optimize the methodology before going for genetic transformation study. Effects of the plant growth regulators (PGRs) like1-Naphthalene-Acetic Acid (NAA), Indole-3-Acetic Acid (IAA) in combination with Benzyl Amino Purine (BAP) and Zeatin at different combinations were investigated on callus induction and regeneration frequency. Results showed that among the combinations of PGRs tested, MS media augmented with 2.5mg l^{-1}BAP + 0.5mg $l^{-1}$of NAA had better result for callus development and regeneration (59.78%) whereas 0.1mg l^{-1}NAA had significant effect on root induction (95%). It was also noticed that combinations of Zeatin with NAA and IAA preferably induce direct regeneration but the number of shoots per ex-plant was significantly low. Regenerated plantlets were further transferred to net house for hardening and acclimatization. Hence it was concluded from this study that different PGRs have distinct response on callus development and regeneration frequency, even minor change in concentrations of PGRs gave dissimilar result.***

Keywords: ***In vitro*** **regeneration, Tomato**

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INTRODUCTION

Tomato (*Solanum lycopersicon* L.) formerly (*Lycopersicume sculentum* Mill.) is a member of Solanaceae family. It is an herbaceous and perennial plant mostly grown intemperate region but also cultivated in different agro-climatic conditions under controlled environment [1-3]. It has great economic importance, stand second in vegetable crops after potato [4]. India account for over 21 million metric tons of production and the leading producers are, Andhra Pradesh, Madhya Pradesh and Karnataka [5]. It is loaded with minerals and bioactive compounds such as alpha- and beta-carotene, lutein, and lycopene which have profound health benefits [6,7]. Short life cycle, herbaceous nature, and simple genomic arrangement of tomato offers advantages to studyas model plant for other solanaceous crops [8,9]. Tomato is modified for various traits such as suitability for adverse environment conditions either biotic and abiotic [10-14] agronomic traits [15,16] and for consumer acceptance etc.

In vitro regeneration technique of plants is inevitable and important tool in biotechnology for genetic transformation study which facilitate introduction of new traits quite efficiently [17]. Standardization of physical (i.e. culture conditions) and biochemical components such as ingredients of MS salts and PGRs for better *in vitro* regeneration of tomato is still an empirical process [18]. The frequency of regeneration in *in vitro* conditions had been found to be controlled by various factors such as genotype, nutrient media, concentrations and combinations of plant growth regulators, explant type, temperature and light [19-24). Numerous studies have been conducted on tomato plant regeneration from different tissues and organs as ex-plant, including leaves, cotyledons, hypocotyl and shoot tip [25,26]. A number of tomato cultivars/genotypes have been scrutinized for their ability of callogenesis and shoot induction in earlier studies [27,28]. Durzan [29] found that different ex-plants have varied degree of response on MS media in the order of leaves, cotyledons and hypocotyls, whereas Plastira and Perdikaris [30] reported in the order of hypocotyl, cotyledon and leaves. However, most researches preferred cotyledonary ex-plant in tomato regeneration over any other ex-plant. *In vitro* regeneration as a prerequisite for plant transformation, it is desirable to regenerate plants by direct regeneration rather than indirect organogenesis not only to saves time but also excludes undesirable somaclonal variations associated with multiple sub-culturing and maintaining callus for long period on culture [31]. Various combinations of plant growth regulators are used to induce callus and adventitious shoots. The most widely used cytokinins for *in vitro* regeneration of shoots from tomato explants are 6-benzylaminopurine (BAP), zeatin, kinetin (Kn) and thidiazorun (TDZ) [32,33]. The concentration of growth regulators employed is dependent on the cultivar being cultured and the particular cytokinin or auxin being employed [25].

Since it was well documented that, cotyledon ex-plant has high regeneration and transformation frequency. Hence in present study we utilized cotyledon as source of ex-plant material to optimize the protocol for the regeneration of tomato cultivar Junagadh Tomato-3 as pre-requisite of transformation. Murashige and Skoog [34] basal media supplemented with different plant growth regulators chosen for standard plant regeneration and rooting, that had the potential to speed up plant development.

