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In Vitro Plant Regeneration of Tomato (*Lycopersicon esculentum*)

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Abstract

The experiment was carried out at the Genetic Engineering Laboratory of the Department of Genetics and Plant Breeding, Sylhet Agricultural University, Sylhet to develop a suitable protocol for high frequency plant regeneration of tomato *(Lycopersicon esculentum)*. An efficient system for high frequency plant regeneration system of *L. esculentum* was developed through investigating various factors such as combination of plant growth regulators; explant type, age and ethylene biosynthesis inhibitor $AgNO_3$. To observe the suitable medium for callus initiation and shoot regeneration, cotyledonary and hypocotyl explants of *L. esculentum* cv. BARI Tomato 5 were cultured on MS medium supplemented with different concentrations of 6-Benzyl Amino purine (BA) and α-Naphthalene Acetic Acid (NAA). The ranges of callus initiation and shoot regeneration frequency of cotyledonary explants were 52.78% to 94.44% and 22.22% to 61.11%, respectively, whereas hypocotyl explants showed 61.11% to 97.22% and 19.44% to 58.33% callus initiation and shoot regeneration frequency, respectively. The highest callus initiation (94.44%) and shoot regeneration (61.11%) frequency were observed on MS medium supplemented with 3.0 mg/L BA and 0.2 mg/L NAA in case of cotyledonary explants. To observe the effect of age of explants source material on shoot regeneration 5 to 9 days old cotyledonary explants were cultured on MS medium supplemented with 3.0 mg/L BA and 0.2 mg/L NAA and 6 days old cotyledonary explants showed the highest shoot regeneration frequency (61.11%). The shoot regeneration frequency was markedly enhanced by the addition of $AgNO_3$. MS medium supplemented with 3.0 mg/L BA, 0.2 mg/L NAA and 2.0 mg/L $AgNO_3$ showed the maximum shoot regeneration frequency (77.78%). To observe the genotypic variation for shoot regeneration potentiality 6 days old explants of six *L. esculentum* genotypes namely BARI Tomato 2, BARI Tomato 5, BARI Tomato 7, BARI Hybrid Tomato 4, BINA Tomato 3 and BINA Tomato 5 were cultured on MS medium supplemented with 3.0 mg/L BA, 0.2 mg/L NAA and 2.0 mg/L $AgNO_3$. Among the six genotypes BARI Tomato 2 showed the highest shoot regeneration frequency (91.67%) and BINA Tomato 3 showed the lowest shoot regeneration frequency (44.44%). MS medium supplemented with 0.1 mg/L NAA showed the highest frequency (100%) of rooting. The regenerated plantlets were transferred in pot soil and grown to maturity.

Keywords: Plant regeneration; Plant growth regulators; NAA; $AgNO_3$; Genotypes

Introduction

Tomato *(Lycopersicon esculentum* Miller*)* is grown throughout the world for its edible fruits and it is one of the most important winter vegetable crops of Bangladesh. It belongs to a large family Solanaceae. The center of origin of tomato is said to be tropical America [1]. The top five leading tomato producing countries are China, India, United States, Turkey and Egypt. Tomato is widely grown in many countries of the world including Bangladesh for its good taste and high nutritional value, and also for its adaptability to wide range of soil and climate [2].

The cultivated tomato is a short-lived diploid (2n =2x = 24) dicotyledonous annual plant, typically growing to l-3 m in height, with a weak woody stern that usually scrambles over other plants.

Complexes of pest and diseases and environmental stress as well as post-harvest loss threaten the stability of the tomato production. Tomato is also sensitive to a number of environmental stresses, especially extreme temperature, salinity, drought, excessive moisture and environmental

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pollution. In vitro technique help to overcome the barrier of self-incompatibility facilitates rapid introduction of new traits [3,4] and development of disease free plant [5]. For raising transgenic crops with useful traits efficient *in vitro* plant regeneration protocol is necessary. Tissue culture techniques can play a significant role for enrichment of genetic variability by creating variation (somaclonal variation) or mutation (by applying radiation or chemical mutagens to *in-vitro* cultured plant materials) at an unexpectedly high rate and may be novel sources of genetic variability in many plant species [6]. But these were found to have limited application in many crop species [7].

