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Germination and *In Vitro* Regeneration Response of Local Nigerian Tomato Cultivar using Different Explant Sources

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Abstract
***In vitro* regeneration of plants is an important tool in both basic and applied studies as well as commercial applications. It can be of use in overcoming the constraints of tomato production. Assessment of seed sterilization procedures and *in vitro* regeneration of tomato cultivar was attempted using different explant sources. Seed sterilization procedures with and without ethanol treatment and varying duration of application were used while explants were cultured on Murashige and Skoog (MS) medium only and MS media with varying concentrations of cytokinins and fixed concentration of auxin as Regeneration media (RM). Seeds sterilized with 10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 5 min gave the highest germination percentage of 57.33% and percentage contamination of 11.11% compared to seeds sterilized with other disinfection procedures. MS media supplemented with 2 mg/l Kinetin and 0.1 mg/l NAA produced average shoot initiation values of 1.71, 1.57 and 1.57 and average root initiation values of 0.57, 0.00 and 2.85 for cotyledon, hypocotyl and leaf explants respectively. The study showed that the tomato cultivar could be amenable for crop improvement and micro propagation purposes.**

Key words: Seed sterilization, *In vitro* regeneration, explant, cultivar, organogenesis.
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Introduction

In Nigeria, tomato is regarded as the most important vegetable after onions and pepper (Fawusi, 1978). It is a key food and cash crop for many low income farmers in the tropics and an important food component (Prior et al., 1994). However, various biotic and abiotic stresses are often the limiting factors in tomato production.

A viable approach to enhance tomato production in view of these limitations is the development of *in vitro* regeneration of the crop from various explant sources. The advent of *in vitro* plant culture which encompasses cell, tissue, organ and embryo culture for crop improvement may lead to the development of useful somaclonal variants, elimination of plant diseases through meristematic tissue culture technique or serve as a precursor for crop improvement through gene transfer (Singh and Chand, 2003; Sarasan et al., 2011). Contamination of plant tissue cultures by different microorganisms, such as bacteria and fungi, reduces their productivity and can completely prevent their cultivation (Sen et al., 2013). Hence, removing contaminants by disinfection from the surface of the explant is of primary concern (Hartmann et al., 1997).

Different disinfection protocols and *in vitro* cultures have been developed for exotic tomato cultivars but there is paucity of information on sterilization protocols and *in vitro* regeneration responses of local Nigerian tomato cultivars. The aim of this study was to develop an appropriate sterilization procedure and also study the shoot and root regeneration potentials via indirect organogenesis of the Nigerian cultivar used in the experiment.

Materials and Methods

Seeds of tomato cultivar (*Solanum lycopersicum* L. cv. Beske) were washed with running water three times and then surfaced sterilized using different disinfection protocols (Table 1). Sterilized seeds were cultured in glass jars containing MS media (Murashige and Skoog, 1962) supplemented with sucrose at 3% (w/v), pH adjusted to 5.7, solidified with agar at 0.7% (w/v) and incubated in the dark for three days to enhance germination. After three days, glass jars were placed under 16 hours' photoperiod through alternating light and dark illumination technique and maintained at 25±2ºC temperature (Mohamed et al., 2010). For indirect organogenesis on regeneration medium (RM), hypocotyls, cotyledons and leaf pieces were selected as explant sources from the 2-3 weeks old *in vitro* seedlings. The explant sources were excised to uniform sizes, approximately 1 cm in length, avoiding the mid vein for both cotyledons and leaf explants. The hypocotyls were cut into a lower, middle and upper segment and placed horizontally on the medium surface. Cotyledons and leaf pieces explants were cultured with the adaxial surface in contact with the medium (Chaudhry et al., 2007). Basic MS medium with various concentrations of cytokinins [Kinetin and 6-benzylaminopurine (BAP)] at 0.5, 1.0, 1.5 and 2.0 mg/l and a fixed concentration of auxin [Naphthaleneacetic acid (NAA)] at 0.1 mg/l were used as regeneration medium with seven explants per medium. Data collected after 28 days includes percentage germination, number of germinating seeds, number of shoots and number of roots. Explant sources and culture media were factors as the factorial experiment was laid out in a completely randomized design, repeated twice and data was analyzed using statistical analysis software (SAS). Analysis of variance (ANOVA) was carried out and means were separated using Duncan's Multiple Range Test (DMRT) at 5% significance level. Data was transformed using arc sine method (Gomez and Gomez, 1984).

