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EFFECT OF GENOTYPE AND EXPLANT TYPE ON *IN VITRO* SHOOT REGENERATION OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.)

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Abstract

In order to select the best tomato cultivar for *Agrobacterium* mediated gene transformation studies, *In vitro* regeneration frequency of hypocotyls, leaf disc and shoot tip of five tomato cultivars (*Lycopersicon esculentum*) was investigated on a regeneration medium supplemented with 1 mg/l zeatin and 0.1 mg/l indole-3-acetic acid. Significant differences in regeneration capacity between genotypes and explant types, expressed as frequency of regeneration, average number of callus, shoot primordial and regenerated shoot primordial were observed. Regeneration in 5 cultivars of tomato using 3 different explant types was achieved. The regeneration capacity was strongly influenced by the cultivar and explant type. The highest regeneration capacity was observed in cultivar Riograndea (80% by using shoot tip, 64.5% by using hypocotyl and 56% by using leaf disc) from all types of explant. Of the explant types shoot tip was found the best explant source for direct shoot formation (80% shoot primordial were regenerated) while hypocotyl was found the best explant source for shoot formation through callogenesis (64.5% shoot primordia were regenerated)

Introduction

Tomato (*Lycopersicon esculentum*) is considered as one of the most important vegetable crops for the genetic engineers because it serves as a model for introduction of agronomically important genes into dicotyledonous crop plants (Wing *et al*., 1994). In tomato, genetic transformation with *in vitro* regeneration has been successfully used for genetic improvement (Lindsey, 1992). Resistance to pests, herbicide tolerance, and production of edible vaccines or other novel bioproducts and quality improvement are the most important goals of genetic plant modification. Callus induction and regeneration from explants of apical meristem, cotyledons, stems, petioles, leaves, anthers and inflorescences have been reported in tomato (Young *et al*., 1987; Branca *et al.,* 1990; Compton & Veilleux, 1991; Sheeja *et al*., 2004). The most frequently used way of regeneration in tomato is via shoot organogenesis from callus, leaf or cotyledon explants or directly from thin cell layers of the inflorescence (Compton & Velleux, 1991). *In vitro* plant regeneration has been found to depend on many factors of which most important are: explant type, genotype, composition of the basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels and vessel covers (Reed, 1999; Sheeja *et al*., 2004). For efficient and reliable transformation, study of the factors such as genotype, explant type which affect the regeneration is very important.

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The present work was carried out to study the effect of cultivar and explant type on *In vitro* regeneration of tomato. This study would be helpfull in providing the best cultivar and explant source of tomato for transformation studies.

Materials and Methods

Five cultivars of tomato (*Lycopersicon esculentum* Mill.) viz., Riograndea, Roma, Money maker, Nagina and Feston were used. The seeds were obtained from a Vegetable Programme of the National Agricultural Research Council (NARC), Islamabad. The dormancy of the seeds was broken at 4^{o}C. The seeds were surface sterilized by using 5% Chlorox (NAOCl commercial grade bleach) for 5-7 min., followed by three times rinses with sterilized distilled water (15 minute each). The seeds were cultured on Murashige & Skoog medium (Murashige & Skoog, 1962) (abbreviated further as MS) and were kept at 25 ± 2^{o}C initially in obscurity and later transferred to conditions with 16 h photoperiod of light intensity of 1500 lux. It was noted that seeds started growing in dark and later they were transferred to light. Germination started after 10 days in Riograndea, Roma and Money maker and after 13 days in case of Feston and Nagina. Hypocotyls, leaf discs and shoot tips from 17-18 day-old *In vitro* raised plantlets were excised under aseptic conditions. The excised explants were cultured on MS-medium supplemented with 1mg/l zeatin (ZEA) and 0.1 mg/l indole-3-acetic acid (IAA) (Ichimura & Oda, 1995; Gubis *et al.,* 2003).