For tomato, plant regeneration protocol has been established from various explants, such as leaf disk, cotyledon and protoplasts. The success of in vitro regeneration from tomato explants has been limited [8]. Regeneration protocol has been established in various commercial and unreleased tomato varieties worldwide. Le et al.,(l99l) [9] stated that, cotyledons of tomato cv. Bony Best produced loose, yellow and rapid growth of callus on MS medium with low levels of BAP and high levels of 2,4-D whereas low levels of 2,4-D gave better results when hypocotyls were used as explants [10]. Use of zeatin for in vitro regeneration is predominant in some report [11,12]. Sizes of explants were also reported to have influence on regeneration capacity [13]. While working with Bangladeshi tomato varieties, good callus was obtained from leaf explants of two (`E-6 and S-l) strains of tomato on MS medium supplemented with 2 mg/l IAA and 2 mg/l kinetin [14]. For regeneration of plants calli were transferred onto MS medium supplemented with 4 mg/ l IAA and 4 mg/l kinetin. But using Indian variety (*Lycopersicon esculentun* cv. PKM. l), Jawahar et al., (l997) [15] induced callus from hypocotyls of tomato on MS medium supplemented with IAA 2mg/L and BAP l mg/L and after subculture in the same medium they got better shoot proliferation. Dwivedi et al., (1990) [16] found that direct shoot bud differentiation was induced in tomato leaf segments by culturing them in the medium containing 0.5 mg/L BAP and 0.25 mg/L NAA for 7 days. Shoot bud grew into shoots by sub culturing in the medium supplemented with 0.25 mg/L BAP and 0.01 mg/L NAA. Yassen etal., (1998) [17] cultured the excised tomato cotyledons and hypocotyls explants on MS medium supplemented with 1.5 mg/L BAP and 0.5 mg/L IAA.

Regenerated shoots rooted on MS medium containing 1mg/L IBA and 50 mg/L kanamycin after transformation. A more detailed work was done by Costa et al., (2000) [12]. Sheeja and his team reported, plain half strength of MS as the best media for rooting when addition of IAA (0.1 mg/L) was found essential to induce longer roots [18]. IBA (0.1-0.5 mg/L) was found to be the best hormonal Supplementation on half strength of MS by Venkartachalam et al., (2000) [19]. Liu et al., (2003) [20] cultured shoot of tomato cv. Peral for root formation in MS medium supplemented with 0.2 mg/L IAA or 0.1 mg/L IAA and 0.I mg/L NAA. IAA at 0.2 mg/L resulted in high rooting and in the production of thick and strong roots.

Materials and Methods

The experiments were conducted at the Genetic Engineering Laboratory of the Department of Genetics and Plant Breeding, Sylhet Agricultural University, Sylhet, Bangladesh during the period of July 2014 to June 2015.

Six genotypes of *L. esculentum* species were used to fulfill the objectives of the present investigations. BARI Tomato 5 was used to standardize the plant regeneration protocol for *L. esculentum* and other genotypes were used to evaluate their plantlet regeneration potentiality. The genotypes used in this study are- BARI Tomato 2, BARI Tomato 5, BARI Tomato 7, BARI Hybrid Tomato 4, BINA Tomato 3 and BINA Tomato 5.

For the present study BA (10 mg/ml), NAA (10 mg/ml) and AgNO3 (10 mg/ml) stock solutions were used. BA and NAA stock solutions separately prepared in laboratory. 1000 ml Murashige and Skoog (MS) (1962) was used as culture medium. To investigate the effect of $AgNO_3$ on shoot regeneration, different concentrations of $AgNO_3$ (0-5 mg/L) (filter sterilized by 0.2 μm filter) were added to the medium.

Culture vessels, beakers, pipettes, measuring cylinders, metal instruments were sterilized in autoclave at 121˚C and 15 PSI for 15 min. The culture room was initially cleaned by gently washing all floors and walls with detergent followed by wiping with 70% ethyl alcohol and savlon. Generally laminar airflow cabinet was sterilized by wiping the working surface with absolute alcohol.

Cotyledons alone with 1-2 mm petioles were very carefully excised from the hypocotyl and apical shoot meristems of 5 days old seedlings. The hypocotyls were then discarded from the root tip and cut into 4-5 mm length segments. Twelve cotyledonary or hypocotyl explants were cultured on each culture vessels. The cultured vessels were then sealed with Para film and marked with permanent marker to indicate specific treatment.