MATERIALS AND METHODS

Seeds and plant materials: The present investigations were conducted during period of October 2018 to December 2019 at Department of Biotechnology, Junagadh Agricultural University, Junagadh (70° 27' 23"E longitude and 21° 30' 55"N latitude) in Western Gujarat (Saurashtra region), India. Seeds of Junagadh Tomato-3 (developed by pure line selection), were obtained from Vegetable Research Station, JAU, Junagadh.

***In vitro* seed germination:** Tomato seeds were dipped in water containing 0.1% carbendazim (a systemic, broad-spectrum fungicide) for 10 min with occasional shaking. Those seeds floating on surface were removed and traces of fungicide removed by washing under running tap water. Seeds were surface sterilized using 70% (v/v) ethanol for 30 sec, followed by 0.1% (w/v) Mercuric chloride ($HgCl_2$) for 4 min, each treatment was immediately followed by washing with autoclaved distilled water for 3-4 times to remove any traces of ethanol and $HgCl_2$. Seeds were blotted on sterile tissue paper to remove water adhered on the surface of seeds and further 4-5 seeds were transferred in each test tube filled with half strength of basal MS media [34]. The medium was adjusted to pH 5.7±0.1 and solidified with 0.4% agar prior to autoclaving at 121ºC and 15 psi for 15 min. The

cultured seeds were incubated at 25±2ºC for 3 days in dark, then transferred to 16 h light/8 h dark photoperiodic cycle. Incubated seeds were regularly monitored for any kind of bacterial and fungal contamination.

***In vitro* shoot regeneration:** After 15-18 days well developed and fully expanded cotyledon explnts were harvested from germinated seedlings, placed on sterile petri dish and cut into 0.5-1.0 cm size with the help of sterile scalpel blade. Explants were inoculated on shoot regeneration media so that the abaxial surface touches the media. The shoot regeneration media formulated by augmenting basal MS media with different concentration and combination of cytokinins and auxins (Tables 1,2). Cultured plates were incubated at 25±2ºC under a 16 h light/8 h dark photoperiod. Explants with regenerated shoots were sub-cultured once after two to three weeks according to visual observations and regularly monitored for growth and development. Around 25 cotyledon explants were cultured in disposable petri plates. For each concentration three replications were used and analyzed statistically using completely randomized design.

Multiplication and elongation of shoots: The same growth regulators are used for shoot multiplication and elongation (Tables 1,2).

***In vitro* rooting and development of complete plantlets:** The regenerated shoots obtained from cotyledon explants were transferred to root induction medium having half strength of MS basal medium in combination with various concentrations of different auxins (IAA and NAA) (Table 3) for root regeneration to get complete plantlets.

Hardening and acclimatization of *in vitro* regenerated plantlets: After proper development of roots, the plantlets were taken out of the culture bottles in such a way that no damage was caused to their root system. The roots were washed gently under running tap water to remove adhering medium and kept in beaker containing Hoagland solution. Plantlets were transferred to plastic cups filled with pre-sterilized potting mixture (equal proportion of sandy soil and cocopeat), watered with 0.5% carbendazim solution and covered with perforated plastic bag to maintain relative humidity (RH). Plantlets were placed inside green house for further development. After 20 days the polythene bags were removed and plants were exposed to direct sunlight for two hours a day. Lastly the plants were placed in natural environment.

The regeneration and rooting percentage of explants will be calculated as follows.

