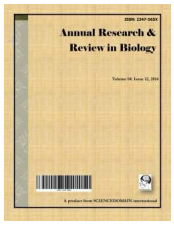

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A Review on Research Progress on *in vitro* Regeneration and Transformation of Tomato

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

After potato tomato is the second major vegetable crop consumed all over the globe as raw and processed food. Due to its high demand its genetic improvement in respect to high yielding, disease resistance, abiotic stress tolerance etc. has been done by several peoples through transformation. Transformation is an emerging tool in crop improvement programme, which expands the source of genes for plant improvement to all species far beyond the gene pool accessible via sexual hybridization. The key component of transformation system is a most functional genomics approaches useful for developing various gene identification strategies and also offers strategies for over expressing or suppressing endogenous genes. The current review provides an overview of the research progress on regeneration and transformation of tomato in the last 15 years.

Keywords: Tomato; transformation.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* L. 2n = 2x = 24), belongs to the family Solanaceae is the second major vegetable crop consumed all over the world directly as raw vegetable, added to other food items or as processed products such as paste, whole peeled, diced, juice, sauces and

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soups., [1]. It is an essential ingredient of most of the vegetarian and non-vegetarian recipes. Tomato is a rich source of Vitamin C, Vitamin B and a good source of β-carotene [2]. It plays a vital role in maintaining human health, vigor and also very helpful in healing wounds due to of the antibiotic properties found in the ripe fruit. The antioxidant lycopene is present in the tomato whose consumption is known to reduce the incidence of many types of cancer [3,4]. Recently, tomato was used as bioreactor in biopharming for the production and oral delivery of vaccines [5] and as functional food for cancer prevention [6]. It is available in almost all seasons of the year and being cultivated throughout the world, in both tropical and sub-tropical regions [7]. It is being cultivated in irrigated areas where salinity of water and soil, especially during summer period, are major constraints limiting productivity and quality of tomato. In the view of these problems, using of classical breeding programmes remains a challenge for breeders due to involvement of many genes with small effects [8]. The biotechnological tools, such as gene transfer technology, which allows the introduction of foreign genes into a germplasm, without modifying the genetic background of elite varieties can be highly benefit in tomatoes breeding programs. However, development of an efficient *in vitro* plant regeneration system play an important role in a breeding program associated to biotechnological tools.

Tomato is considered as one of the most important vegetable crops for genetic engineering due to its small genome (0.7-1.0 pg) and well developed classical and molecular genetics maps [9,10] and serves as a model plant for introduction of agronomically important genes into dicotyledonous crop plants [10]. The first resistant gene (Pto) that elicits a hypersensitive response to disease resistance was cloned in tomato [11]. The *Agrobacterium tumefaciens* has the natural ability of infecting only dicotyledonous plants due to the signaling of acetosyringone, phenolic compounds released from the wounds of the plant cells, which provide the way to the researchers in getting more understanding and a precise manner of working upon this process. However, other methods like particle gene gun technology, electroporation, and protoplast mediated, polyethylene glycol mediated transfer, and microinjection etc. is also used for genetic manipulation [12]. The preferential integration of defined T-DNA into transcriptionally active regions of the chromosome of a plant with exclusion of vector DNA [13,14], unlinked integration of co-transformed T-DNA [15] provide a remarkable advantages to the *Agrobacterium* mediated transformation over other transformation methods [16,15]. Though several reports were found on transformation of tomato, it is still far from routine methods. This Current review summarizes the tomato regeneration and Transformation during the last 15 years.

2. REGENERATION

For an efficient transformation in a particular species, a reliable regeneration protocol is very much essential. Therefore prior to transformation work plant regeneration protocol have to be optimized for a given plant species and type of explants. The regeneration system is a three steps procedure, i.e. induction phase (culture of the explants in the medium), elongation phase (culturing of shoot buds in the media containing low concentration of cytokinins) and rooting phase (Culturing of elongated shoots in the media containing auxins). In regeneration system the complex interaction of cytokinins and auxins will determine the development of organogenesis, caulogenesis and somatic embryogenesis of the explants, which may be synergic, antagonistic or additive depending on the type of tissues and on the plant species. Although the molecular mechanisms of the auxin-cytokinin interactions are mostly unknown, they are thought to include mutual control of auxin and cytokinin metabolism, interactions in the control of gene expression and posttranscriptional interaction. Advances are being made towards better understanding of metabolic process co-related with regeneration [17], but determining the conditions for better *in vitro* plant regeneration is still an empirical process. Thus *in vitro* regeneration of some plant species or particularly genotypes within a species can be difficult. *Lycopersicon peruvianum* is considered to be highly organogenetic among the different species of *Lycopersicon* studied. Regeneration of a particular plant depends upon the explants type, likely highest shoot regeneration was observed from the hypocotyls explants (53.2%) in the MS medium supplemented with 2 mg/lt BA and 0.2 mg/l IBA [18]. The media composition has also a significant role in a regeneration system. Maximum rate of callus formation and shoot regeneration was observed in the MS medium supplemented with 2 mg/l BA and 0.2 mg/l NAA [19]. Similarly Sundararajan [20] has