After 15 days of incubation of explants the calli were excised from the explants inside the laminar airflow cabinet. The celli were cultured in culture vessels containing freshly prepared shoot regeneration medium. When the shoots grew about 2-3 cm in length, these were rescued from the culture vessels aseptically inside the laminar airflow cabinet and were again cultured in culture vessels containing freshly prepared root induction medium to develop root.

When the rooted plantlets became 5-7 cm in length with sufficient root system, these were very carefully taken out from the culture vessels with undisturbed rooting system and then transplanted to moistened soil in pots containing potting mixture. To reduce sudden shock the pots were kept in growth room for 5 days under controlled environment. The polythene bags were completely removed after 10-15 days when the plantlets were seemed to be self-sustainable. To investigate the effect of different treatments and response of different genotypes to callus initiation, shoot regeneration and root formation, the data were collected for the following parameters- Days of callus initiation, Number of explants with callus (% of callus initiation), Number of callus with shoot (% of shoot regeneration), Number of shoots per explant, Number of shoots with root (% of root formation). The experiment was arranged in Completely Randomized Design (CRD) with 3 replications. The mean and standard deviation for all treatments were calculated by using MS Excel 2007. The significance and difference between means were evaluated at 5% level of significance by Duncan's Multiple Rang Test [21] using MSTATC statistical software [22].

Results and Discussion

The aim of the present investigation was to develop an efficient, stable and reproducible regeneration system of *L. esculentum*. The effects of different explants of *L. campestris* under different hormonal combinations were tested in the research to find out the optimal

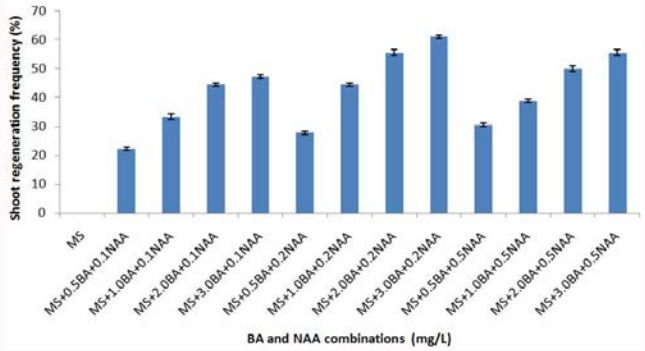

Figure 1: Frequency of callus initiation from 6 days old cotyledonary explants of *L. esculentum* cv. BARI Tomato 5 on MS media supplemented with various concentrations of BA and NAA. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of means.

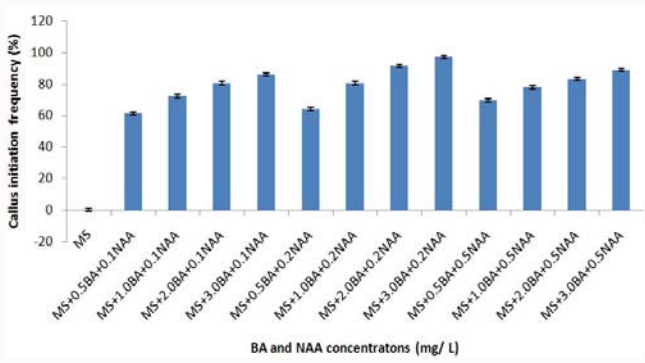

Figure 2: Frequency of callus initiation from 6 days old hypocotyl explants of *L. esculentum* cv. BARI Tomato 5 on MS media supplemented with various concentrations of BA and NAA. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of means.

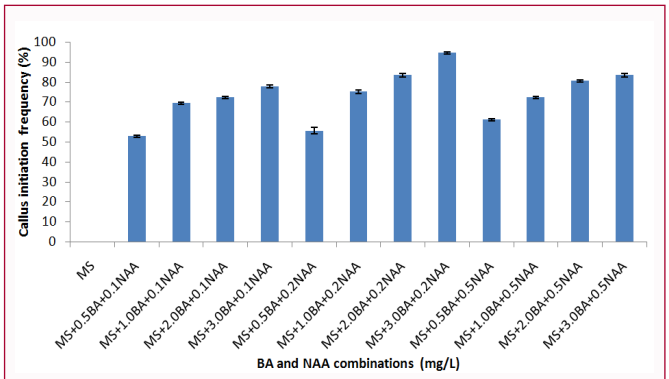

Figure 3: Frequency of shoot regeneration from 6 days old cotyledonary explants of *L. esculentum* cv. BARI Tomato 5 on MS media supplemented with various concentrations of BA and NAA. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of means.