Regeneration response (%) = (Number of explants showing@ regeneration response) / (Total number of explants @inoculated) X 100

Rooting (%) =
(Total No.of shoot that @produce healthy roots)
/ (Total no. shoots transferred@ to rooting medium) X 100

RESULTS AND DISCUSSION

Effect of BAP and IAA on shoot regeneration in tomato (*Solanum lycopersicum*) JT-3: The effect of different levels of BAP and IAA on *in vitro* shoot regeneration is shown in (Table 1). Both BAP and IAA had significant interaction and exhibited varied response on the cotyledon ex-plants of tomato. The highest shoot regeneration (50.01%) and number of shoots per explant(1.55) was obtained on medium containing 2.0 mg l^{-1} of BAP and 0.5 mg l^{-1} of IAA (BI15) in compare to the other combinations (Table 4). Among the different treatments, BI5, BI6, BI10 were statistically significant with BI15. The lowest shoot regeneration (18.42%) was obtained on media containing 0.5 mg l^{-1} of BAP and 0.0 mg l^{-1} of IAA (BI2) whereas lowest number of shoots per explant (0.36) was reported on media containing 1.5 mg l^{-1} of BAP and 0.1 mg l^{-1} of IAA (BI4). Lowest was recorded in control which is devoid of any growth regulator.

Effect of BAP and NAA on shoot regeneration in tomato (*Solanum lycopersicum*) JT-3: Study of different combinations and concentrations of BAP and NAA on *in vitro* regeneration of shoots are depicted in (Table 1). The combined effect of BAP and NAA had noteworthy interaction and exhibited varied response on the cotyledon ex-plants of tomato. MS media supplemented with 2.5 mg l^{-1} of BAP and 0.5 mg l^{-1} of NAA (BI16) showed highest shoot regeneration (59.78%) and number of shoots per explant (0.92) in compare to the other combinations (Table 5). The lowest shoot regeneration (17.82%) was reported on media containing 0.5 mg l^{-1} of BAP and 0.0 mg $l^{-1}$of NAA (BN2) whereas least number of shoots per explant was reported on media containing 1.5 mg l^{-1} of BAP and 0.2 mg $l^{-1}$of NAA (BI9).

Effect of zeatin and IAA on shoot regeneration

Table 1: Different combinations and concentrations of BAP along with IAA and NAA used in MS medium for shoot regeneration from cotyledon explant of tomato (*Solanumlycopersicum*) JT-3

Sl. No.	Medium composition				Medium composition			
	Media Code	Basal MS media	BAP (mg l^{-1})	IAA (mg l^{-1})	Media Code	Basal MS media	BAP (mg l^{-1})	NAA (mg l^{-1})
1	BI1	MS	0.0	0.0	BN1	MS	0.0	0.0
2	BI2	MS	0.5	0.1	BN2	MS	0.5	0.1
3	BI3	MS	1.0	0.1	BN3	MS	1.0	0.1
4	BI4	MS	1.5	0.1	BN4	MS	1.5	0.1
5	BI5	MS	2.0	0.1	BN5	MS	2.0	0.1
6	BI6	MS	2.5	0.1	BN6	MS	2.5	0.1
7	BI7	MS	0.5	0.2	BN7	MS	0.5	0.2
8	BI8	MS	1.0	0.2	BN8	MS	1.0	0.2
9	BI9	MS	1.5	0.2	BN9	MS	1.5	0.2
10	BI10	MS	2.0	0.2	BN10	MS	2.0	0.2
11	BI11	MS	2.5	0.2	BN11	MS	2.5	0.2
12	BI12	MS	0.5	0.5	BN12	MS	0.5	0.5
13	BI13	MS	1.0	0.5	BN13	MS	1.0	0.5
14	BI14	MS	1.5	0.5	BN14	MS	1.5	0.5
15	BI15	MS	2.0	0.5	BN15	MS	2.0	0.5
16	BI16	MS	2.5	0.5	BN16	MS	2.5	0.5

Table 2: Different combinations and concentrations of Zeatin along with IAA and NAA used in MS medium for shoot regeneration from cotyledon explant of tomato (*Solanumlycopersicum*) JT-3

