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EFFECT OF GA_3 ON REGENERATION RESPONSE OF THREE TOMATO CULTIVARS (*LYCOPERSICON ESCULENTUM*)

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Abstract

This study was conducted for developing a high frequency regeneration system in short time span using GA_3, as a pre-requisite for the genetic transformation in tomato cultivars. Effects of GA_3 were investigated on regeneration efficiencies and days to maturity of three varieties of tomato *Lycopersicon esculentum* (using hypocotyls and leaf discs as explant source). 0.5 mg/l Indole acetic acid (IAA) and 0.5-2.5 mg/l of benzyl amino purine (BAP) were used alone or in combination with GA_3 2mg/l on MS media. Regeneration was significantly higher with different treatments used in combination with GA_3. It was increased from 57.33% to 70% in Avinash, followed by Pusa Ruby 51.66% to 67.22% and from 53.2% to 60% in case of Pant Bahr when hypocotyls were used as explant source. Same trend was followed in case of leaf disc derived regeneration, although it was less pronounced. Regeneration was increased from 68% to 73% in Avinash followed by Pusa Ruby 68.5% to 72.33 %. Inclusion of GA_3 in the media also significantly reduced the days to regeneration (20-25) as against 40-45 days when GA_3 was excluded from media in all three varieties of tomato cultivars.

Introduction

Tomato is an important solanaceous crop grown on a wide range throughout the world. It is amenable to physiological and cytogenetic investigation due to its ease of *In vitro* handling and genetic uniformity resulting from autogamy (Rick, 1980). Significant advances have been made during the past decades in the development of *In vitro* culture techniques which have been extensively applied to more than 1000 different crop species (Bigot, 1987).

In vitro culture is used in tomato in different biotechnological applications i.e., production of virus free plants (Moghaieb *et al*., 2004), genetic transformation (Park *et al*., 2003) and studies about the effect of variety and plant growth regulators on callus proliferation and regeneration of three tomato cultivars (Chaudhry *et al*., 2007). Most of the reports about adventitious regeneration in tomato deal with induction of regeneration in hypocotyls or cotyledon explants (Moghaieb *et al*., 2004, Brichkova *et al*., 2002, Raiziuddin *et al*., 2004). Shoot formation from different explants as apical meristem, cotyledons, stems internodes , leaves, anthers and inflorescences has been reported in tomato (Jatoi *et al*., 1999, 2001; Young *et al*., 1987; Branca *et al*., 1990; Compton & Veilleux 1991).

Gibberellin is a naturally occurring plant hormone that affects cell enlargement and division which leads to internodes elongation in stems. They have a dwarf reversing response e.g., it allows certain dwarf cultivars to grow to normal height when treated with gibberellin. It also affect many developmental processes, particularly those controlled by temperature and light such as seed and plant dormancy, germination, seed stalk and fruit development are controlled by GA_3 (Janick, 1979).

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Dwarf mutants that respond to gibberellins (GA_3) by restoring normal phenotypic growth have played a major role in establishing GA biosynthetic pathways and in identifying the active GA in plant species such as maize, rice, pea and Arabidopsis (Ried *et al*., 1990).

GA_3 (0.58 µM $GA_{3)}$ enhanced the shoot elongation by stem internode extension (Dielien *et al*., 2001). The rate of growth of *Xanthium* plants treated with GA_3 was at least twice when compared with control one as stated by (Maksymowych *et al*., 1984).

A novel protocol has been developed for inducing highest number of somatic embryogenesis from leaf cultures of *Decalepis hamiltoni*, when transferred to MS media containing zeatin (13.68 µ*M*) / gibberellic acid (GA_3) and BA (10.65 µ*M*), (Giridhar *et al*., 2004).

Inclusion of higher concentration of GA_3 may have an opposite effect from that desired. Low concentration may require the plant to be repeatedly treated to sustain desired level. It also promotes male flower initiation in cucumbers when pollen are desired for hybrid seed production and may overcome the cold requirement for flowering of some perennial plants (Hartman, 1981).

Sheeja & Mandel (2004) reported that GA found to be the best among all treatments for producing calli with very good growth from leaf and stem explants of tomato cultivars. Callus induction was observed within 8-10 days of culturing the leaf explants source.

Regeneration of whole fertile plant from appropriate tissues *in-vitro* is of prime importance in different biotechnological studies. Prolong period of calli to initiate shoot primordia and consequently the delayed formation of whole plants of tomato had reduced the efficiency of regeneration in various ways.

In the view of above facts the present research was designed to evaluate the effect of GA_3, and explant source in MS medium on callus proliferation and regeneration from leaf discs and hypocotyls of there tomato cultivars Pusa Ruby, Pant Bahr and Avinash.

