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DIRECT REGENERATION FROM EMBRYO CULTURES OF *LYCOPERSICON ESCULENTUM* MILL CV PUSA RUBY

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ABSTRACT

Embryos of Lycopersicon esculentum Mill cv Pusa Ruby cultured on MS medium supplemented with auxins such as IAA (1.0mg/l) and NAA (1.0mg/l) alone produced whole plantlets. However, embryos cultured on 2,4-D (1.0mg/l) produced callus with roots. BAP (1.0mg/l) and Kn (1.0mg/l) alone are able to induce shoots with callus. Among various combinations of auxins and cytokinins i.e., (1.0 mg/l) IAA + BAP (2.0 mg/l) and IAA (1.0 mg/l) + Kn (2.0 mg/l) gave good response and produced green nodular callus with shoots. Whereas, NAA (1.0mg/l) + BAP (2.0mg/l) and Kn (2.0mg/l) produced callus only. The combinations of IAA (0.5mg/L) + BAP (4.0 mg/L) was found to be better medium compared to all other concentrations of IAA + Kn medium analyzed.

KEY-WORDS: ***Lycopersicon esculentum Mill*** **cv Pusa Ruby Direct regeneration, Embryoculture, Morphogenic response growth regulators.**

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INTRODUCTION

Tomatoes originated in South America and were domesticated in Mexico. They were introduced to Europe by the Spaniards in the 1500's and were initially regarded with grave suspicion because of the reputation of Solanum like fruit as being poisonous. Even by 1800 people in Northern Europe were anti-tomato, whereas in Spain it had become the most commonly eaten vegetable. Tomato is an important crop species and is being used as a model plant in molecular studies. In addition to a kitchen plant, it is considered as a source of economic traits. The explant tissues of tomato from different organs are totipotent to regenerate into plants either through callogenesis or organogenesis.' However, their morphogenic responses are affected by different components of the culture media. Hence it is important to evaluate their effects on plant regeneration. Different explants tissues like cotyledons, hypocotyl, embryos, ovules, and protoplasts of tomato have been used to regenerate plants (Zapata & Sink, 1981; Koblitz & Koblitz, 1982 ; Uddin & Berry, 1988 ; Chen & Adachi, 1998 ; Gill et al, 1995 ; Newman et al , 1996 ; Ling et al, 1998 ; Costa et al, 2000). In the present investigations, the plants were regenerated through zygotic embryo culture in tomato cv Pusa Ruby.

MATERIALS AND METHODS

The seeds of *L esculentum* cv Pusa Ruby were obtained from Maharastra Hybrid Seeds Co. Seeds were surface sterilized with ethanol (70%) for 30 seconds followed by mercuric chloride (0.2%) for 5 minutes and then rinsed several times with distilled water. Seeds were germinated aseptically on MS basal medium. The medium was gelled with 0.8% agar and cultures were incubated at 25±2°c under 16 hr. photoperiod. Mature embryos were excised aseptically from the sterilized seeds soaked for 24 hrs.

RESULTS & DISCUSSIONS

Embryos of *L esculentum* cv Pusa Ruby cultured on MS medium containing various growth regulatorsauxins such as IAA (1.0mg/l) and NAA (1.0mg/l) alone showed good response and produced complete plantlets. However embryo cultures on 2, 4-D (1.0mg/l) produced callus with roots (Table-1). When embryo explants cultured on MS medium supplemented with BAP (1.0mg/l) and Kinetin (1.0mg/l) alone was able to induce shoots with callus. However, Kinetin showed less response and induced little amount of compact callus in comparison to BAP. Among various combinations of auxins and cytokinins like IAA (1.0mg/l) +BAP (2.0mg/l) and IAA (1.0mg/l) +Kn (2.0mg/l) gave good response and produced green nodular callus with more shoots, whereas NAA (1.0mg/l) +BAP (2.0mg/l) and Kinetin (2.0mg/l) produced callus only. Embryos cultured on MS medium fortified with various concentrations of IAA+BAP and IAA+Kinetin were analyzed to observe the plant regeneration response (Table 1 & 2) (fig.1-a,b,c,d). The data showed that the percentage of cultures and mean number of shoots per explant recorded at 0.5mg/L IAA + 5.0 mg/L BAP medium were higher than IAA+Kn combination. Thus the combination of IAA+BAP was found to be the best medium compared to all other concentrations ofIAA+Kn medium analyzed. This work was undertaken to develop a protocol for embryo culture, which will be used for rescuing embryos in interspecific hybridization experiments. Most of the work related to embryo culture was done on the embryo rescue of interspecific and intergeneric crosses, such as *L.esculentum* X *L.peruvianum* (Smith, 1944), *L.esculentum* X *L. Chiense* (Riek and Smith, 1953 ; Riek 1963) and *L.esculentum* X *Solanum Lycopersicoides* (De Vernea et. all, 1987). The present investigations are helpful in rescuing the embryos of interspecific and intergeneric crosses in tomato.