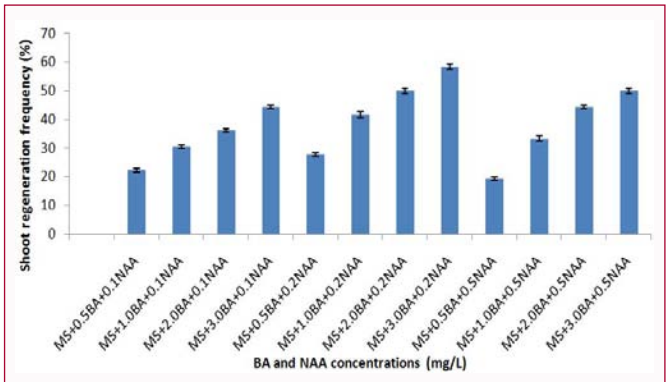

Figure 4: Frequency of shoot regeneration from 6 days old hypocotyl explants of *L. esculentum* cv. BARI Tomato 5 on MS media supplemented with various concentrations of BA and NAA. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of means.

combination for callus initiation and shoot regeneration. Later on effects of explant age and $AgNO_3$ on shoot regeneration as well as number of shoots per explant were tested to establish a suitable protocol for high frequency plant regeneration of *L. esculentum*. To achieve the objectives six *L. esculentum* varieties were tested to screen the best variety for callus induction and plantlet regeneration.

The optimal medium for callus initiation

The optimal medium for callus initiation from cotyledonary and hypocotyl explants of *L. esculentum* cv. BARI Tomato 5was determined by using various combinations of BA (0.5, 1.0, 2.0 and 3.0 mg/L) and NAA (0.1, 0.2 and 0.5 mg/L) (Figure 1 and 2). After 3-4 days of culture, the explants became swollen and callus formation started within a week. When explants were cultured in hormone free MS basal medium (control), they did not produce any callus and died after a few days. From a total of 12 combinations of BA and NAA tested, cotyledonary explants showed the highest (94.44%) callus initiation frequency in MS+3.0 mg/L BA+0.2 mg/L NAA combination and the lowest (52.78%) in MS+0.5 mg/L BA+0.1 mg/L NAA combination whereas hypocotyl explants showed the highest (97.22%) callus initiation frequency in MS+3.0 mg/L BA+0.2 mg/L NAA combination and the lowest (61.11%) in MS+0.5 mg/L BA+0.1 mg/L NAA combination. The callus initiation frequency increased with the increase of BA concentration up to 2 mg/L and further increase of BA concentration decrease the callus initiation frequency when NAA concentration remain same. When the MS medium containing the lowest BA (0.5 mg/L) concentration and the lowest (0.1 mg/L) NAA concentration, the callus initiation frequency drastically reduced from 94.44% to 52.78% in case of cotyledon explants and 97.22% to 61.11% in case of hypocotyl explants.

There was significant difference between cotyledonary and hypocotyl explants for callus initiation frequency. Hypocotyle explants showed better performance than the cotyledonary explants in terms of callus initiation in the same concentrations. Chaudhuryet al.,(2004) [23] and Gubis et al.,(2004) [24] also reported that hypocotyl explants produced higher frequency of celli than the cotyledons.

The optimal medium for shoot regeneration

To obtain the optimal medium for shoot regeneration, calli obtained from 6 days old cotyledonary and hypocotyl explants of *L. esculentum* cv. BARI Tomato 5 were cultured on the media containing same combinations of BA (0.5, 1.0, 2.0, and 3.0 mg/L) and NAA (0.1, 0.2 and 0.5 mg/L) (Figure 3 and 4) as used for callus initiation. After two weeks of explants culture, shoot bud formation started from the calli (Figure 5 and 6). The calli alone with the shoot buds were cultured on shoot regeneration media to obtain complete shoot buds. From a total of 12 combinations of BA and NAA tested, cotyledonary explants showed better shoot regeneration frequency than the hypocotyl explants. In few combinations like MS+0.5 mg/L BA+0.1 mg/L NAA, MS+0.5 mg/L BA+0.2 mg/L NAA both the hypocotyls and cotyledonary explants very small number of shoots. The highest (61.11%) and the lowest (22.22%) shoot formation