Materials and Methods

Seeds of three varieties of tomato (*Lycopersicon esculentum*) cvs, Avinash, PusaRuby and Pant Bahr were kindly provided by Awan seed center and from National Agriculture Research Centre, Islamabad.

Disinfection: Seeds were washed with tap water and surface sterilized in a sequential manner with 0.8 % (v/v) "Clorox" bleach (Sodium hypochlorite) for 10 minutes and rinsed three times (5 min each) with autoclaved distilled water under aseptic conditions in a laminar flow cabinet (Chaudhry *et al*., 2001).

Culture media and inoculation: Seeds were germinated on full strength MS medium at 5.76 pH by keeping them initially in dark for two days at 25 ± 1°C and then maintained under 16h photoperiod at 50 u mol/ m^2/s, with day night temperature at 25°C ± 2 respectively.

Culture media for callus induction and regeneration: The hypocotyls and leaf discs of about 1 cm in length were taken from 2-3 weeks old *In vitro*, seedlings, these were utilized as explants source for callus induction and regeneration on ten different media

combinations as shown in Table 1. Media formulations consisted of different concentrations of Indole acetic acid and Benzyl amino purine either in combination with 2 mg/l GA_3 or without GA3 along with other adjuvant having agar as the solidifying agent. Experiments were conducted with four replicates for both types of explants. Data was recorded on number of calli induced and number of days to maturity for seedlings.

Rooting medium: As the tomato shoots began to regenerate from calli, they were transferred to rooting media supplemented either with IBA, NAA, or IAA, 0.1 and 0.2 mg/l respectively. The number of shoots that produced roots were recorded after three weeks of incubation. All media contained 3% sucrose with pH adjusted at 5.76 and were solidified with 4g/l of gel rite.

Plants establishment in the soil: Thirty rooted plants derived from each of the hypocotyls and the leaf discs after one week of root formation were shifted into small pots in the glass house. They were covered with the polythene bag for 10-12 days to control the temperature and humidity and watered at 4-5 days intervals. Data was analyzed by MSTATC.

Results and Discussion

In vitro culture were used in tomato in different biotechnological applications, production of virus free plants (Moghaieb *et al*., 1999), genetic transformation (Ling *et al*., 1998) and in many fundamental researches programmes (M Arriliaga *et al*., 2000). We also used hypocotyls and leaf discs for establishment of a high frequency and quick regeneration in tomato cultivars (Avinash, Pusa Ruby and Pant Bahr).

Seeds were inoculated on MS medium to observe the behavior of varieties for *In vitro* seed germination. Pant Bahr 68%, Pusa Ruby 75% and Avinash showed 78.54% growth on MS plane medium (Fig. 1a, b, c, Table 1). The results showed that Avinash gave the maximum percentage of seed germination. The reason of difference in germination rate might be linked to the genotypes of the varieties (JaeBok *et al*.,2001). This result confirmed that germination rate depends upon the genetic makeup of the varieties.

These *In vitro* derived plants under aseptic conditions were cut into small pieces to select the leaf discs and hypocotyls as explant source. During present studies, callus formation leading to regeneration was achieved on MS medium supplemented with IAA, BAP and GA_3. Ten regeneration media viz., T1 (treatments) to T10 were utilized for *In vitro* culture induction (Table 2). Our results were varying on the basis of treatments and genotype of the cultivars to some extent. Earlier, many workers also reported the hypocotyls (Jabeen *et al*., 2005, Chaudhry *et al*., 2004) and leaf disc (Raj *et al*., 2005 and Roy *et al*., 2006) as best explants source for *In vitro* studies. Soniya *et al*., (2001) used leaf explants of *Lycopersicum esculentum* cv. Sakthi from a field grown plants (mother plant). Reda *et al*., (2004) used 6 days old seedling for callus induction and regeneration. In our studies explants were taken from 2-3 week old seedlings.

De-differentiation of tomato (*Lycopersicon esculentum*) leaf explants into callus followed by shoot formation was dependent on genotype, culture medium and physiological stage of the donor plant (Guillerno *et al*., 2003).