The pH of the medium was adjusted to 5.8 after the addition of hormones. The size of leaf disc was almost 5 x 5 cm^2 and those of hypocotyls and shoot tips were 4-5 cm. The experiment was repeated two times and for every experiment 24 explants of each type from all varieties were cultured. After two weeks, each explant was shifted to the fresh culture medium. All the media mentioned earlier were sterilized at 120^{o}C for 20 minutes. After sub-culturing, the cultures were kept at 25 ± 2^{o}C for regeneration. Regeneration and callus formation started after two weeks. The regeneration ability of explants was assessed six weeks later. The following parameters were evaluated: frequency (%) of shoot formation, average number of shoot primordia formation and regenerated shoot formation. Statistical significance of the data was analysed by Pooled Completely Random Design (CRD Pooled).

Results and Discussion

In all the tomato varieties yellow green calli appeared within two weeks from leaf disc and hypocotyls on which shoot primordia and later on shoots appeared, but when shoot tip was used as an explant source direct shoots were formed. Percent regeneration varied among the cultivars and explant type (Fig. 1). In almost all the cultivars, a maximum regeneration was observed from shoot tips, while leaf disc showed poor response. Cultivar Riograndea had the highest regeneration capacity from all the explants while Feston had the lowest regeneration capacity from all the explants. Cultivars Roma and Money maker also possessed good regeneration capacity. A similar type of comparison for genotype and explant type selection was also reported earlier (Moghleb *et al*., 1999; Gubis *et al.,* 2003). Our results are in agreement with the study conducted by Moghleb *et al*., (1999) in which they observed 70% regeneration from hypocotyls. They studied regeneration of three tomato cultivars using hypocotyls and cotyledons as an explant source. Our results are not in agreement with the study conducted by Gubis *et al*., (2003). In their study, highest regeneration was 100% from hypocotyls but in our case

highest regeneration was 80% and was from shoot tip. Shoot tip has already been found the most compatible for whole plantlet regeneration (Sheeja *et al*., 2004). We were unable to get 100% regeneration from all explant sources and varieties used. This difference in regeneration frequency is due to the difference in genotype used.

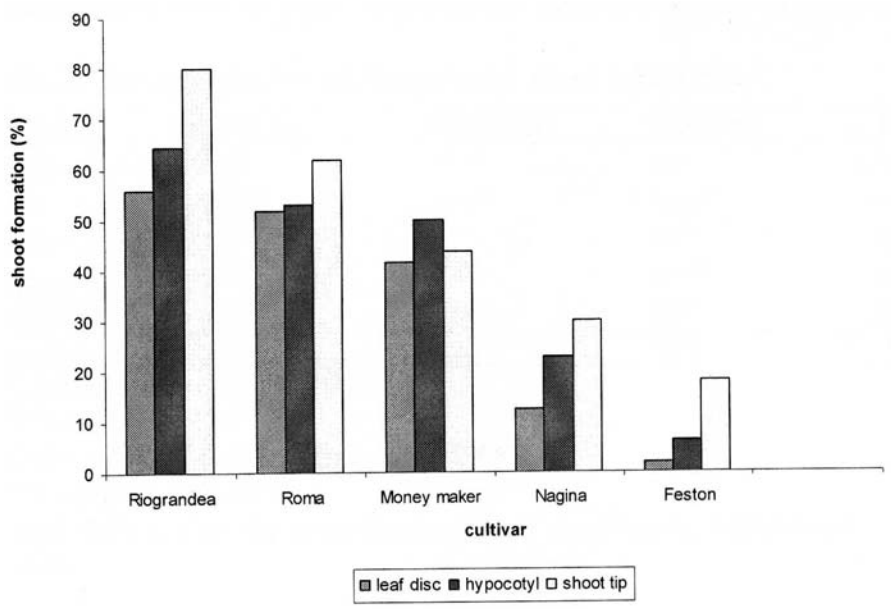

Fig. 1. Percentage of shoot formation in three different explants of five different varıetıes.

