

Effect of Cultivar and Explants Type on Tissue Culture Regeneration of Three Nigerian Cultivars of Tomatoes

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Abstract

In order to assess the suitable explant(s) for *in-vitro* regeneration of three local cultivars of Nigerian tomatoes, Ibadan local (IbL), Ife and JM94/46, cotyledon, hypocotyls and radicle explants were cultured in shoot regeneration medium consisting of MS containing 30 g L⁻¹ sucrose and 8 g L⁻¹ agar with no exogenous plant growth hormones. Forty-five of each explant type was cultured on the medium in triplicate experiments and results showed varied percentage survival and shooting for the various explants. Hypocotyl explants had the highest percentage of shooting explants at 13.3% for IbL; 6.67% for Ife and 20% in JM94/46. IbL cotyledon explants had 4.44% of shooting explants with no shoots recorded in Ife and JM94/46 cotyledon explants. IbL radicle explants had 2.22% shooting explants and no shoots recorded in Ife and JM94/46. Student Newman Keuls (SNK) statistical analysis of cultivar-media interaction showed there was no significant difference ($P > 0.05$) among the three cultivars in number of calli and shooting calli. There was however significant difference among the cultivars in the number of shoots recorded. SNK values for explants-media interaction showed that cotyledon and radicle explants were significantly different ($P < 0.05$) from hypocotyl explants in the number of shoots produced.

Keywords: *Solanum lycopersicon*, Nigerian, cultivar, cotyledon, hypocotyl, radicle

1. Introduction

Tomato (*Solanum lycopersicon* L.) belongs to the family Solanaceae and is the world's most widely cultivated vegetable (Villarael, 1980) with an estimated global production of over 159,023,383 tons (FAOSTAT, 2011). It is a fleshy berry, globular to oblate, smooth or furrowed about 2-15cm in diameter, usually red when ripe, sometimes pink, orange or yellow and many seeded (Van der Vossen & Nono-Womdim, 2004). Tomato is rich in vitamins A and C and fibre; it is also cholesterol-free (Hobson & Davies, 1971) and contains approximately 20-50 mg lycopene/100 g of fruit weight (Kalloo, 1991). Tomato fruits are a significant source of nutrition for substantial portions of the world's human population because this vegetable crop is widely cultivated and consumed extensively as both a fresh vegetable and concentrated processed products. A single small tomato is sufficient to supply about a quarter of the vitamins A and C recommended for humans to consume daily (Hamner & Maynard, 1942; Beecher, 1998).

Nigeria is the largest producer of tomatoes in tropical Africa, accounting for an annual production of 1,504,670 tons (FAOSTAT, 2011). Nigeria's production is about 1.5 million tons out of the estimated annual production of 16.55 million tons in Africa (FAOSTAT, 2011). In Nigeria alone, annual total area of one million hectares is reportedly used for its cultivation (Anonymous, 1989; Bodunde, Erinle, Eruotor, & Amans, 1993). The use of tomato is about 18 percent of the average daily consumption of vegetables in Nigeria (Olayide, Olatunbosun, Idusogie, & Abiagom, 1972). A large volume of the tomatoes consumed in Nigeria are usually transported over long distances from the Northern part of the country to other parts. In Nigeria, as in most other developing countries, efficient storage, packaging, transport and handling techniques are practically non-existent for perishable crops (Babalola, Makinde, Omonona, & Oyekanmi, 2010) resulting in considerable loss of produce.

Local cultivars of tomatoes in Nigeria suffer from a myriad of problems ranging from post harvest losses due to biochemical processes and the humid climate; diseases caused by bacteria, fungi, viruses and nematodes. The introduction of genes that confer these qualities to commercial cultivars by conventional breeding techniques often encounters serious difficulties due to high incompatibility barriers to hybridization (Kaul, 1991). To overcome these problems certain more recent approaches of gene manipulation might be required. Therefore the application of biotechnology to bring about a number of improvements may be necessary in the locally cultivated cultivars such as longer shelf-life, resistance to biotic and abiotic stresses, nutrient enhancement, higher soluble solids, etc.