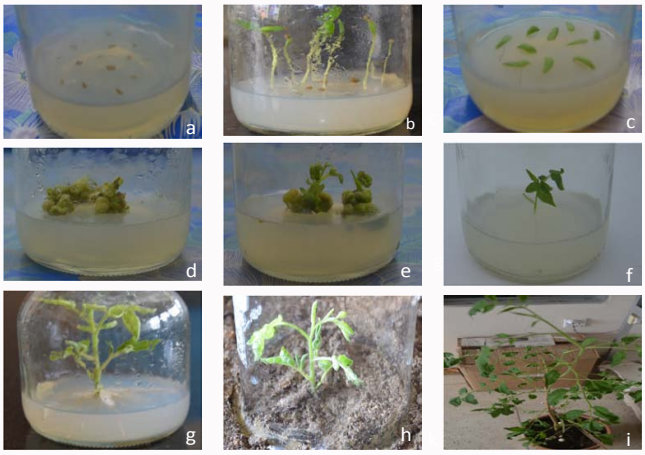

Figure 5: The regeneration process of *L. esculentum* cv. BARI Tomato 5. (a) seeds on ½ MS media at 1st day of culture,(b) seedlings of 6 days old, (c) one day old cotyledonary explants on callus induction media, (d) 15 days old cotyledonary explants on callus regeneration media,(e) shoot regeneration on shoot induction media at 21 days of culture, (f) shoot elongation in MS media supplemented with 3.0 mg/L BA, 0.2 mg/LNAA and 2.0 mg/L $AgNO_3$, (g) root formation of regenerated shoot , (h) plantlets transferred on soil and (i) flowered plant in pot soil.

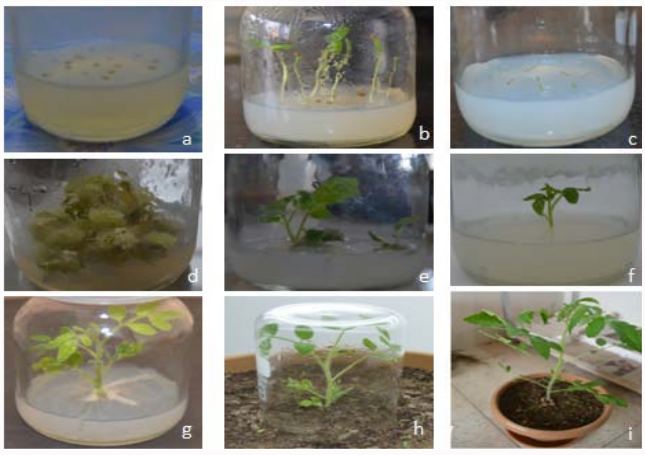

Figure 6: The regeneration process of *L. esculentum* cv. BARI Tomato 5. (a) seeds on ½ MS media at 1st day of culture, (b) seedlings of 6 days old, (c) one day old hypocotyl explants on callus induction media, (d) 15 days old cotyledonary explants on callus regeneration media, (e) shoot regeneration on shoot induction media at 21 days of culture, (f) shoot elongation in MS media supplemented with 3.0 mg/L BA, 0.2 mg/L NAA and 2.0 mg/L $AgNO_3$, (g) root formation of regenerated shoot , (h) plantlets transferred on soil and (i) flowered plant in pot soil.

frequency were obtained by using cotyledonary explants in MS+3.0 mg/L BA+0.2 mg/L NAA and MS+0.5 mg/L BA+0.1 mg/L NAA combinations respectively. On the other hand hypocotyl explants showed the highest (58.33%) shoot regeneration frequency in MS+3.0 mg/L BA+0.2 mg/L NAA combination and the lowest (19.44%) in MS+0.5 mg/L BA+0.5 mg/L NAA combinations respectively. It was observed that when BA concentration increased up to 3.0 mg/L along with same NAA concentration showed the highest shoot regeneration frequency but further increase of NAA above 0.2 mg/L concentration decreased the shoot regeneration frequency.