Statistically significant differences in average number of calli formed (Table 1), number of shoot primordia forming explants (Table 2) and number of regenerated shoot primordia (Table 3) were observed among cultivars and also among explants. The cultivar x explant interaction was also significant. The highest number of callus and shoot primordia were formed in cv. Riograndea from all types of explants (Table 1). Callus and shoot primordial formation in cv. Roma and Money maker was also good but significantly lower number of calli, shoot primordia and shoots were formed in cvs. Feston and Nagina (Table 1, Table 2 and Table 3). Number of calli formed was insignificantly different from hypocotyl and leaf disc but from shoot tip no callus formation was observed (Table 1). However, number of shoot primordia and regenerated shoot primordia was significantly different among the cultivars. It was observed that calli obtained from the hypocotyls formed more shoot primordia and shoots as compared to leaf disc. The order of callus formation was leaf disc> hypocotyls> shoot tip in all the cultivars except from cv. Feston and the order of shoot primordia regeneration was shoot tip> hypocotyl> leaf disc (Table 3). It was observed that although more number of calli was formed by using leaf disc but their regeneration ability was low. These results are supported by the results of previous study conducted by Faria & Illg (1996) in which they reported a maximum shoot formation from the hypocotyls derived calli among all the explant sources used. Similar differences in callogenic and organogenic ability among the various cultivars and explants of tomato were reported earlier by using the same hormones (Hille *et al*., 1989, Arrilaga *et* al., 2001).

Table 1. Average number of calli formed.

Cultivar	Leaf disc	Hypocotyl	Shoot tip	Average
Riograndea	21.5^{a}	19^{ab}	^{0g}	13.5
Roma	19.5^{ab}	17.5^{bc}	0^{g}	12.33
Money maker	20.5^{a}	15^{c}	0^{g}	11.83
Nagina	6.5^{f}	11^{d}	0^{g}	5.83
Feston	2.5^{g}	8.5^{ef}	0^{g}	3.67
Average	**14.1**	**14.2**	**0**	

C.D for cultivar=1.47
C.D for explant=1.14
C.D for cultivar x explant interaction=2.55
*C.D= critical difference
*Values followed by the same letters are not significantly different at α= 0.05

Table 2. Average number of shoot primordia forming explant.

Cultivar	Leaf disc	Hypocotyl	Shoot tip	Average
Riograndea	15^{bcd}	16.5^{b}	22^{a}	17.83
Roma	13.5^{bcd}	13.5^{bcd}	12^{d}	13
Money maker	12^{d}	13^{cd}	15.5^{bc}	13.5
Nagina	4.5^{fg}	7.5^{ef}	5.5^{ef}	5.83
Feston	1.5^{g}	4.5^{fg}	8^{e}	4.67
Average	**9.3**	**11**	**12.6**	

C.D for cultivar=1.87
C.D for explant=1.45
C.D for cultivarx explant interaction= 3.23
*C.D= critical difference
*values followed by the same letters are not significantly different at α= 0.05

Table 3. Average number of regenerated shoot primordia

Cultivar	Leaf disc	Hypocotyl	Shoot tip	Average
Riograndea	13.5^{b}	14.5^{a}	19^{a}	15.67
Roma	12.5^{bc}	12.5^{bc}	10.5^{cd}	11.83
Money maker	10^{cd}	12^{bc}	14^{b}	12
Nagina	3^{fgh}	5.5^{ef}	4.5^{efg}	4.33
Feston	0.5^{h}	1.5^{gh}	6.5^{e}	2.83
Average	**7.9**	**9.2**	**10.9**	

C.D for cultivar=1.87
C.D for explant=1.45
C.D for cultivarx explant interaction= 2.64
*C.D= critical difference
*values followed by the same letters are not significantly different at α= 0.05

Cultivar, explant type and medium composition are considered the three main factors affecting *in vitro* plant regeneration in many plant species. In this work, we observed statistically significant differences in regeneration capacity among genotypes and explant types, expressed as frequency of regeneration, average number of callus, shoot primordial and regenerated shoot primordia. Regeneration in 5 cultivars of tomato using 3 different explant types was achieved. The regeneration capacity strongly depended on the cultivar

and explant type. The highest regeneration capacity was observed in cultivar Riograndea using all types of explant. Among the explant types shoot tip showed highest regeneration ability directly while calli obtained from hypocotyls showed the highest regeneration ability in all the cultivars.

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