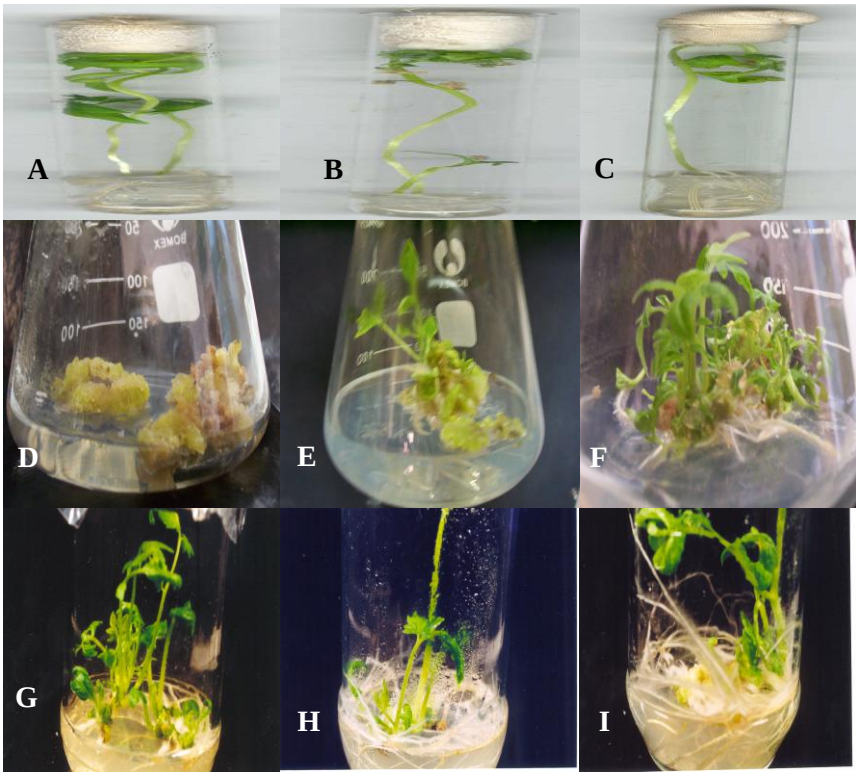

Fig. 1. Regeneration in tomato cultivars (A)*In-vitro* seedlings of Pusa Ruby (B) Pant Bahr (C)Avinash (D) leaf disc derived calli of Pusa Ruby.(E) leaf disc derived calli of Avinash. (F)Hypocotyls derived calli of Pant Bahr. (G) Leaf disc derived regeneration of Pant Bahr. (H) Hypocotyls derived regeneration of Pant Bahr. (I) Hypocotyls derived regeneration of Avinash.

Table 1. Germination percentage of the three varieties of tomato on MS plain medium (*Lycopersicon esculentum*).

Varieties	Total number of seeds	Germinated seeds	Percentage germination
Pusa ruby	144	108	75
Pant bahr	144	98	68
Avinash	144	114	78.54

Table 2. List and composition of media used for regeneration.

Media	Composition
RM1	MS salts and vitamins +IAA 0.5 mg/l+ BAP 0.5 mg/l+ 3% sugar +Ph 5.7-5.8
RM2	MS salts and vitamins + IAA 0.5 mg/l+ BAP 1mg/l+ 3% sugar + Ph 5.7-5.8
RM3	MS salts and vitamins + IAA 0.5 mg/l+ BAP 1.5 mg/l+ 3% sugar + Ph 5.7-5.8
RM4	MS salts and vitamins + IAA 0.5 mg/l+ BAP 2mg/l+ 3% sugar + Ph 5.7-5.8
RM5	MS salts and vitamins + IAA 0.5 mg/l+ BAP 2.5 mg/l+ 3% sugar + Ph 5.7-5.8
RM6	MS salts and vitamins +IAA 0.5 mg/l+ BAP 0.5 mg/l+ 2mg/l GA3+ 3% sugar +Ph 5.7-5.8
RM7	MS salts and vitamins + IAA 0.5 mg/l+ BAP 1mg/l+ 2mg/l GA3+3% sugar + Ph 5.7-5.8
RM8	MS salts and vitamins + IAA 0.5 mg/l+ BAP 1.5 mg/l+ 2mg/l GA3+3% sugar + Ph 5.7-5.8
RM9	MS salts and vitamins + IAA 0.5 mg/l+ BAP 2mg/l+ 2mg/l GA3+3% sugar + Ph 5.7-5.8
RM10	MS salts and vitamins + IAA 0.5 mg/l+ BAP 2.5 mg/l+ 2mg/l GA3+3% sugar + Ph 5.7-5.8

Hypocotyls and leaf discs derived calli regeneration varied significantly among the treatments and varieties used as shown in Table 3, 4 and 5. Other parameters used to study were maturity period or (time taken by explants to regenerate). There were significant difference among the treatments for the regeneration frequencies.

Hypocotyls derived regeneration: Varieties and explant sources showed significant difference in results from ten media combinations. Hypocotyls derived regeneration varied significantly among the treatments used. The maximum regeneration was observed on regeneration medium (RM8) that contains MS salts in combination with vitamins and hormones like IAA 0.5 mg/l + Kinetin 1.5 mg/l + GA_3 0.5 mg/l. Maximum regeneration frequency (70%) was observed for *cv*. Avinash, followed by 67.22 % for cultivar Pusa Ruby and 60% for *cv*. Pant Bahr (Table 3).

