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Research article

STUDIES ON THE EFFECT OF AGE OF *IN VITRO* GROWN SEEDLINGS ON REGENERATIVE RESPONSE OF EXPLANTS IN TOMATO (*Solanum lycopersicum*)

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ABSTRACT: Studies on the effectiveness of age of *in vitro* grown seedlings on regenerative response of explants derived from them were conducted in tomato (*Solanum lycopersicum* L.) cv. PKM-1. The results revealed that cotyledons and hypocotyls of 10 days old seedlings were found to be superior compared to explants collected from 8, 12, 14 days old seedlings when MS medium is supplemented with BAP 1.5 mg/L + Kinetin 1.0 mg/L. These findings could be well exploited for further development of quick regenerative and transformation protocols for the tomato cv.PKM-1.

Key Words: Toamto, *in-vitro* cultures, cotyledons, hypocotyls and MS medium.

INTRODUCTION

Tomato (*Solanum lycopersicum* L. 2n=24) is one of the most important vegetable crop and known as "protective food" because of its special nutritive value. Tomato is a rich source of minerals, vitamins and organic acids (healthy acids). Though, tomato has been subjected to genetic improvement using classical breeding methods for many years, full extent of exploitation of this crop has not been achieved as it is succumbed to several biotic and abiotic factors. Among several factors, the two important factors which limit the progress of breeding efforts are the availability of source of interest in sexually related plants and the duration of the reproductive cycle. The wild relatives of cultivated tomato especially *L.peruvianum* are a rich source of vitamin C. But, it is difficult to transfer these specific traits to cultivated tomato as they are governed by polygenes and existence of specific barriers in inter-specific hybridization with wild relatives. In this context, development of regeneration and transgenic protocols are highly essential. Tomato is very amenable to tissue culture and highly responsive to *in vitro* cultures. Standardization of in-*vitro* propagation protocols in this crop is also essential for the development of efficient transformation procedures. Until now different explants such as leaf discs (Mc Cormick *et al*, 1986), cotyledon and hypocotyls etc., (Park *et al*, 2003) were used for in vitro regeneration and transformation. However, regenerative response is not only dependent on the genotype but also on the age of seedlings while choosing explants. Though several protocols were developed for different varieties, work on regeneration protocol for PKM-1 is so far has not been accomplished. PKM-1 is an adaptable high yielding cultivar widely grown in A.P for its high acidity and is ideally suitable for long distance transport and is also mostly used as a parent for the development of green shoulder hybrids. Hence, studies on the effect of age of *in vitro* seedlings on regenerative response of different explants of tomato cv.PKM-1 has been considered for further development and standardization of in-*vitro* propagation protocols in this variety.

MATERIAL AND METHODS

The seeds of PKM-1 procured from Dept. of Horticulture, TNAU, Coimbatore were used for investigation. The seeds were immersed in sterile double distilled water for 15 minutes and treated with Bavistin 1% solution for 20 minutes followed by thorough rinsing with sterilized water. One drop of Tween-20 was added to the seeds and shaked thoroughly for 5 min and thoroughly rinsed with sterile distilled water for 4-5 times. The seeds were taken in to laminar air flow cabinet, and treated with 5 % NaOCl for 20 minutes of time with occasional swirling.

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They were washed with 4-5 changes of sterile distilled water and were treated with 70% ethyl alcohol for 30 sec followed by washing for 4-5 times with double distilled water. After sterilization the seeds were germinated on MS medium with out sucrose with dark incubation. Cotyledon and hypocotyl explants obtained from 8, 10, 12 and 14 days of *in vitro* seedlings were cultured on MS basal medium supplemented with different combinations of BAP, Kinetin, Zeatin, IAA and IBA. The twenty eight different combinations viz., MS + BAP 1.0, MS + BAP 1.5, MS + BAP 2.0, MS + BAP 2.5, MS + Kinetin 0.5, MS + Kinetin 1.0, MS + Kinetin 1.5, MS + Kinetin 2.0, MS + Zeatin 0.5, MS + Zeatin 1.0, MS + Zeatin 1.5, MS + Zeatin 2.0, MS + BAP 1.0 + Kinetin 0.5, MS + BAP 1.5 + Kinetin 1.0, MS + BAP 2.0 + Kinetin 1.5, MS + BAP 2.5 + Kinetin 2.0, MS + BAP 0.25 + IBA 0.1, MS + BAP 0.5 + IBA 0.1, MS + BAP 1.0 + IBA 0.1, MS + BAP 2.0 + IBA 0.1, MS + BAP 0.5 + IAA 0.1, MS + BAP 1.0 + IAA 0.1, MS + BAP 1.5 + IAA 0.5, MS + BAP 2.0 + IAA 0.5, MS + Zeatin 0.5 + IAA 0.1, MS + Zeatin 1.0 + IAA 0.1, MS + Zeatin 1.5 + IAA 0.5, MS + Zeatin 2.0 + IAA 0.5 were tried on the explants obtained from 8, 10, 12 and 14 days of *in vitro* seedlings for identifying better explant regeneration. The inoculated cultures were incubated in culture rack provided with white fluorescent tubes with a light intensity of 30-40 μ moles under a 16 hour light and 8 hr dark photoperiod regime in a culture room whose temperature was maintained at 25 ± 2°C. The observations for explants response were recorded visually based on bulging or becoming flaccid on transfer to the different combinations of the regenerative medium. The statistical design for studying the better explants response to regeneration among 28 treatments a completely randomized design was used. For each treatment, 10 bottles/plates/test-tubes constituting 4 replications were made. The data were analyzed for standard analysis of variance for various comparisons of the treatment differences.