reported maximum rate of shoot regeneration in the MS medium supplemented with 12.3 µM BA, but by addition of triacontand (2.28 µM) and Ascorbic acid (0.24 mM) to the medium has increased the regeneration frequency. Till date several reports were found for *in vitro* regeneration of tomato with response to the explants types, species and media composition, which was briefly summarized in Table 1.

Table 1. A brief of work done on effect of cultivars explants type and media used on percentage of regeneration in different time by different people

Name of the cultivars	Explants type	Media used	% of regeneration	References
cv.ES58 WC156	L	MS+2.5 mg/lt BA	70.00	[21]
cv. WC156	L	MS+2.5 mg/lt BA	50.00	[21]
cvs 'SantaClara', and 'Firme'	C	MS+ 1.0 mg/lt zeatin 0.1 mg /lt IAA+ 300 mg/lt timentin	90 -100	[22]
cv. Marglou	LD	MS+1 mg/lt Zeatin	53.3	[23]
cv. T-146	LD	MS+1 mg/lt Zeatin	89.3	[23]
cvs. Hana and Premium	H	1 mg L−1ZEA+0.1 mg L−1 IAA	100	[24]
ND	L	N6+3 mg/lt BA+0.2mg/l IAA	100	[25]
cv.KalG, Su2207	C	MS+2 mg/lt Zeatin+2mg/lt BA	ND	[26]
cv. CastleRock	L	MS +3 mg/lt BA+ 2.5mg/lt IAA	65.12	[27]
cv. 981 XTY-6	C, H	MS + 0.5 mg/lt Zeatin + 0.1mg/lt IAA	ND	[28]
cv. CastleRock	H	MS+1 mg/lt BA+1 mg/lt Zeatin + 1 mg/ltAgNo3	92.00	[29]
cv.	CL, H	MS+2.5 mg/lt BA	ND	[30]
cv. Rio Grande	C	MS+1 mg/lt Zeatin+0.1 mg/lt IAA	ND	[31]
cv. Pusa Ruby		MS +0.5 mg/lt BA+ 0.1 mg/lt IAA	96.0	[32]
cv. Pusa Ruby	C	MS+2 mg/lt BA	ND	[33]
cv. Punjab upma	H	MS+0.mg/lt BA+0.5 mg/lt Kn+ 0.2 mg/ltIBA	86.02	[34]
cv. IPA-3	H	MS+0.mg/lt BA+0.5 mg/lt Kn+ 0.2 mg/lt IBA	82.57	[34]
cv. Pusa rubby	C	MS+B5vitamin+0.5 mg/lt BA+ 0.5 mg/lt IAA	90.90	[35]
cv. Pusa uphar	C	MS+B5vitamin+0.5 mg/lt BA+ 0.5 mg/lt IAA	82.20	[35]
cv. DT-39	C	MS+B5vitamin+0.5 mg/l BA+ 0.5 mg/lt IAA	55.50	[35]
cv. megha (L15)	CL, H	MS+3 mg/lt Kn+0.3 mg/lt IAA	ND	[7]
cv. Riogrande	LD	MS + 0.5 mg/lt IAA+ 0.5 mg/lt GA3+1.5 mg/lt Kn	90.60	[36]
cv. Riogrande	H	MS + 0.5 mg/lt IAA+ 0.5 mg/lt GA3+1.5 mg/lt Kn	82.50	[36]
cv. Roma	LD	MS + 0.5 mg/lt IAA+ 0.5 mg/lt GA3+1.5 mg/lt Kn	72.60	[36]
cv. Roma	H	MS + 0.5 mg/lt IAA+ 0.5 mg/lt GA3+1.5 mg/lt Kn	65.40	[36]
cv Smart-18	L	MS+3 mg/lt BA+ 1 mg/lt IAA	87.00	[37]
cv Pusa ruby	I	MS+2 mg/lt BA+ 0.2 mg/lt NAA	100.00	[38]
cv PKM-1	L	MS+12.µM BA	94.03	[39]