Sl. No.	Medium composition				Medium composition			
	Media Code	Basal MS media	Zeatin (mg l^{-1})	IAA (mg l^{-1})	Media Code	Basal MS media	Zeatin (mg l^{-1})	NAA (mg l^{-1})
1	ZI1	MS	0.0	0.0	ZN1	MS	0.0	0.0
2	ZI2	MS	0.5	0.1	ZN2	MS	0.5	0.1
3	ZI3	MS	1.0	0.1	ZN3	MS	1.0	0.1
4	ZI4	MS	1.5	0.1	ZN4	MS	1.5	0.1
5	ZI5	MS	2.0	0.1	ZN5	MS	2.0	0.1
6	ZI6	MS	2.5	0.1	ZN6	MS	2.5	0.1
7	ZI7	MS	0.5	0.2	ZN7	MS	0.5	0.2
8	ZI8	MS	1.0	0.2	ZN8	MS	1.0	0.2
9	ZI9	MS	1.5	0.2	ZN9	MS	1.5	0.2
10	ZI10	MS	2.0	0.2	ZN10	MS	2.0	0.2
11	ZI11	MS	2.5	0.2	ZN11	MS	2.5	0.2
12	ZI12	MS	0.5	0.5	ZN12	MS	0.5	0.5
13	ZI13	MS	1.0	0.5	ZN13	MS	1.0	0.5
14	ZI14	MS	1.5	0.5	ZN14	MS	1.5	0.5
15	ZI15	MS	2.0	0.5	ZN15	MS	2.0	0.5
16	ZI16	MS	2.5	0.5	ZN16	MS	2.5	0.5

Table 3: Composition of root regeneration media having various concentrations of IAA and NAA

Sl. No.	Media Code	IAA (mg l^{-1})	Media Code	NAA (mg l^{-1})
1	RI1	½ MS basal (control)	RN1	½ MS basal (control)
2	RI2	0.1	RN2	0.1
3	RI3	0.5	RN3	0.5
4	RI4	1.0	RN4	1.0
5	RI5	1.5	RN5	1.5
6	RI6	2.0	RN6	2.0

in tomato (*Solanum lycopersicum*) JT-3: The effect of various levels of zeatin and IAA was studied for *in vitro* shoot regeneration in tomato, the different concentration of zeatin and IAA used listed in (Table 2). Both zeatin and IAA had significant interaction and exhibited varied response on the cotyledon explants. The highest shoot regeneration (46.50%) was obtained on medium containing 2.0 mg l^{-1} of zeatin and 0.2 mg l^{-1} of IAA (ZI10) and highest number of shoots per explant (1.22) was obtained in combination of 2.5 mg l^{-1} of zeatin and 0.5 mgL^{-1} of IAA (ZI16) (Table 6). The lowest shoot regeneration (18.62%) was obtained on media containing 0.5 mg l^{-1} of zeatin and 0.0 mg l^{-1} of IAA (ZI2) whereas lowest number of shoots per explant (0.58) was reported on media containing 1.0 mgL^{-1} of zeatin and 0.2 mg l^{-1} of IAA (ZI8).

Table 4: Effect of different level of BAP and IAA on shoot regeneration from cotyledon explants in tomato(JT-3)

Sr. No.	Media Code	BAP (mg l^{-1})	IAA (mg l^{-1})	Callus formation	Average number of shoots per explant	Per cent shoot regeneration
1	BI1	0.0	0.0	-	0.00	0.00(0.00)
2	BI2	0.5	0.0	+	1.13	18.42(9.98)
3	BI3	1.0	0.1	+	1.44	32.89(29.49)
4	BI4	1.5	0.1	+	0.36	42.73(46.04)
5	BI5	2.0	0.1	+	0.82	46.29(52.25)
6	BI6	2.5	0.1	+	1.36	46.40(52.45)
7	BI7	0.5	0.2	+	1.02	28.12(22.21)
8	BI8	1.0	0.2	+	1.00	32.33(28.61)
9	BI9	1.5	0.2	+	1.13	38.38(38.54)
10	BI10	2.0	0.2	+	0.93	44.55(49.21)
11	BI11	2.5	0.2	+	1.19	40.74(42.59)
12	BI12	0.5	0.5	+	0.66	27.43(21.22)
13	BI13	1.0	0.5	+	0.89	36.79(35.86)
14	BI14	1.5	0.5	+	1.08	40.64(42.42)
15	BI15	2.0	0.5	+	1.55	50.01(58.71)
16	BI16	2.5	0.5	+	0.99	38.73(39.15)
SEm±						2.37
CD (p=0.05)						6.83