Table 1

Effect of auxins, cytokinins and auxin-cytokinin combination on Morphogenic response of embryo culture of L esculentum cv Pusa Ruby

Hormonal Concentration (mg/L)	% of response	Morphogenic response
IAA 1.0	42	Complete Plant
NAA 1.0	45	Complete Plant
2,4-D 1.0	33	Callus + Roots
BAP 1.0	48	Callus + Shoot buds
Kinetin 1.0	45	Callus + Shoot buds
IAA 1.0 + BAP 0.5	52	Callus + Shoots
IAA 1.0 + BAP 1.0	60	Callus + Shoots
IAA 1.0 + BAP 2.0	69	Green Nodular, Callus + Shoots
IAA 1.0 + Kinetin 0.5	51	Callus + Shoots
IAA 1.0 + Kinetin 1.0	56	Callus + Shoots
IAA 1.0 + Kinetin 2.0	58	Green Nodular, Callus + Shoots
NAA 1.0 + BAP 0.5	60	Callus
NAA 1.0 + BAP 1.0	68	Callus
NAA 1.0 + BAP 2.0	70	Callus
NAA 1.0 + Kinetin 0.5	55	Callus
NAA 1.0 + Kinetin 1.0	52	Callus
NAA 1.0 + Kinetin 2.0	58	Callus

Data scored after six weeks of culture based on 10 repliates.

Table 2

Effect of IAA in combination of BAP and Kinetin on direct regenertaion in embryo cultures of L esculentum cv Pusa Ruby

Growth hormones (mg/l)			Morphogenic response	
IAA	BAP	Kinetin	% of cultures with shoots	Mean No. of shoots per explant
0.5	1.0	--	38	1.2 ± 0.21
0.5	1.5	--	32	1.8 ± 0.28
0.5	2.0	--	47	2.2 ± 0.31
0.5	2.5	--	41	2.5 ± 0.22
0.5	3.0	--	46	3.3 ± 0.39
0.5	3.5	--	50	4.8 ± 0.12
0.5	4.0	--	59	5.4 ± 0.27
0.5	4.5	--	52	6.2 ± 0.21
0.5	5.0	--	62	8.2 ± 0.23
1.0	0.5	--	52	0.8 ± 0.15
1.0	1.0	--	57	1.3 ± 0.11
1.0	1.5	--	58	2.2 ± 0.12
1.0	2.0	--	66	2.5 ± 0.25
1.0	2.5	--	62	2.8 ± 0.32
1.0	3.0	--	61	3.3 ± 0.26
1.0	3.5	--	65	3.6 ± 0.21
1.0	4.0	--	72	5.2 ± 0.16
1.0	4.5	--	58	3.1 ± 0.12
1.0	5.0	--	67	2.4 ± 0.23
0.5	--	1.0	48	0.2 ± 0.23
0.5	--	1.5	52	0.6 ± 0.13
0.5	--	2.0	56	1.1 ± 0.25
0.5	--	2.5	58	1.8 ± 0.21
1.0	--	0.5	53	0.3 ± 0.24
1.0	--	1.0	56	0.8 ± 0.20
1.0	--	1.5	58	1.0 ± 0.21
1.0	--	2.0	60	1.1 ± 0.35
1.0	--	2.5	63	2.0 ± 0.27

Data scored after six weeks of culture based on 10 replicates.

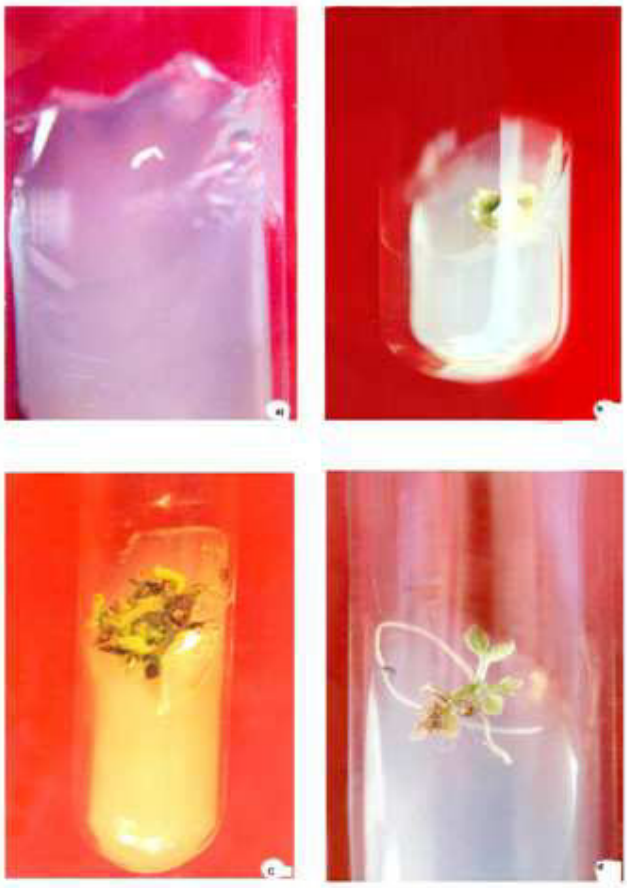

Figure 1

a. Isolated embryo of L. esculentum cv Pusa Ruby on MS medium containing IAA (1mg/L) + BAP (4.5 mg/L).
b. White nodular callus formed from embryo cultured on MS medium fortified with IAA (1.0 mg/L)
c. Adventitious shoot buds formation from embryo on MS medium with IAA (0.5 mg/L) + BAP (5.0 mg/L).
d. Direct plantlet formation from embryo cultured on MS medium supplemented with IAA (1.0 mg/L) + BAP (2.0 mg/L) showing roots.

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