The development of protocols for *in-vitro* selection can provide new advances for the production of stress tolerant cultivars (Bhatia, Ashwath, Senaratna, & Midmore, 2004). *In-vitro* regeneration of cultivated tomato (*Solanum lycopersicon*) has been a subject of research because of the commercial value of the crop and its potential of amenability to further improvement through genetic engineering strategy (Evans, 1989). Yet, studies on *in-vitro* regeneration of local cultivars of tomato in Nigeria have not been extensive. An efficient tissue culture protocol is an essential pre-requisite for harnessing the advantage of cell and tissue culture for genetic improvement. Efficient plantlet regeneration in tomato was reported from meristems (Mirghis, Mirghis, & Lacatus, 1995), leaf (Padmanabhan, Paddock, & Sharp, 1974; Behki & Lesley, 1976; Kartha, Gamborg, Shyluk, & Constable, 1976; Ajenifujah-Solebo, Isu, Olorode, Ingelbrecht, & Abiade, 2012), stems, anthers (Zamir, Jones, & Kedar, 1980) and hypocotyls (Ohki, Bigot, & Mousseau, 1978).

This study is therefore to assess the *in-vitro* regeneration response of various explant types of three local cultivars of tomatoes, Ibadan local, Ife and JM94/46 which are farmer preferred varieties in the south-western part of Nigeria obtained from the National Institute for Horticultural Research and Training (NIHORT). These cultivars are reported to be resistant to certain diseases and relatively high yielding (Badra, Denton, & Anyim, 1984; Anno-Nyako & Ladunni, 1984).

2. Materials and Methods

Seeds of three local cultivars of tomatoes namely Ibadan local, Ife and JM94/46 were obtained from the National Institute for Horticultural Research and Training (NIHORT), Ibadan, Nigeria. Cotyledon, hypocotyls and radicle explants were taken from each of the cultivars and used for *in-vitro* regeneration studies. Fifteen pieces of the different explant types from each cultivar were cultured on medium in petri-dish and set in complete randomized design (CRD) with three replications. Data was pooled from the experiments and subjected to analysis of variance (ANOVA).

2.1 *In-vitro* Seed Establishment

The tomato seeds were surface sterilized with NaOCl (3.5% v/v) containing a drop of tween 20 for 20 min without an ethanol treatment. The seeds were then rinsed with sterile distilled water at least three times. About 50-100 ml of MS (Moorashige & Skoog, 1962) medium consisting of 30 g L⁻¹ sucrose and 8 g L⁻¹ agar gel without any growth hormones and pH adjusted to 5.8 was filled into culture bottles and sterilized by autoclaving at 121 °C at 15 psi for 15 min. Efficiency of sterilization was ascertained using Bowie Dick auto clave tape which changed from blue to white. The medium was allowed to cool and solidify prior to seed inoculation. Each culture jar was inoculated with ten surface sterilised seeds and were placed in the dark at 25± 2 °C for 3-5 d to germinate and then transferred to growth conditions of 16 h photoperiod with light intensity of 1500 lux for 7-10 d at the same temperature.

2.2 *In-vitro* Regeneration Through Direct Shoot Organogenesis

Cotyledon (5x5 mm²), hypocotyls and radicle (5-6 mm) explants from 10-13 d old *in-vitro* tomato seedlings of Ibadan local (IbL), Ife and JM94/46 cultivars were excised under aseptic conditions and were cultured on shoot regeneration medium (SRM) consisting of MS with 30 g L⁻¹ sucrose and 8 g L⁻¹ agar gel. After about 3-4 weeks in the shoot regeneration medium, regenerated shoots were sub-cultured into the rooting medium (RM) consisting of MS with 15 g L⁻¹ sucrose and 8 g L⁻¹ agar gel and 0.1 mg L⁻¹ NAA. After 10 d in RM, rooted plants were taken to the screen house for hardening.