Table 5: Effect of different combinations of BAP and NAA on shoot regeneration from cotyledon explants in tomato (JT-3)

Sr. No.	Media Code	BAP (mg l^{-1})	NAA (mg l^{-1})	Callus formation	Average number of shoot per explant	Per cent shoot regeneration
1	BN1	0.0	0.0	-	0.00	0.0(0.00)
2	BN2	0.5	0.0	+	0.82	17.82(9.37)
3	BN3	1.0	0.1	+	0.87	31.11(26.70)
4	BN4	1.5	0.1	+	0.80	39.23(40.01)
5	BN5	2.0	0.1	+	0.79	42.28(45.26)
6	BN6	2.5	0.1	+	0.89	49.62(58.03)
7	BN7	0.5	0.2	+	0.76	29.76(24.63)
8	BN8	1.0	0.2	+	0.58	34.03(31.31)
9	BN9	1.5	0.2	+	0.42	39.84(41.05)
10	BN10	2.0	0.2	+	0.54	43.61(47.58)
11	BN11	2.5	0.2	+	0.68	45.19(50.33)
12	BN12	0.5	0.5	+	0.91	26.74(20.25)
13	BN13	1.0	0.5	+	0.84	32.72(29.21)
14	BN14	1.5	0.5	+	0.65	39.98(41.28)
15	BN15	2.0	0.5	+	0.84	55.14(67.33)
16	BN16	2.5	0.5	+	0.92	59.78(74.67)
SEm±						2.92
CD (p=0.05)						8.42

Table 6: Effect of different level of Zeatin and IAA on shoot regeneration from cotyledon explants in tomato (JT-3)

Sr. No.	Media Code	Zeatin (mg l^{-1})	IAA (mg l^{-1})	Callus formation	Average number of shoot per explant	Per cent shoot regeneration
1	ZI1	0.0	0.0	-	0.00	0.00(0.00)
2	ZI2	0.5	0.0	+	1.02	18.62(10.19)
3	ZI3	1.0	0.1	+	1.12	26.82(20.36)
4	ZI4	1.5	0.1	+	0.73	44.60(49.30)
5	ZI5	2.0	0.1	+	0.78	45.50(50.88)
6	ZI6	2.5	0.1	+	0.61	46.02(51.78)
7	ZI7	0.5	0.2	+	1.05	32.24(28.46)
8	ZI8	1.0	0.2	+	0.58	39.69(40.78)
9	ZI9	1.5	0.2	+	0.91	42.28(45.26)
10	ZI10	2.0	0.2	+	0.73	46.50(52.62)
11	ZI11	2.5	0.2	+	0.87	41.55(44.00)
12	ZI12	0.5	0.5	+	0.85	29.81(24.72)
13	ZI13	1.0	0.5	+	0.77	30.26(25.39)
14	ZI14	1.5	0.5	+	0.92	43.25(46.95)
15	ZI15	2.0	0.5	+	1.04	42.14(45.01)
16	ZI16	2.5	0.5	+	1.22	41.39(43.71)
SEm±						2.13
CD (p=0.05)						6.13

Table 7: Effect of various combinations of Zeatin and NAA on shoot regeneration from cotyledon explants in tomato (JT-3)

