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Short Communication

In vitro studies in capsicum (*Capsicum annum* L.)

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In the present investigation the effect of different growth regulators on callus induction and plantlet proliferation was studied in capsicum (*Capsicum annum* L.). The seeds (Sungro Wonder) were first washed with a detergent teepol under running tap water, dipped in 70% ethanol for 45 seconds and surface sterilized with 0.05 % $Hgcl_2$ for 5 minutes. Seeds were finally washed thoroughly with sterile distilled water 4-5 times and inoculated on MS medium [1] without any growth regulators. After one-month stem (0.5 cm long), petiole (0.5 cm long) and leaf (0.5 cm square) explants were taken for callus induction, while shoot tip and axillary node (0.5 cm long) explants for plantlet proliferation. The MS medium was supplemented with different levels of 2,4-D and Kinetin for callus induction, while with Kinetin and BAP for plantlet proliferation. The cultures were kept in darkness for callus induction, while under light (1600 lux) for plantlet proliferation in the culture room with controlled temperature (25±2°C) and humidity (70 %). The number of days required from inoculation till the first callus appearance was recorded as days to callus initiation and fresh callus weight was recorded after 30 days. The number of days required from inoculation till the first shoot bud appearance was recorded as days to shoot regeneration. The plantlet height, percent regeneration and shoots produced per explant were recorded after 30 days.

The 2,4-D and Kinetin levels had pronounced effect on days to callus initiation and fresh callus weight (Table 1). The mean variation ranged from 9.33 to 14.00 days in stem, 9.66 to 16.66 days in petiole and 10.33 to 18.00 days in leaf explant. The minimum days were required with 0.5 mg/l Kinetin in stem and 4.0 mg/l Kinetin in petiole and leaf explants. The fresh callus weight ranged from 0.34 to 0.98 gm, 0.18 to 0.96 gm and 0.33 to 0.99 gm in stem, petiole and leaf explants, respectively. The maximum fresh callus weight was observed with 3.5 mg/l 2,4-D in petiole and 5.0 mg/l Kinetin in stem and leaf explants. Thus explant and growth regulators play important role in callus initiation and growth [2].

The Kinetin and BAP levels created variable responses for plantlet proliferation (Table 2). The mean variation for days to shoot proliferation ranged from 4.66 to 12.00 in shoot tip and 8.33 to 14.00 in axillary node explants. The minimum days were required with Kinetin levels of 2.5 and 1.0 mg/l in shoot tip and axillary node explants, respectively. The plantlet height ranged from 1.50 to 5.00 cm in shoot tip and 1.33 to 3.66 cm in axillary node proliferated plantlets. In shoot tip proliferated plantlets maximum height was observed with 0.5; while in axillary node proliferated plantlets with 0.5 and 2.0 mg/l BAP. More plantlet height was recorded in shoot tip proliferated plantlets as compared to axillary node proliferated plantlets [3].

Table 1. Effect of 2,4-D and Kinetin on days to callus initiation and fresh callus weight

MS + Growth regulator	Days to callus initiation			Fresh callus weight (gm)		
	Stem	Petiole	Leaf	Stem	Petiole	Leaf
2,4-D (mg/l)						
0.5	10.00	15.66	15.33	0.34	0.25	0.33
1.0	14.00	14.33	15.00	0.62	0.66	0.33
1.5	12.66	15.33	13.66	0.44	0.61	0.43
2.0	12.66	16.66	14.00	0.65	0.64	0.25
2.5	12.00	14.00	18.00	0.64	0.73	0.74
3.0	13.00	13.33	12.66	0.28	0.47	0.50
3.5	11.33	12.00	12.00	0.60	0.96	0.72
4.0	12.33	11.66	12.00	0.52	0.67	0.44
4.5	12.33	13.66	13.66	0.59	0.66	0.47
5.0	15.33	14.33	13.66	0.67	0.57	0.38
Kinetin (mg/l)						
0.5	09.33	12.33	13.00	0.48	0.72	0.95
1.0	11.00	13.33	12.00	0.67	0.82	0.96
1.5	11.33	11.33	11.00	0.57	0.54	0.95
2.0	11.33	14.33	11.33	0.86	0.66	0.67
2.5	10.33	11.33	11.66	0.41	0.49	0.65
3.0	11.00	11.33	11.66	0.58	0.61	0.78
3.5	11.00	10.33	10.66	0.58	0.47	0.33
4.0	11.33	09.66	10.33	0.59	0.41	0.38
4.5	11.00	12.66	11.66	0.53	0.18	0.96
5.0	14.00	11.00	11.00	0.98	0.32	0.99

Table 2. Effect of Kinetin and BAP on different parameters of plantlet proliferation.

MS + Growth regulator	Days to shoot proliferation		Plantlet height (cm)		Plantlet proliferation (%)		Shoots produced/ explant	
	Shoot tip	Axillary node	Shoot tip	Axillary node	Shoot tip	Axillary node	Shoot tip	Axillary node
Kinetin (mg/l)								
0.5	12.0	13.33	4.00	3.33	95.00	91.67	1.00	1.00
1.0	6.66	8.33	4.33	3.16	93.33	88.33	1.00	1.00
1.5	6.00	14.00	4.16	3.83	91.67	86.67	1.00	1.00
2.0	5.00	9.00	1.50	1.33	88.33	85.00	1.00	1.00
2.5	4.66	9.00	2.66	1.66	85.00	78.33	1.00	1.00
BAP (mg/l)								
0.5	6.33	11.00	5.00	3.66	98.33	95.00	1.33	1.00
1.0	6.00	10.00	4.33	3.00	96.67	90.00	1.67	1.33
1.5	7.33	8.66	4.00	3.66	95.00	91.67	2.00	1.67
2.0	6.66	10.00	2.66	2.00	93.33	85.00	2.00	1.67
2.5	8.66	11.33	2.33	1.83	90.00	86.67	2.33	2.00

The percent plantlet proliferation ranged from 85.00 to 98.00 and 78.00 to 95.00 in shoot tip and axillary node explants, respectively. In both the explants, maximum proliferation was recorded with 0.5 mg/l BAP. The shoots produced per explant varied from 1.00 to 2.33 in shoot tip and 1.00 to 2.00 in axillary node. The BAP level of 2.5 mg/l recorded maximum shoots in both explants. Though plantlet proliferation differs with different levels of Kinetin and BAP, the successful proliferation was observed with both the growth regulators [4]. The micropropagation of pepper is on record through shoot tip culture [5].

References

1. **Murashige T. and Skoog F.** 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant., **15**: 473-497.
2. **Arroyo R. and Reuilla M. A.** 1991. *In vitro* plant regeneration from cotyledon and hypocotyls segments in two bell pepper cultivars. Pl. Cell Res., **10**: 414-416.
3. **Kale V. P.** 2005. Studies on variation in tissue culture plants in capsicum (*Capsicum annum* L.). *In*: UGC-CSIR sponsored International conference on modern trends in plant sciences with special reference to the role of biodiversity in conservation. 20-22 Feb. 2005. PP.117. Amravati, India.
4. **Christopher T. and Rajan M. U.** 1994. *In vitro* clonal propagation of Capsicum spp. Pl. Cell Tiss. Org. Cul., **38**: 25-29.
5. **Agrawal S., Chandra N. and Kothari S. L.** 1988. Shoot tip culture of pepper for micropropagation. Cur. Sci., **57**: 1347-1349.