I-leaf, C- Cotyledon, H- Hypocotyl, I-Internodes, LD- Leaf disc. CL- Cotyledonary leaf, LL-Leaf let, R-Radicle, ND- Not defined

Table 2. A brief of work done on effect of cultivars explants type (ET), mode of transformation (MT), bacterial strain (BS), plant vector (PV), selection markers (SM) on transformation efficiency (TE) and the gene transferred (TG) to tomato plant in different time by different people

Name of the cultivars	ET	MT	BS	PV	SM	TE	TG	References
cv WC156	C, H	AT	EHA105	pGUSINT	kan	ND	uidA nptII	[21]
cv. Starfire	L, C, H	AT	LBA4404	pBI121	kan	12, 60, 75	pcht28	[52]
cv. UC82	C	AT	pGV 2260	pBI121	kan	25	uidA nptII	[53]
cv. Bailichun	LD	AT	LBA4404	pBin438	kan	ND	BADH	[54]
cv. Marglou	LD	AT	GV3Ti11SE R1601	pMON200	kan	1.1 80	nptII	[23]
cv. T-146	LD	AT, AR	GV3Ti11SE R1601	pMON200	kan	0 90	nptII	[23]
cv. Pusa Ruby	C	AT	LBA 4404	pROK-ITCP17	kan	11.4	TLCV–CP	[55]
Cv CL5915	C	AT	LBA4404	pCAMBIA1301	Kan hyg	ND	NPR1	[56]
cv. Pusa Ruby	L	AT	EHA 105	pBIG-HYG-bspA	hyg	ND	bspA	[57]
cv.KalG, Su2207	C, H	AT	pGV3850	pBI121	kan	35	nptII	[26]
cv.Kal-early	C, H	AT	pGV3850	pBI121	kan	17	nptII	[26]
cv. 981 XTY-6	C, H	AT	LBA 4404	FGC5941	kan	4.3-7.4	nptII	[21]
cv. CastleRock	H	AT, PB	LBA 4404	pMONRTG	bar	26.5	uidA	[29]
cv. UC82B	C	AT	LBA 4404	pMBP1	kan	ND	Aβ	[58]
cv. UC82B								
cv. Rio Grande	C	AT	LBA 4404	pCB 302.2	cef	14 13	TVP1 TNHX1	[31]
cv. Pusa Ruby	CL	AT	LBA4404	pBI121	kan	ND	nptII uidA	[32]
cv. Pusa Ruby	C	AT	AGL1	pCTBEZL	Cef	40.7	CtxB, uidA	[33]
cv. Castle Rock	H, C	AT	LBA4404	pITB-AFP	hyg	ND	AFP	[59]
cv. Pusa rubby	C	AT	CV3101	pBII01	kan	71.6	npt-II	[35]
cv. Pusa uphar	C	AT	CV3101	pBII01	kan	67.5	npt-II	[35]
cv. megha (L15)	CL,H	AT	GV2260	pCAMBIA 1301	hyg	ND	uidA	[7]
cv. Riogrande	H,LD	AT	EHA101	pTCL5	hyg	24	uidA	[36]
cv. Roma	H,LD	AT	EHA101	pTCL5	hyg	8	uidA	[36]
cv Micro-Tom	H	AT	EHA10	pCAMBIA 2301	Kan	19.1	gusA	[60]
cv Arka	C,H	AT	ND	pCAMBIA 2301	Kan	34	Rd29A	[18]

Name of the cultivars	ET	MT	BS	PV	SM	TE	TG	References
vikas								
cv Micro-Tom	C	AT	GV3101, EHA1O5, AGL1, MP90	pBI121	Kan	60 40 35,15	uidA	[50]
cv Arka vikas	H	AT	LBA4404	pCAMBIA 2301	Kan	11	AFP	[51]