Sr. No.	Media Code	Zeatin (mg l^{-1})	NAA (mg l^{-1})	Callus formation	Average number of shoot per explant	Per cent shoot regeneration
1	ZA1	0.0	0.0	+	0.0	4.05(0.50)
2	ZA2	0.5	0.0	+	0.88	14.63(6.38)
3	ZA3	1.0	0.1	+	0.94	30.88(26.34)
4	ZA4	1.5	0.1	+	0.89	44.17(48.55)
5	ZA5	2.0	0.1	+	0.97	50.53(59.60)
6	ZA6	2.5	0.1	+	0.95	41.09(43.19)
7	ZA7	0.5	0.2	+	0.89	25.59(18.66)
8	ZA8	1.0	0.2	+	0.86	33.99(31.26)
9	ZA9	1.5	0.2	+	0.53	39.36(40.22)
10	ZA10	2.0	0.2	+	0.63	46.96(53.42)
11	ZA11	2.5	0.2	+	0.68	48.57(56.21)
12	ZA12	0.5	0.5	+	0.76	26.83(20.37)
13	ZA13	1.0	0.5	+	0.71	29.73(24.60)
14	ZA14	1.5	0.5	+	0.80	38.54(38.82)
15	ZA15	2.0	0.5	+	0.84	46.67(52.92)
16	ZA16	2.5	0.5	+	0.69	41.08(43.18)
SEm±						2.65
CD (p=0.05)						7.64

Table 8: Effect of IAA on root induction

Sl. No.	Media Code	IAA (mg l^{-1})	Percent root induction
1	RI1	Half strength of MS media	27
2	RI2	0.1	90
3	RI3	0.5	73
4	RI4	1.0	77
5	RI5	1.5	64
6	RI6	2.0	71

Table 9: Effect of NAA on root induction

Sl. No.	Media Code	NAA (mg l^{-1})	Percent root induction
1	RN1	Half strength of MS media	32
2	RN2	0.1	95
3	RN3	0.5	89
4	RN4	1.0	72
5	RN5	1.5	83
6	RN6	2.0	68

Effect of zeatin and NAA on shoot regeneration in tomato (*S. lycopersicum*) JT-3: The effect of different combinations and concentrations of zeatin and NAA on *in vitro* regeneration of tomato shoots are shown in (Table 2). The combined effect of zeatin and NAA had significant interaction and exhibited varied response on the cotyledon ex-plants. The highest shoot regeneration (50.53%) and number of shoots per explant was found on medium containing 2.0 mg l^{-1} of zeatin and 0.1 mg l^{-1} of NAA (ZA5) in compare to the other combinations (Table 7). The lowest shoot regeneration (14.63%) was reported on media containing 0.5 mg l^{-1} of zeatin and 0.0 mg $l^{-1}$of NAA (ZA2) whereas least number of shoots per explant (0.53) was reported on media containing 1.5 mgL^{-1} of zeatin and 0.2 mgL^{-1} of NAA (ZA9).

From the above study it was concluded that among the different combination and concentration of auxins and cytokinins used, BAP (2.5mg l^{-1}) and NAA (0.5mg l^{-1}) had significantly positive result with respect to callus morphology, number of shoots per explant and percent shoot regeneration. It was also observed that although BAP (2.0mg l^{-1}) and IAA (0.5mg l^{-1}) had higher number of shoot per explant (1.55) compare to 2.5mg l^{-1}BAP +0.5mg l^{-1}NAA (0.92) but texture of callus was better in later combinations *i.e*. less friable and greenish in nature and appearance. Zeatin as cytokininin combination with IAA and NAA favour direct shoot regeneration with little callogenesis and percent shoot regeneration was less.

The previous reports show that the callus formed in the medium having the equimolar concentration of auxin (IAA) and cytokinins (BAP) had good and faster response [35], which found to be contrary to our findings. Present findings showed that callogenesis can be induced when higher concentration of

cytokinins was used in compared to auxin [36]. The auxin/cytokinin ratio of (1/5), used in the present study (2.5 mg l^{-1} BAP+ 0.5 mg l^{-1} NAA) significantly increased the callus induction and with higher frequency. Treatments in which neither of the PGRs added show little callogenesis but there was no significant shoot regeneration occurs. It indicated that the presence of cytokinins is essential for shoot organogenesis. Gubis et al. [20] asserted that, the presence of high cytokinin without or with equal amount of auxins was necessary for *in vitro* regeneration.