Table 1: List of sterilization procedures used to facilitate *in vitro* seed germination

Treatment	Sterilization Procedure
T1	10% (v/v) solution of sodium hypochlorite for 20 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 10 min.
T2	10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 5 min.
T3	70% ethanol for 5 min, 10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added.

Results

Two weeks after culturing on MS media, optimum germination of 57.33% was obtained from seeds sterilized with 10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 5 min. However, 11% contamination was obtained which was higher than values obtained from other treatments (Table 2). A poor percentage seed germination of 22.67% was obtained from seeds sterilized whose disinfection procedure includes 70% ethanol treatment (Figure 1).

For indirect shoot and root organogenesis on regeneration media (RM), explants cultured on RM4 [MS medium supplemented with Kinetin (2 mg/l) and NAA (0.1 mg/l)] had shoot formation mean values of 1.71, 1.57 and 1.57 for cotyledon, hypocotyl and leaf explants respectively. All explants cultured on RM4 responded positively to shoot formation compared to RM8 [MS medium supplemented with BAP (2 mg/l) and NAA (0.1 mg/l)] which had a shoot formation mean value of 0.43 for cotyledon explants (Table 3). The absence of shoots on RM1 [MS medium with Kinetin (0.5 mg/l) and NAA (0.1 mg/l)] and RM5 [MS medium supplemented with BAP (0.5 mg/l) and NAA (0.1 mg/l)] may have been caused by the low amount of cytokinin

[Kinetin and 6-benzylaminopurine (BAP) at 0.5 mg/l] present in these media while RM9 (MS medium) had no plant growth hormones to facilitate shoot formation.

RM5, RM6 [MS medium supplemented with BAP (1.5 mg/l) and NAA (0.1 mg/l)] and RM1 enhanced root formation from cotyledon, hypocotyl and leaf explants, respectively as mean values of 6.14, 5.57 and 11.57 was observed for root formation respectively.

Table 2: Effect of seed sterilization treatments on germination in MS media

Treatment	Percentage germination (%)	Percentage contamination (%)
T1	50.67	0.00
T2	57.33	11.11
T3	22.67	5.56
LSD (0.05)	13.84	15.70

Means were separated using Fisher's LSD at 5% significance level.
T1: 10% (v/v) solution of sodium hypochlorite for 20 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 10 min.
T2: 10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 5 min.
T3: 70% ethanol for 5 min, 10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added.

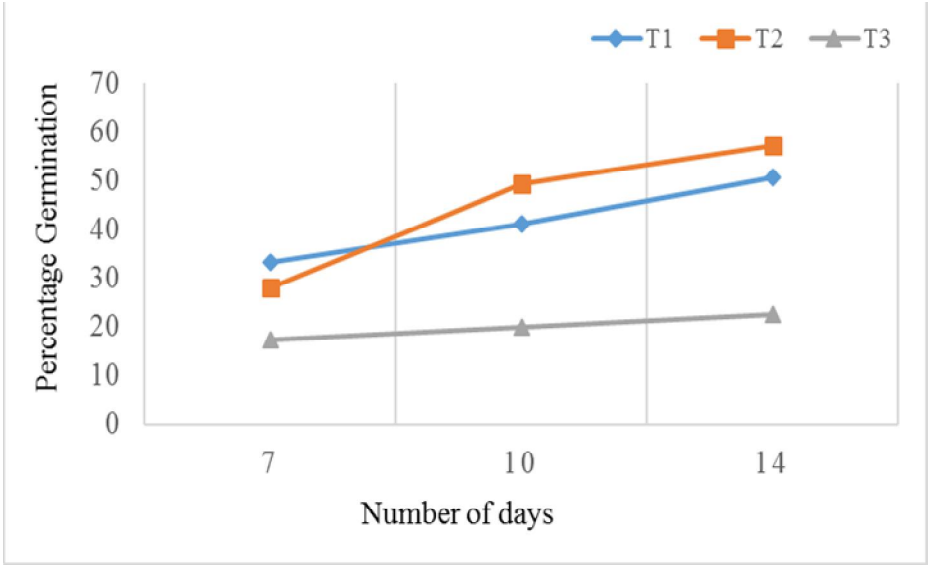

Figure 1: Germination of tomato seeds after treatment with different disinfection procedures

T1: 10% (v/v) solution of sodium hypochlorite for 20 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 10 min.
T2: 10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added without ethanol treatment and repeated for 5 min.
T3: 70% ethanol for 5 min, 10% (v/v) solution of sodium hypochlorite for 10 min with two to three drops of non-ionic surfactant (Tween 20) added.