RESULTS AND DISCUSSION

The data for better explant response in terms of age of the explant for 8, 10, 12, 14 days both in cotyledons and hypocotyls on MS medium with different concentrations of BAP, Kinetin, Zeatin alone and BAP, Kinetin, Zeatin, IAA, IBA in combinations were evaluated and presented in Table 1. Explant response was recorded visually based on bulging or becoming flaccid on transfer to the different combinations of the regenerative medium. The results indicated that the significant differences between treatments were observed only with cotyledonary explants of age 8, 10, 12 days and hypocotyls of 10 days old. Among different combinations used, cotyledons of 10 days old seedlings showed the highest explant response followed by 8 days, 12 days and least response has been observed at 14 days. In terms of hormonal treatments, MS medium + BAP 1.5 mg/L + Kinetin 1.0 mg/L reported high explant response (8.6) when 10 days old cotyledons were used as explant followed by MS medium + BAP 1.0 mg/L + Kinetin 0.5 mg/L (7.3), MS medium + BAP 0.25 mg/L + IBA 0.1 mg/L (6.33) (Plate 1). However there was no significant difference between these treatments. Incase of hypocotyls also 10 days old showed better explant response followed by 8days, 12days and 14 days. In terms of treatments MS medium supplemented with Kinetin 1.0 mg/L and BAP 1.5mg/L + Kinetin 1.0 mg/L (3.6) showed better explant response when 10 days old hypocotyls were used as explant followed by Zeatin 1.0 mg/L + IAA 0.1 mg/L (3.0) and Kinetin 2.0 mg/L (3.0) (Plate 2). However, there was no significant difference between these treatments.

In vitro plant regeneration frequency depends on the age of the explant, type of explant and culture conditions. Response of seedling age in terms of explant response is known to be existed and it has been reported by many workers (Hamza and Chupeau, 1993, Gubis *et al.*, 2004 and Rao *et al.*, 2007). In the present study also cotyledons and hypocotyls of 10 days old were found to be superior compared to explants collected from 8, 12, 14 days old seedlings when MS medium is supplemented with BAP 1.5 mg/L + Kinetin 1.0 mg/L. Among the cotyledons and hypocotyls better regenerative response has been observed in case of cotyledons. In terms of explant response Duzyaman *et al.* (1994), Muthuvel *et al.* (2005) and Grigoriodis *et al.* (2005) reported the superiority of cotyledon explants over the hypocotyl explants in tomato. In contrast, Jabeen *et al.* (2004), Gubis *et al.* (2004), Borge *et al.* (2005) and Rao *et al.* (2007) reported that hypocotyls were superior to cotyledon explants in tomato.

By and large, the present investigation revealed that genotypic variations existed in the regenerative response of explants and the age of the seedlings. Since, seedling vigour and uniformity of germination depends on the seed vigour of the genotype, these results vary with the genotype selected for the study. Similarly variability in regenerative response of the explants were already reported by several workers and also recorded in this study. Hence, these results further support the fact that the regeneration protocols are strictly genotype specific one and the tissue culture regeneration responses vary from genotype to genotype to a greater extent. These findings could be well exploited for further development of quick regenerative and transformation protocols for the tomato cv.PKM-1.

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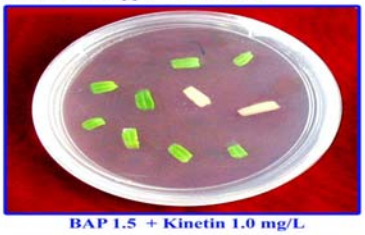
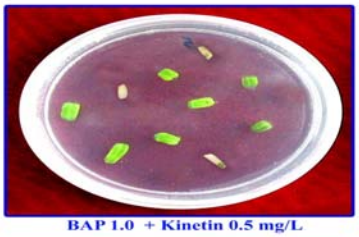
Better explant response in cotyledons of 10 days old seedlings when MS medium is supplemented with



BAP 1.5 + Kinetin 1.0 mg/L

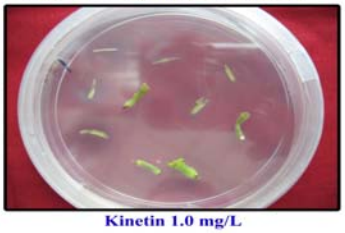


BAP 1.0 + Kinetin 0.5 mg/L

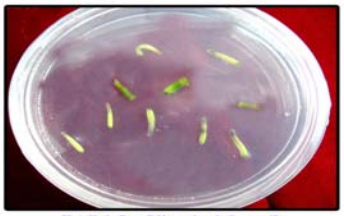
BAP 0.25 + IBA 0.1 mg/L.