2.3 *Ex-situ* Seedling Establishment

Rooted plants were transferred to hardening medium consisting of coconut fibre pellets (peat) which were soaked in water for about 3 h to loosen up and vermiculite was added to make the medium sturdy enough to keep the plants upright. The mixture was poured into polythene bags and the rooting plants were transferred into the medium. They were kept in humidity chamber for 2 weeks before planting in soil in pots for another 2 weeks, still under humidity chamber and gradually acclimatized in the screen house environment.

2.4 Statistical Analysis of Regeneration Data

Data was pooled from the regeneration experiments and subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of Statistical Analysis Software (SAS)-(SAS Institute, Cary, NC) (Version 8.2, 2001). Least significant difference (LSD) test was based on Student Neuman Keuls' (SNK) procedure after a significant F-test in the analysis of variance.

3. Result

3.1 Direct Shoot Regeneration

Seeds of cultivar JM94/46 germinated 3 days after planting (d.a.p.) while Ife and IbL germinated about 5d.a.p. For cultivar IbL, only 4.44% of cotyledon explants developed shoots; while no shoot was recorded for Ife and JM94/46 cultivars. IbL survival rate on SRM was 84.44% (Table 1). Only 2 from the surviving thirty-eight cotyledon explants from the initial 45 produced shoots but the shoots produced were not transferred as individual shoots. The rate of survival of Ife and JM94/46 cotyledon explants on the medium was 93.33% and 71.11% respectively, but no shoots were produced.

Table 1. Regeneration of cotyledon explants of the three tomato cultivars

Cultivar	No. of explant	Surviving Explants	% survival	Shooting explant	% shooting explant	No of shoots	Average shoot/explant
IbL	45	38	84.44	2	4.44	0	0
Ife	45	42	93.33	0	0	0	0
JM94/46	45	32	71.11	0	0	0	0

From the 45 hypocotyl explants cultured in triplicate experiments for IbL, Ife and JM94/46 cultivars, only 6, 3 and 9 explants produced shoots respectively (Table 2). JM94/46 had the highest number of shooting hypocotyl explants of 9 from 28 surviving explants. Percentage shooting explants was 13.3, 6.67 and 20 respectively for IbL, Ife and JM94/46. No shoots were produced from Ife; average shoots/explant for IbL and JM94/46 was 2.17 and 2.89 respectively.

Table 2. Regeneration of hypocotyl explants of the three tomato cultivars

Cultivar	No. of explant	Surviving Explants	% survival	Shooting explant	% shooting explant	No. of shoots	Average shoot/explant
IbL	45	26	57.78	6	13.33	13	2.17
Ife	45	27	60	3	6.67	0	0
JM94/46	45	28	62.22	9	20	26	2.89

For radicle explants, only IbL produced one shoot; while Ife and JM94/46 cultivars did not produce any shoots. Percentage survival of radicle explants of the cultivars was 57.7, 48.89 and 71.11 for IbL, Ife and JM94/46 cultivars respectively (Table 3).

Table 3. Regeneration of radicle explants of the three tomato cultivars

Cultivar	No. of explant	Surviving Explants	% survival	Shooting explant	% shooting explant	No of shoots	Average shoot/explant
IbL	45	26	57.78	1	2.22	1	0.026
Ife	45	22	48.89	0	0	0	0
JM94/46	45	32	71.11	0	0	0	0