The primary mode of regeneration is shoot organogenesis, which can be obtained directly or indirectly from explants [21, 35]. Most of the reports about shoot organogenesis in tomato are related to the induction of regeneration from hypocotyls or cotyledon explants [37, 38].

Effect of different concentrations of IAA on root initiation from *in vitro* developed shoots

Shoots regenerated from calli of cotyledon explants were excised with sharp scalpel blade and cultured on half strength of MS media supplemented with different concentrations of IAA. Single intact shoot was cut out from the rest of the callus. Regeneration of roots started within two week of inoculation. Very profuse and cottony type of rooting was observed. Basal portion of shoot showed little callogenesis. Almost in all concentration rooting was achieved, but first of all it was observed on 0.5mg l^{-1} IAA (Table 8), followed by in other concentration.

Effect of different concentrations of NAA on root initiation from *in vitro* developed shoots: *In vitro* regenerated shoots were removed from the cluster of shooting callus and transferred on half strength MS medium contain different concentrations of NAA. Root initiation started within two week of culturing. All regenerated shoots showed root initiation first on 0.1mg l^{-1} NAA (Table 9). After one and half month well developed fibrous roots were developed.

Root induction: In this study we found that half strength of MS media supplemented with 0.1mg l^{-1} NAA has better rhizogenic effect (root induction ability) than the other treatments. [39] reported that roots induction occurred in regenerated shoots when MS media supplemented with various combination of NAA, their result showed 0.1 mg l^{-1} NAA showed profuse rooting. In contrast [40] reported that, tomato does not require any exogenous plant growth regulators. There are some reports which confirm that tomato has sufficient endogenous auxin for root induction [18,41]. Root formation took place within second week after culturing on the rooting media. There was very less significant difference observed between IAA and NAA with respect to root induction, but the best root induction 95% and 89% was observed on ½ MS medium supplemented with NAA at concentration of 0.1 and 0.5mg l^{-1} respectively.

Hardening and acclimatization of *in vitro* regenerated plantlets of tomato (*S. lycopersicum*) JT-3: The percentage of survived plantlets after transferring to potting mixture was almost 100%. All plants reach reproductive stage of flowering and fruiting. Some researchers, who worked on tomato regeneration have also reported the good response of plantlets to acclimation [18,35,42]. From the present investigation it was concluded that a dedifferentiated propagation route *via* de novo shoots development in *Solanum lycopersicum* L. var. Junagadh Tomato-3 could be used for large scale multiplication and genetic transformation techniques.

From this study, optimization of regeneration protocol of tomato cotyledon leaves, it was found that cotyledon explants have distinct response on different combination and concentration on regeneration capacity. Combination of BAP:IAA and BAP:NAA showed regeneration preferably through callogenesis whereas zeatin:IAA and zeatin:NAA showed direct regeneration response. BAP as cytokinin induced higher number of shoot regeneration as compare to zeatin in tomato (JT-3)

Conclusion: The shoot induction media supplemented with 2.5mg l^{-1} BAP and 0.5mg l^{-1} NAA was most effective for shoot regeneration whereas ½ MS media supplemented with 0.1mgL^{-1} NAA showed better root induction. The study signified the differential *in vitro* indirect regeneration of the investigated tomato