Plate-1

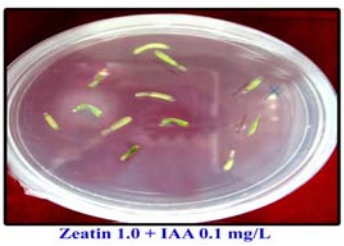
Better explant response in hypocotyls of 10 days old seedlings when MS medium is supplemented with



Kinetin 1.0 mg/L



BAP 1.5 + Kinetin 1.0 mg/L



Zeatin 1.0 + IAA 0.1 mg/L

Plate-2

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Table 1: Effect of Age of *in vitro* seedlings on higher explant response (No. of explants bulged or flaccid) in different hormonal media.

S. No.	Hormonal combination	Cotyledons				Hypocotyl			
		8 days	10 days	12 days	14 days	8 days	10 days	12 days	14 days
1	MS + BAP 1.0	4.0	5.0	3.3	1.0	1.6	2.0	1.6	0.3
2	MS + BAP 1.5	5.0	6.0	4.0	1.0	1.6	2.3	1.3	0.3
3	MS + BAP 2.0	6.0	5.0	4.6	1.0	1.6	1.6	1.3	0.6
4	MS + BAP 2.5	5.3	3.6	4.3	0.6	1.3	1.3	1.3	0.3
5	MS + Kinetin 0.5	3.3	4.3	2.3	0.6	1.3	2.0	1.6	1.0
6	MS + Kinetin 1.0	3.0	4.0	3.0	0.6	2.3	3.6	2.0	0.6
7	MS + Kinetin 1.5	5.0	6.0	4.0	1.0	1.6	2.6	1.3	1.3
8	MS + Kinetin 2.0	3.6	4.6	3.3	0.6	1.6	3.0	1.3	0.6
9	MS + Zeatin 0.5	3.6	4.6	2.6	1.0	1.6	2.0	1.3	0.6
10	MS + Zeatin 1.0	3.6	4.6	3.3	1.3	2.0	2.6	1.3	1.0
11	MS + Zeatin 1.5	3.6	5.0	3.6	0.3	1.6	2.6	1.3	1.0
12	MS + Zeatin 2.0	4.0	4.0	3.0	1.0	1.6	3.0	1.6	1.0
13	MS + BAP 1.0 + Kinetin 0.5	6.3	7.3	5.3	1.3	1.6	2.0	1.3	1.3
14	MS + BAP 1.5 + Kinetin 1.0	7.6	8.6	5.6	1.6	1.6	3.6	1.6	0.6
15	MS + BAP 2.0 + Kinetin 1.5	5.3	5.3	4.3	1.0	2.0	2.0	1.3	1.0
16	MS + BAP 2.5 + Kinetin 2.0	6.0	5.6	5.0	0.6	1.0	1.6	1.3	0.6
17	MS + BAP 0.25 + IBA 0.1	5.0	6.3	4.6	1.0	2.0	2.0	1.6	0.3
18	MS + BAP 0.5 + IBA 0.1	4.3	5.6	4.0	0.6	1.6	1.3	1.3	0.6
19	MS + BAP 1.0 + IBA 0.1	3.6	4.0	3.3	0.6	1.3	1.6	1.3	0.6
20	MS + BAP 2.0 + IBA 0.1	3.3	3.3	2.3	0.3	1.3	1.6	1.3	0.6
21	MS + BAP 0.5 + IAA 0.1	4.3	5.0	4.3	1.0	1.3	2.6	1.6	0.3
22	MS + BAP 1.0 + IAA 0.1	4.0	5.6	3.6	0.6	2.0	2.0	2.3	1.0
23	MS + BAP 1.5 + IAA 0.5	3.6	4.3	3.6	0.3	1.6	1.6	1.3	0.6
24	MS + BAP 2.0 + IAA 0.5	3.3	4.0	2.6	0.6	1.3	1.3	1.3	1.0
25	MS + Zeatin 0.5 + IAA 0.1	4.3	5.3	3.6	1.0	1.6	2.0	1.3	0.6
26	MS + Zeatin 1.0 + IAA 0.1	5.0	6.0	4.3	1.0	2.0	3.0	2.0	1.0
27	MS + Zeatin 1.5 + IAA 0.5	4.0	4.6	3.3	0.6	1.6	2.3	1.3	0.6
28	MS + Zeatin 2.0 + IAA 0.5	3.6	3.6	2.3	1.0	1.6	2.0	1.3	1.0
	(±) S.Em	0.44	0.47	0.40	0.45	0.38	0.41	0.36	0.35
	C.D at 5%	1.25	1.34	1.14	NS	NS	1.18	NS	NS

Note: Observations were taken from ten explants

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