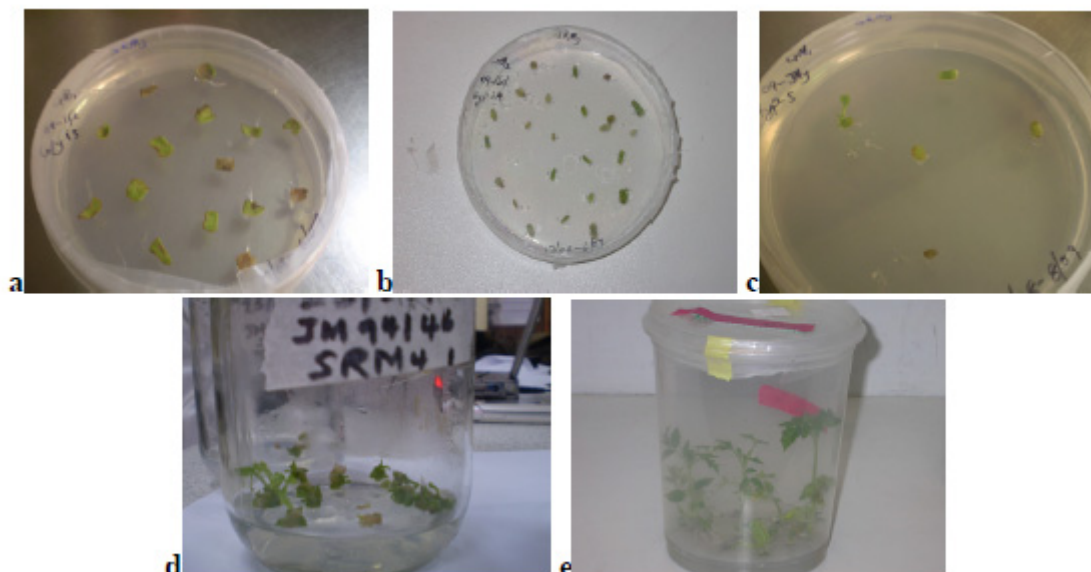


Figure 1. (a) Cotyledon explants (3 wks); (b) Hypocotyl explants (3 wks); Radicle explants (3 wks); (d) Shooting hypocotyl explants (6 wks); (e) Rooting seedlings from hypocotyl explant (8-10 wks)

Student Neuman Keuls (SNK) values for cultivar-media interaction showed that there was no significant difference at $P > 0.05$ in the response of the cultivars for number of calli and shooting calli (Table 4); there was however significant difference ($P < 0.05$) between Ife and JM94/46 in the number of shoots produced. SNK values for explant-media interaction indicated that hypocotyl and radicle explants response were not significantly different ($P > 0.05$) for number of calli formed, but was significantly different from cotyledon. Cotyledon and radicle explants response in the medium for shooting calli and number of shoots produced were not significantly different ($P > 0.05$) while hypocotyls response was significantly different (Table 5).

Table 4. Mean values of SNK test for cultivars-media interaction (cultivar * media)

Cultivar	IbL	Ife	JM94/46
No calli	0.667a	0.674a	0.681a
Shoot calli	0.067a	0.022a	0.067a
Shoots	0.104ab	0.000b	0.193a

Mean values with same letters on the same row are not significantly different at $\alpha = 0.05$.

Table 5. Mean values of SNK test for explants-media interaction (explants * media)

Explants	Cotyledon	Hypocotyl	Radicle
No calli	0.8296a	0.600b	0.593b
Shoot calli	0.0148b	0.133a	0.0074b
Shoots	0.000b	0.289a	0.0074b

Mean values with same letters on the same row are not significantly different at $\alpha = 0.05$.

The Least Significant Difference (LSD) values (Table 6) indicates that there was significant difference ($P < 0.05$) in the survival rate of calli, shooting explants and shoots in SRM. There was significant difference ($P < 0.05$) among the cultivars for no. of shoots produced; while there was no significant difference ($P > 0.05$) in calli formation and shooting explants among the three cultivars. There was significant difference ($P < 0.05$) among the explants in calli formation, shooting explant and shoot formation in the medium.

Table 6. LSD Values for cultivars and explants in SRM at $P \leq 0.05$

Parameter	LSD Values		
	Survive	Cultivar	Explant
Calli	0.0001	0.9654	<0.0001
Shoot explant	<0.0001	0.1459	<0.0001
Shoot	<0.0001	0.0071	<0.0001

P values ≤ 0.05 are not significantly different.

3.2 Rooting and Hardening of Regenerated Shoots

Elongated shoots from IbL and JM94/46 hypocotyl explants were transferred to rooting medium (RM) after 4-6 weeks in SRM. Rooted plants were transferred to hardening medium for 3-4 weeks comprising of 2 weeks in peat and vermiculite; and then planted in soil pots for another 2 weeks under controlled humidity conditions.