Table 3: Effect of Kinetin, 6-Benzylaminopurine (BAP) and Naphthaleneacetic acid (NAA) on shoot and root formation.

	Hormone (mg/l)			Cotyledon		Hypocotyl		Leaf	
Regeneration Media (RM)	Kinetin	BAP	NAA	ASN	ARN	ASN	ARN	ASN	ARN
RM1	0.5	0.0	0.1	0.00^{b}	1.29^{b}	0.00^{b}	4.42^{ab}	0.00^{b}	11.57^{a}
RM2	1.0	0.0	0.1	1.14^{ab}	1.29^{b}	0.00^{b}	1.14^{bc}	0.43^{ab}	5.29^{abc}
RM3	1.5	0.0	0.1	0.43^{ab}	1.57^{b}	0.29^{b}	1.14^{bc}	1.86^{a}	2.14^{bc}
RM4	2.0	0.0	0.1	1.71^{a}	0.57^{b}	1.57^{a}	0.00^{c}	1.57^{a}	2.85^{bc}
RM5	0.0	0.5	0.1	0.00^{b}	6.14^{a}	0.00^{b}	3.86^{abc}	0.00^{b}	10.29^{a}
RM6	0.0	1.0	0.1	0.00^{b}	0.57^{b}	0.14^{b}	5.57^{a}	0.00^{b}	11.43^{a}
RM7	0.0	1.5	0.1	0.86^{ab}	2.00^{b}	0.00^{b}	0.86^{bc}	1.57^{a}	8.14^{ab}
RM8	0.0	2.0	0.1	0.43^{ab}	0.86^{b}	0.00^{b}	2.14^{abc}	0.00^{b}	2.71^{bc}
RM9	0.0	0.0	0.0	0.00^{b}	0.00^{b}	0.00^{b}	0.57^{bc}	0.00^{b}	0.00^{c}

ASN = Average Shoot Number
ARN = Average Root Number
Means with the same letter in columns are not significantly different at $p < 0.05$ using Duncan's Multiple Range Test.

Plate 1: Germinating tomato seeds at two weeks after culturing on Murashige-Skoog (MS) media