L-leaf, C- Cotyledon, H- Hypocotyl, LD- Leaf disc. CL- Cotyledonary leaf, LL-Leaf let, R-Radicle, AT-Agrobacterium tumefaciens, AR-Agrobacterium rhizogenes, PB-Particle bombardment,ND-Not defined

3. TRANSFORMATION

A significant increase in the farmer's income in many developing countries is due to the intensive cultivation of tomatoes, but a multiple complex type of pests, diseases and post harvest lose the stability and production of tomato. Besides, abiotic factors like salinity, heavy metal stress etc are also create significant problems in the conventional tomato cultivation, so transgenic plants may be a better alternative. The development of transgenic plants having a new trait is difficult to obtain via traditional breeding programmes due to the tedious methods and lack of suitable gene pool. Advances biotechnological tools and techniques particularly genetic transformation can lead to the development of transgenic plants in both monocot and dicot plants with desired characters using proper transgene. Transformation involved the transfer/introduction of the foreign DNA into the plant cells followed by incorporation of foreign DNA into the chromosomes and its successful expression in cells, which will induced to regenerate the transgenic plants [40,41]. Mainly there are two methods available for the introduction of gene into the plant i.e. via particle bombardment method and *Agrobacterium* mediated transformation. In the first method the gene of interest was first coated in gold/tungsten particle then bombarded to the targeted tissue with high pressure by using a gene gun. In the second method *Agrobacterium* a soil born gram negative bacteria is used as the vector for transformation. Though *Agrobacterium* has different species but mainly 2 species i.e. *A. tumefaciens* and *A. rhizogenes* were used for the purpose of transformation, but *A. tumefaciens* accounts for about 80% of the transgenic plants that produced so far plays a major role in the development of plant genetic engineering and the basic research in the molecular biology [42]. *A. tumefaciens* via its Ti plasmid has transferred the gene of interest present in the T-DNA region of Ti plasmids into the genome of the targeted plants [43]. *A. rhizogenesis* responsible for hairy root disease in plants due to the induction of adventitious root formation by the integration and subsequent expression of portion of the bacterial DNA (T-DNA) from the root inducing (Ri) plasmid of the bacterium. This is particularly valuable for the transformation of genes acting in the root system. Four loci identified in the T-DNA of Ri plasmid and designated gene loci (rol) A, B, C and D were responsible for root formation.

Transformation in tomato was first achieved by [44] via *Agrobacterium* mediated transformation. Since transformation in tomato is well established worldwide many reports were found on tomato transformation for a variety of purposes, including characterization of gene function, production of insects and disease resistance, herbicide tolerance, abiotic stress tolerance, improved fruit quality, delay in fruit ripening and production of foreign protein etc [45]. Various factors like variety/genotype, explants types, plant growth regulators, selection system used, concentration of acetosyringone, bacterial density, duration of infection etc. has determined the efficiency of *Agrobacterium* mediated transformation [46,47]. Irrespective of several difficulties in tomato transformation numbers of reports have been published on time to time by solving the problems and taking different genes in account as summarized in Table 2. Above all the transformation frequency is the most important in transformation experiment, which can be calculated in various ways, as percentage of explants regenerating on selection medium or percentage of co-cultivated explants producing transgenic plants representing independent transformation events and the presence of transgene has also validated by PCR and Southern analysis [48,49]. The transformation efficiency of four *Agrobacterium* strain i.e GV3101, EHA105, AGL1 and MP90 was evaluated in the transformation of tomato cv

Micro-Tom using plant vector pBI121. A maximum efficiency of 65% was observed in the strain GV3101 followed by 40%, 35% and 15% in EHA105, AGL1 and MP90 respectively [50]. *Agrobacterium* mediated transformation in Tomato cv Arka Vikas using CaMV35S promoter and pCAMBIA2301 plant vector revealed highest regeneration frequency in the MS medium supplemented with BA (2 mg/lt) and TDZ (1 mg/lt) with 11% transformation efficiency [51].

4. CONCLUSION

Although several reports were found on in vitro regeneration and transformation of tomato, it is still far from routine methods. The rate of success of regeneration depends upon the explants type, species/cultivars and media composition where as the success of transformation depends upon a suitable *Agrobacterium* strain and antibiotic selection system. On the basis of the *in vitr*o behavior of genotypes of tomato and working out an efficient protocol of transformation in a given set of genotypes is necessary to harness the benefit of candidate genes for tomato improvement programme.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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