4. Discussions

Due to the lack of information on explants to use for regeneration in local tomato cultivars, different parts (cotyledon, hypocotyl and radicle) of tomato seedlings were used as explants in shoot regeneration media (SRM) without exogenous growth hormones to determine the most suitable to these local cultivars. Both roots and cotyledons of young seedlings actively produce PGRs involved in the control of organogenesis (Hicks, 1994). Pozueta-Romero, Houlne, Canas, Schantz, and Chamarro (2001) reported the successful regeneration of tomato and bell peppers on medium without exogenous growth hormones from decapitated explants with radicle, hypocotyl and with or without cotyledon explants.

4.1 Direct Shoot Regeneration

Various researchers have reported the successful use of different explants; hypocotyl (Davis, Breiland, Frear & Secor, 1994; Venkatachalam, Geetha, Priya, Rajaseger, & Jayabalan, 2000); cotyledon (Costa, Nogueira, Otoni & Brommonschenkel, 2000; Sun, Uchii, Watanabe, & Ezura, 2006); radicle, hypocotyls and cotyledon (Pozueta-Romero et al., 2001). The results of this study did not however support the literature as only hypocotyl explants of IbL and JM cultivars responded in SRM without growth hormones and the response of the three different explants of IbL to the medium were significantly different at $P > 0.05$. Average shoots/explant from hypocotyls explants in IbL and JM94/46 in SRM were 2.17 and 2.89 respectively. Only few of the surviving explants produced shoots. These results are comparable to that reported by Pozueta-Romero et al. (2001) of 2.8 shoots per explant in tomato cultivar Rutgers in similar medium with no exogenous growth hormones. JM94/46 hypocotyl explants therefore had the best response in SRM followed by IbL. Cotyledon and radicle explants showed poor response in the medium. Plastira and Perdikaris (1997) reported differential regeneration frequency of various explants in the order hypocotyls > cotyledon > leaf. Gunnay and Rao (1980) also demonstrated preferential regeneration from hypocotyls plants than cotyledon explants; which supports the results of these experiments. Hypocotyl explants from the three cultivars responded at varying degrees in SRM. The superiority of hypocotyls explant derived callus in terms of plantlet regeneration corroborates the findings of Locky (1983).

4.2 Root Induction

Rooting medium according to Sun et al. (2006) with no exogenous plant growth hormone (PGH) was adopted, which is in line with report by Mensuali-Sodi et al. (1995) that tomato does not usually require any (PGR) for rooting. The cultures however did not produce roots after three weeks in the rooting medium. The modification of the rooting medium by the addition of 0.1 mg L^{-1} of NAA (auxin) as reported by Davis et al. (1994) resulted in the development of roots which was observed after 8-10 days. Compton and Veilleux (1991) also reported the use of NAA at concentrations of 0.02 mg L^{-1} to induce rooting from leaf and hypocotyl explants.

5. Conclusions

Results of these experiments show the influence and importance of growth regulators on the number of shoots regenerated from tomato explants (cotyledons, hypocotyls and radicles). Although plants have endogenous growth hormones, they are sometimes required to be supplemented under *in-vitro* conditions to obtain optimal results. This is corroborated by the reports of Jozef, Zuzana, and Zuzana (2004) that the addition of growth regulators in media enhanced the number of shoots regenerated from tomato cotyledons and hypocotyls. The *in-vitro* morphogenic responses of *in-vitro* cultured plant tissues are therefore affected by the different

components of the culture media, especially by concentration of growth hormones. These responses are also dependent on cultivar and explants types. The addition of plant growth hormones to the shoot regeneration medium could therefore enhance shoot regeneration in these cultivars and explants. Other experiments with the same cultivars using cotyledon explants in shoot regeneration medium supplemented with 0.1 mg L⁻¹ IAA and 1 mg L⁻¹ zeatin (Ajenifujah-Solebo et al., 2012) gave higher percentage of shooting (64-97%) from the three cultivars.

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