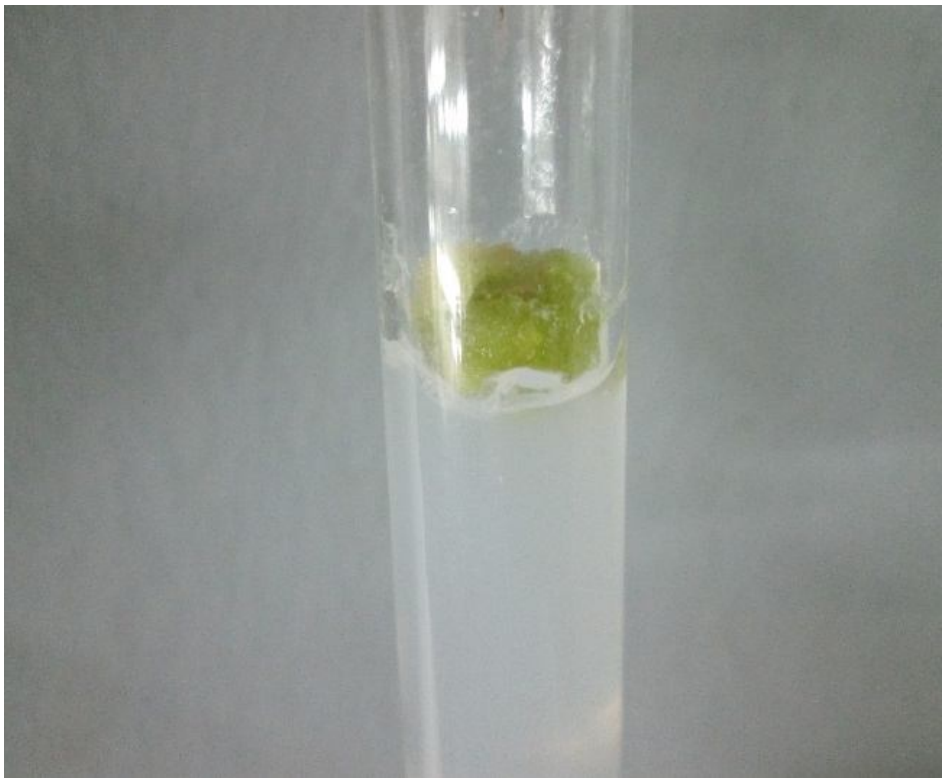

Plate 2: Callus formation from cotyledon explant at 3 weeks after culturing on Murashige-Skoog (MS) media

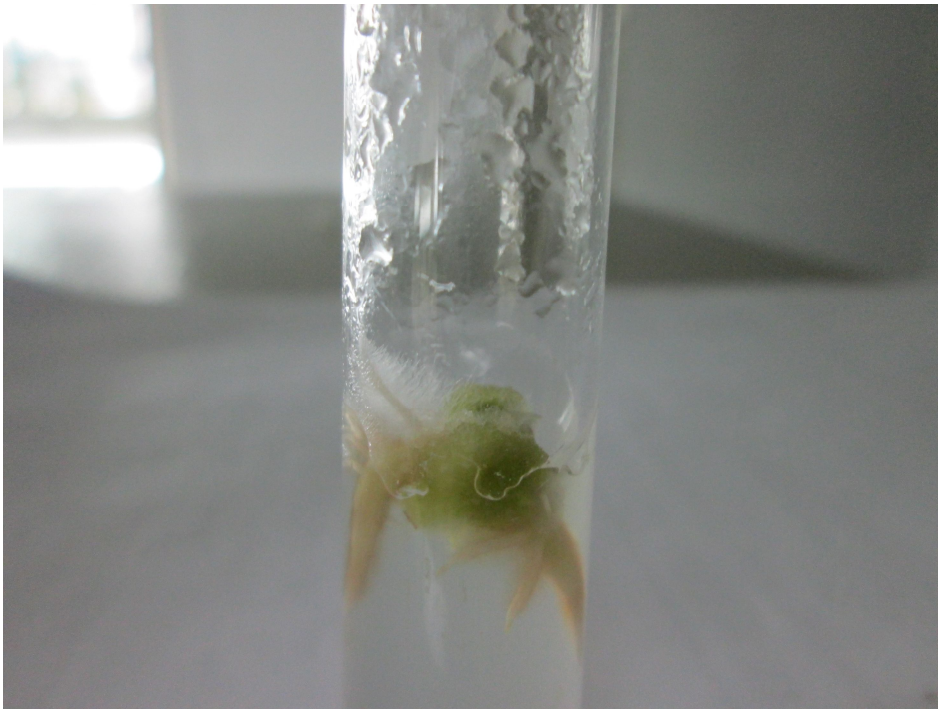

Plate 3: Root growth from callus at 5 weeks after culturing on Murashige-Skoog (MS) media