From the above results, It was determined that the use of cotyledonary explants showed more shoot regeneration frequency than the use of hypocotyl explants cultured on MS medium

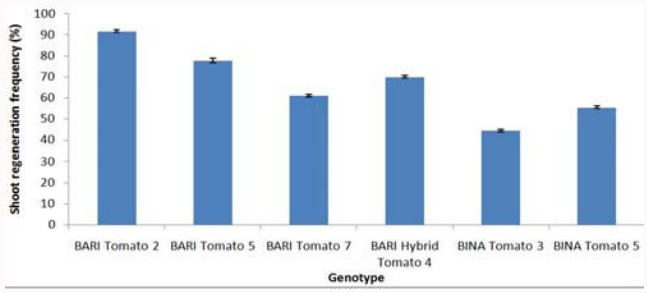

Figure 7: Effect of explant age on shoot regeneration from cotyledonary explants of *L. esculentum* cv. BARI Tomato 5. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of means.

supplemented with 12 combinations of BA and NAA. Osman et al., (2010) [25] noted that the highest number of callus was obtained on hypocotyls explants and the highest number of shoot per explant was also obtained from cotyledon. Ashakiran et al., (2011) [26] conducted a study to develop a rapid, efficient and genotypic specific shoot regeneration system suitable for the transformation of tomato *(Solanum lycopersicum)* and he found that cotyledonary nodes showed a higher shoot formation capacity than hypocotyls. Gubis et al., (2004) [24] reported that using cotyledonary explants of *L. lycopersicum* cv. Hana produced the maximum number of shoots (0.43 or 0.37 shoot per explant with 2.0% and 3.0% sucrose respectively.

Effect of explant age

Cotyledonary explants of different ages (5 to 9 days) was cultured on callus initiation media (MS+3.0 mg/L BA+0.2 mg/L NAA). Explants from 2 to 4 days old seedlings were too small and was not used in this experiment. Cotylenodary explants of 6 days old seedlings showed the highest (61.11%) shoot regeneration frequency and explants of 9 days old seedlings showed the lowest (16.67%) shoot regeneration frequency after two weeks of explant incubation. The shoot regeneration frequency of 5 days (50%) and 7 days (47.22%) old seedling showed no significant difference, but a rapid decrease in shoot regeneration frequency was observed in the explants derived from 7 days to 9 days old seedlings. The result showed that maximum number of shoot is produced from 6 days old seedling explants and further increase in the seedling age decreased the frequency of shoot regeneration (Figure 7). Rai et al., (2012) [27] also reported that Cotyledons excised from 6-days old seedlings germinated on half-strength MS medium containing 8.9 μM benzyladenine (BA) produced the most suitable explant material.

Influence of $AgNO_3$

To investigate the effect of $AgNO_3$ on shoot regeneration and number of shoots per explant, 6 days old cotyledonary explants of *L. esculentum* cv. BARI Tomato 5 were cultured on shoot regeneration media (MS+3.0 mg/L BA+0.2 mg/L NAA) supplemented with different concentration of $AgNO_3$ (1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) (Figure 8). The highest (77.78%) shoot regeneration frequency was observed in shoot regeneration media supplemented with 2.0 mg/L $AgNO_3$ and the lowest (30.56%) shoot regeneration frequency was observed in shoot regeneration medium supplemented with 5.0 mg/L $AgNO_3$. The shoot regeneration frequency enhanced with the increase of $AgNO_3$ concentration up to 2 mg/L but further increase of $AgNO_3$ concentration decreased the regeneration frequency.

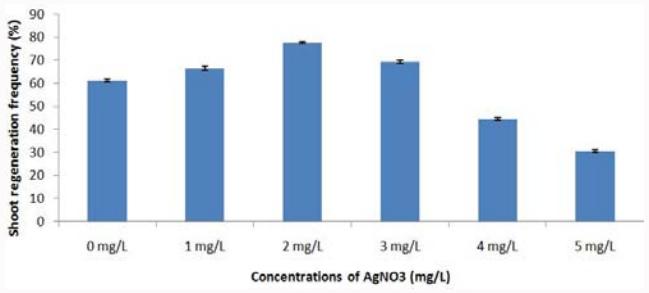

Figure 8: Effect of $AgNO_3$ concentrations on shoot regeneration from 6 days old cotyledonary explants of *L. esculentum* cv. BARI Tomato 5. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of the means.

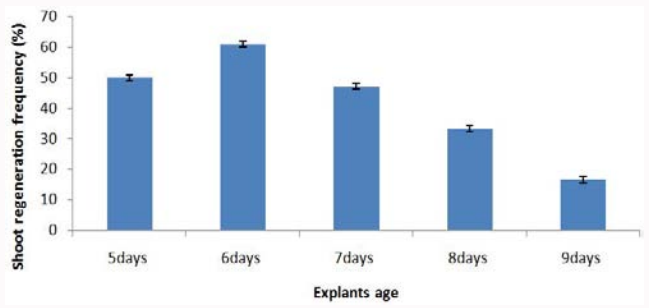

Figure 9: Influence of genotypes on shoot regeneration from 6 days old cotyledonary explants of *L. esculentum*. Data consist of three replications and 12 explants were used for each replication. Bars represent SD of means.