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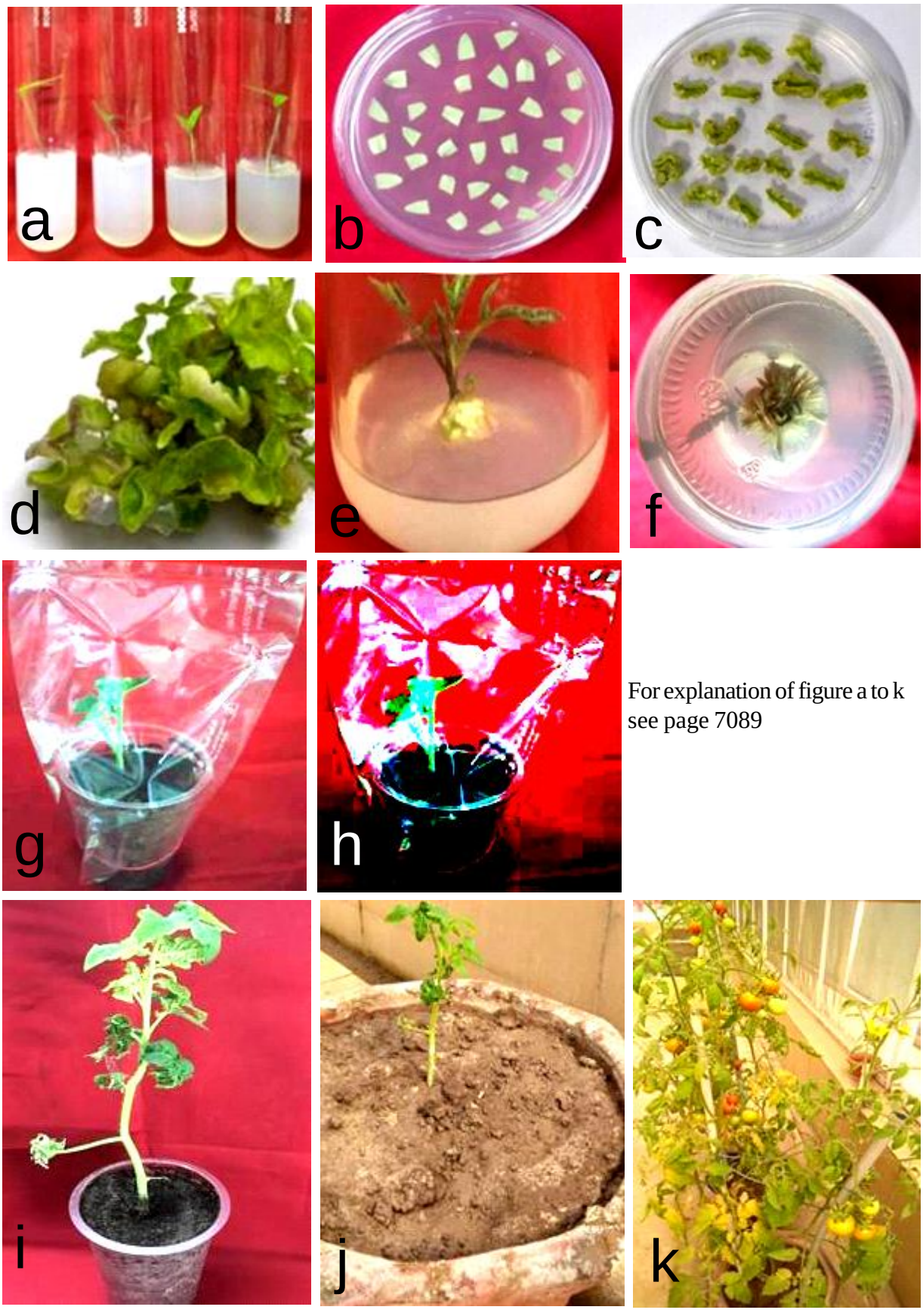

For explanation of figure a to k see page 7089

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Explanation of figures

Fig. a) 15 Days old *In vitro* germinated tomato seedlings; b) Cotyledon ex-plants cultured on shoot regeneration medium (MS + 2.5mg l^{-1} BAP + 0.5mg l^{-1} NAA); c) Callogenesis and regeneration after two week of re-culturing; d) Multiple shoot regeneration from cotyledon explants on shoot regeneration medium (MS + 2.5mg l^{-1} BAP + 0.5mg l^{-1} NAA); e) Elongation of *in vitro* regenerated shoots; f) Root initiation in *in vitro* developed shoots; g) *In vitro* regenerated tomato plants with well-developed root system; h-i) Planting of *in vitro* regenerated plantlet on potting mixture for hardening; j) Transplanting of regenerated plants in earthen pot for growth and development; k) Flowering and fruiting in *invitro* regenerated tomato plants.

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