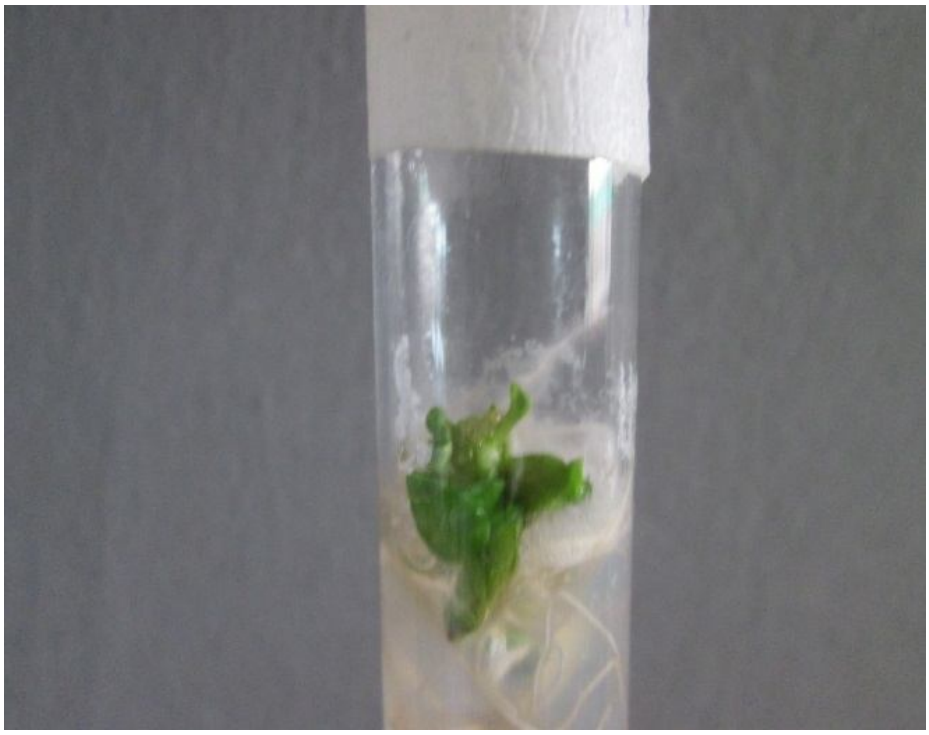

Plate 4: Shoot and root growth from leaf callus cultured on Murashige-Skoog (MS) medium with 1.5 mg/l Kinetin and 0.1 mg/l NAA

Discussion

To establish an efficient *in vitro* regeneration protocol, an efficient sterilization procedure needs to be developed. Contamination of plant tissue cultures by different sources can completely prevent their successful culture. Himabindu et al. (2012) used different surface sterilization chemicals such as mercuric chloride, Hydrogen peroxide and sodium hypochlorite for the sterilization of tomato seeds and found out that 5% sodium hypochlorite for 20 min was more effective resulting in high germination rate of 77.07% with no contamination. In contrast, the absence of contamination in this study was observed with 10% sodium hypochlorite for 20 min, 10% sodium hypochlorite for 10 min with 2 - 3 drops of tween 20 as disinfection procedure for tomato seeds giving rise to 50.67% germination. A lower germination of 22.67% was recorded for seeds subjected to the same treatment with ethanol treatment for 5 min. The result shows that exposure of seeds to ethanol during ethanol treatments for a period of time as used in this study may be detrimental to the germinability of tomato seeds used for tissue culture.

The *in vitro* morphogenetic responses of explants on callus induction media was also evaluated in this study. The use of Kinetin and 6-benzylaminopurine (BAP) at 0.5 mg/l in combination with 0.1mg/l NAA did not favor the development of shoots across the explant sources of the tomato cultivar selected for the experiment. This further supplements existing reports from previous studies by Osman et al. (2010) on the use of 0.5 mg/l BAP to regenerate shoots from cotyledon and hypocotyl explants. The uses of Kinetin and BAP at 2 mg/l favored shoot regeneration across the explant sources of the selected tomato variety used in the experiment while the use of BAP at 2 mg/l favored the development of shoots for cotyledon explants only. Contrary to reports by Osman et al. (2010), shoot induction was possible with 2 mg/l BAP for cotyledon explants. The contrast could be due to genotypic differences. Reports obtained from this study is also in contrast to other reports by various authors who have proved that 2 mg/l BAP produces optimum shoot regeneration with hypocotyl and cotyledon explant sources (Mohammed et al., 2010; Ntui et al., 2009; Sheeja et al., 2004). Ajenifujah et al. (2013) reports direct shoot organogenesis from three Nigerian cultivars of tomatoes with the use of MS media without exogenous hormones obtaining shoot formation values as high as 20% for the three cultivars

using hypocotyl explants. Results obtained for shoot organogenesis on MS only medium across the explants of the local cultivar used are poor for this study. Also, reports obtained for rooting shows the cultivar's high performance for rooting with the various callus induction media used. Regeneration media supplemented with 2 mg/l Kinetin gave the best performance for shoot formation and moderate root formation across the explant sources of the cultivar used. These results are similar to results obtained by Shadin et al. (2009).

Conclusion

The study has shown that contaminant free cultures could be obtained with an efficient sterilization protocol as well as the derivation of an *in vitro* regeneration protocol for indirect shoot and root organogenesis from local tomato cultivar which could be useful for crop genetic improvements and micro propagation purposes.

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