The result showed that the frequency of shoot regeneration is markedly enhanced with the addition of ethylene biosynthesis inhibitor $AgNO_3$. It is observed that the level of enhancements of shoot regeneration depends on the level of concentrations of $AgNO_3$. The positive response of $AgNO_3$ was compliant with the previous results of Shah et al., (2014) [28] who found that $AgNO_3$ interacts with ethylene and enhances callus induction and *In vitro* shoot regeneration in tomato. Du et al., (2000) [29] also noted that addition of 2mg/L $AgNO_3$ to the medium could greatly increase the frequency of shoot regeneration and the shoot regeneration rate reached up to 86.5% in *L. esculentum*.

Genotypic variation

Cotyledonary explants from 6 days old seedlings of six *L. esculentum* genotype namely BARI Tomato 2, BARI Tomato 5, BARI Tomato 7, BARI Hybrid Tomato 4, BINA Tomato 3 and BINA Tomato 5 were cultured on shoot regeneration medium (MS+3.0 mg/L BA+0.2 mg/L NAA) in addition with2 mg/L $AgNO_3$ to determine their shoot regeneration ability and number of shoots per explants. Shoot regeneration frequency is 91.67%, 77.78, 61.11%, 69.94, 44.44% and 55.56% in BARI Tomato 2, BARI Tomato 5, BARI Tomato 7, BARI Hybrid Tomato 4, BINA Tomato 3 and BINA Tomato 5, respectively (Figure 9). Result indicated that shoot regeneration frequency was greatly influenced by the genotypic variation. From result, it is found that BARI Tomato 2 showed the highest (91.67%) shoot regeneration frequency whereas BINA Tomato 3showed the lowest (44.44%) shoot regeneration frequency (Figure 9). *In vitro* regeneration of tomato varieties using various explants, *viz.* cotyledons, hypocotyls, meristem, leaf, stems, roots, internodes, petiole, anthers and inflorescences has been reported. Among these explants cotyledonary leaf segments have reported to

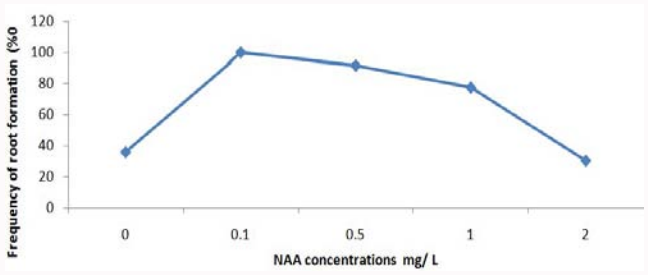

Figure 10: Influence of NAA concentrations on rooting of regenerated shoots from cotyledonary explants of *L. esculentum* cv. BARI Tomato 5. Data consist of three replications and 12 regenerated plants were used for each replication. Bars represent SD of means.

be the most responsive explants for tomato regeneration in various tomato varieties including BARI Tomato 2, BARI Tomato 3, BARI Tomato 5, BARI Tomato 7, BARI Tomato14, BARI Tomato 15, BINA Tomato 3, Bahar, Pussa Rubby and Maple [30-32].

Initiation of roots

The shoots regenerated from different explants required root formation to establish them in soil. When regenerated shoots attained about 2-3 cm length, these were excised and transferred to rooting media. For root initiation the regenerated shoots were placed into MS medium supplemented with different concentration of NAA (0, 0.1, 0.5,1 and 2 mg/L). Within 4 days root formation was started and the highest (100%) root formation frequency was observed in MS medium supplemented with 0.1 mg/L NAA and the lowest (30.56%) was observed in MS + 2 mg/L NAA combination (Figure 10). It was found that root formation frequency varies with the different concentrations of NAA. Plantlets produced well developed root system within 12 to 15 days.