Same trend of regeneration was followed in the case of T3 (0.5 mg/l IAA, 1.5 mg/l of Kinetin and 2 mg/l of GA_3). On this combination maximum regeneration percentage was observed for Avinash (57.3 %), 51.6 % for Pusa Ruby and 53.2% for Pant Bahr (Table 3).With T4 and T5, hypocotyls derived regeneration percentage was 45.6% and 32.7% for Avinash, 34.1% and 30.1% in Pusa Ruby, 33.2% and 21.4% in Pant Bahr respectively (Table 3). Regeneration response observed was media specific and having no relation with the variety used. The maximum regeneration frequency was observed on T8 when GA_3 was included in comparison with T3 in which GA_3 was excluded.

Botau *et al.,* (2002) and Chandel & Katiyar (2000) reported that cotyledons and hypocotyls of tomato cultivars showed best response with NAA and BAP. In our studies GA_3 has shown pronounced effect on regeneration as compared to NAA and BAP.

Leaf disc derived regeneration: Avinash and Pusa Ruby had maximum regeneration value at T8 that is 73% and 72.33%. After T8, Avinash gave its maximum regeneration percentage at T3 (0.5 mg/l IAA, 1.5 mg/l of Kinetin and 2 mg/l of GA_3) that is 68%. Pusa Ruby gave second highest value at T3 that is 68.5%. Pant bahr gave highest value at T3 that is 61.83%. Pant Bahr gave its 2nd highest value at T8 that is 58.17%. With T4 and T5, leaf disc derived regeneration percentage was 60.67% and 32.33% for Avinash .It was 53.5% and 32.43 % for Pusa Ruby, 45.67% and 22.67 % for Pant Bahr.

Varieties showed significant variations in results obtained for different media combination. IAA 0.5 mg/l and BAP 1.5 mg/l was proved most efficient for regeneration when GA_3 was included in the medium, whereas Raj *et al*., (2005) used 0.1 mg/l of IAA and 0.1mg/l of zeatin on leaf explants of the tomato cultivar Pusa Ruby. Instead of zeatin we used BAP and GA_3 for regeneration in tomato cultivars.

Leaf disc proved to be a better explant source as compared to the hypocotyls for the regeneration for all media tested irrespective of the varieties used. Sheeja & Mendel 2003 found best regeneration with BAP. Chaudhry *et al*., (2004) obtained the regeneration percentage of 45.8% and 30.8% for the hypocotyls and leaf discs respectively by using relatively higher level of auxin and higher amount of cytokinin e.g., IAA 2mg/l, BAP 5mg/l, NAA 2mg/l and kinetin 4mg/l.

Days taken for regeneration: Another factor studied was days taken for regeneration. Days to maturity varied significantly among the different media combinations used. Time to regenerate the plantlets was significantly different among the ten treatments used. It was not significantly different among the three varieties used.

From T1-T5 in which different concentrations of IAA and BAP were used without inclusion of GA_3, took 31.66-47.3 days to regenerate the plants (Table 5). From T5-T10 the days to regenerate the plantlets was 21-27.3 days. In the first five treatments in which the IAA and BAP were used in different combinations, time taken for regeneration was not very significant. From T6-T10 in which GA_3 was included, days to regenerate the plants was reduced to half, although did not vary significantly among them. and the survival rate of the regenerated plants was also comparatively high than the plants in which GA_3 was not used. So we have reported in this study that the use of GA_3 has increased the regenerative potential and also reduced the required time (Table 5).

Sheeja *et al*., (2004) worked for optimum callus induction and plantlet regeneration in tomato. Regeneration was observed in 30 days across varieties by the use of Kinetin (2.0 mg/l) and IAA (0.5 mg/l), glucose and sucrose (1.5% each), folic acid (0.25 mg/l), biotin (0.5 mg/l), coconut water (5%) and use of young hypocotyl explants were found to enhance plantlet regeneration and length of plantlets. Coconut water is a complex growth regulator whose constitution is not fully known but GA_3 is an important component of coconut water.

Results were unique in the sense that regeneration using GA_3 had not been reported before in the *Lycopersicon esculentum* cultivars Pusa Ruby, Pant Bahr and Avinash.

Plant establishment in the soil: Thirty rooted plants derived from each of the hypocotyls and the leaf discs after one week of root formation were shifted into small pots in the glass house. They were covered with the polythene bag for 10-12 days to control the temperature and humidity and watered at 4-5 days intervals. About 80-90% of the plants survived in the soil which were produced from medium containing GA_3. All survivors flowered normally. About 85% of the seeds collected from these plants were viable.

Conclusion

A rapid high frequency regeneration system was established by using GA_3 in the treatments for three tomato cultivars. The time to regenerate the plants is reduced to half in all tested varieties. As with GA_3, not only it is faster to produce the plants derived from calli but also the plants are more viable. Tomato cv. Avinash responded well among the three varieties used.

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