Establishment of plantlets

After sufficient development of root system the small plantlets were taken out from the culture vessels without damaging roots. Excess agar around the roots was washed off by running tap water to prevent microbial infection. Healthy well developed rooted plantlets were successfully transplanted into plastic pot having soil, sand and cow dung in 1:2:3 ratio. The plants were covered by plastic bags with holes for 5 days to maintain humidity. After that the plants were transferred to acclimatization room and irrigated every alternate day with normal tap water. The success rate of this transfer was 100%. After 7-8 weeks the plants produce flowers and no difference was observed in the morphology of these regenerated plants compared with seed derived control plants.

Conclusion

To determine optimal medium for callus initiation, 6 days old cotyledonary and hypocotyl explants of *L. esculentum* cv. BARI Tomato 5 was cultured on callus initiation medium (MS media supplemented with different concentration of BA and NAA. A total of 12 different combinations of BA and NAA were used for callus initiation. From these different combinations, hypocotyl explants showed the highest (97.22%) callus initiation frequency in MS+3.0 mg/L BA+0.2 mg/L NAA combination and the lowest (61.11%) callus initiation frequency in MS+0.5 mg/L BA+0.1 mg/L NAA combination whereas cotyledonary explants showed the highest (94.44%) callus initiation frequency in MS+3.0 mg/L BA+0.2 mg/L NAA combination and the lowest (52.78%) callus initiation frequency

in MS+0.5 mg/L BA+0.1 mg/L NAA combination.

To determine optimal combination for shoot regeneration, calli obtained from cotyledonary and hypocotyl explants of *L. esculentum* cv. BARI Tomato 5 was cultured on the same medium (MS media supplemented with different concentration of BA and NAA as used for callus initiation for shoot regeneration. From these combinations, cotyledonary calli showed the highest (61.11%) and the lowest (22.22%) shoot regeneration frequency in MS+3.0 mg/L BA+0.2 mg/L NAA and MS+0.5 mg/L BA+0.1 mg/L NAA combinations respectively. On the other hand hypocotyl calli showed the highest (58.33%) and the lowest (19.44%) shoot regeneration frequency in MS+3.0 mg/L BA+0.2mg/L NAA and MS+0.5 mg/L BA+0.5 mg/L NAA combinations respectively.

To investigate the effect of age of explants on shoot regeneration, cotyledonary explants of 5 to 9 days old seedlings of BARI Tomato 5 were cultured on shoot regeneration medium (MS+3.0 mg/L BA+0.2 mg/L NAA) followed by callus initiation medium (MS+3.0 mg/L BA+0.2 mg/L NAA). 6 days old explants showed the highest (61.11%) shoot regeneration frequency.

In this experiment, we used different concentrations of $AgNO_3$ to investigate the effect of $AgNO_3$ on shoot regeneration and number of shoots per explant. The frequency of shoot regeneration and number of shoots per explants were markedly enhanced by ethylene biosynthesis inhibitor AgNO3. The level of enhancement of shoot regeneration was dependent on the concentration of $AgNO_3$. The maximum shoot regeneration rate (77.78%) was observed when the 6 days old cotyledonary explants of BARI Tomato 5 were cultured on shoot regeneration medium (MS+3.0 mg/L BA+0.2 mg/L NAA) in addition with 2.0 mg/L $AgNO_3$ followed by callus initiation medium (MS+3.0 mg/L BA+0.2 mg/L NAA) in addition with 2.0 mg/L $AgNO_3$.

Callus initiation medium and shoot regeneration medium (MS+3.0 mg/L BA+0.2 mg/L NAA) added with 2.0 mg/L $AgNO_3$ were used to investigate the shoot regeneration ability of six *L. esculentum* genotypes. Shoot regeneration frequency ranged from 91.67% in BARI Tomato 2, 77.78% in BARI Tomato 5, 61.11% in BARI Tomato 7, 69.94% in BARI Hybrid Tomato 4, 44.44% in BINA Tomato 3 and 55.56% in BINA Tomato 5. The shoot regeneration frequency is greatly influenced by genotypic variation and BARI Tomato 2 showed better performance among the genotypes.

Hypocotyl explants of *L. esculentum* showed higher callus initiation but in case of shoot regeneration frequency cotyledonary explants showed higher frequency. MS medium supplemented with 3.0 mg/L BA, 0.2 mg/L NAA and 2.0 mg/L $AgNO_3$ is the appropriate medium for high frequency shoot regeneration. MS medium added with 0.1 mg/L NAA is the best rooting medium of *L. esculentum*. Among the six genotypes of *L. esculentum*, BARI Tomato 2 showed the highest frequency (91.67%) of shoot regeneration.

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