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Short communication

Studies on *in vitro* seed germination of chilli

J.K. Ranjan*, A.K. Chakrabarti, S.K. Singh and Pragya*****
Division of Vegetable Science, Indian Agricultural Research Institute, New Delhi 110 012

Chilli is one of the most important vegetable crops grown worldwide. In India, it is the leading spice crop contributing maximum in production and export. Several biotic and abiotic stresses confront its cultivation and hence genetic transformation may offer some solutions. Tissue culture is an ideal technique for rapid clonal multiplication and complete regeneration of transformed cell/tissue in any plant species. It is most difficult to regenerate under *in vitro* conditions (Ranjan *et al*., 6). Tissue culture permits asexual multiplication of elite or difficult to isolate genotypes, selection for useful variants and recovery of transformed plant from genetically engineered cell (Ranjan *et al*., 7). Since the *ex vitro* raised plantlets are reservoir of several latent microorganisms like viruses, bacteria and fungus, the explants obtained from these plants are met with the problems of infection during *in vitro* culture. One of the solutions of this problem is to take the explant from *in vitro* raised seedlings. Since the information on *in vitro* germination of chilli is meager and also varies from genotype to genotype, hence, information generated on this aspect will be of immense help for those who wish to establish axenic cultures for regeneration healthy plantlets.

Present study was conducted with four cultivars of chilli namely Pusa Sadabahar, KtPL-19, ArCH-1 and Salem, at Division of vegetable science and Central Tissue culture laboratory of Indian Agricultural Research Institute, New Delhi. The experiment was divided in two parts (I) Standardization of techniques for surface sterilization and (II) Standardization of culture medium for enhanced seed germination

For first experiment, two different concentration of $HgCl_2$ (0.1% and 0.2%) were tested for different durations (1, 2 and 3 min.). After sterilization, seeds were rinsed three times with sterile double distilled water under aseptic conditions in laminar air flow chamber and then inoculated in full-strength Murashige and Skoog (4) medium. The per cent uncontaminated growing culture was recorded after 10 days of inoculation. For second experiment, seeds were treated with three treatments *viz*., T_1, T_2 and T_3 (T_1=distilled water soaked for 24 h; T_2= 50mg L^{-1} GA_3 soak for 24 h and T_3 =100mg L^{-1} GA_3 soak for 24 h) and then inoculated on to two media, M_1 (Full strength MS + 2.5%sucrose + 0.7% agar) and M_2 (M_1 + 2 mg L^{-1} GA_3). The percentage germination was calculated on the basis of number of seeds showing germination. The days required for seed germination was based on the number of days required by the uncontaminated cultures to germinate from the day of inoculation.

All the experiments were repeated thrice with a lot of 65-75 tubes per treatment. The data were analyzed as per the factorial completely randomized design (Gomez and Gomez, 3).

The results on uncontaminated growing cultures are presented in table 1. It was found that 0.1 % of $HgCl_2$ for 2 minutes was best for all the genotypes and maximum per cent uncontaminated culture was observed in this treatment. Furthermore, maximum percent of uncontaminated growing culture was observed in KTPL-19. The treatment with 0.1% $HgCl_2$ for 1 minute might not be able to sterilize completely and hence, more contamination was observed in this treatment. The longer duration (3 min.) or use higher concentration (0.2% $HgCl_2$) caused less contamination however the culture establishment was also low. This may be due to the fact that higher concentration of $HgCl_2$ or longer duration of treatment might have damaged the growing embryos of seeds. The results are in close conformity with the earlier results of Christopher *et al.* (2) and Agrawal *et al.* (1).

The percentage of germination was found maximum (73.88) on MS medium supplemented with 2 mg $L^{-1}GA_3$ and when seed were given 24 h of soak in 100 mg $L^{-1}GA_3$ as compared to other media and treatments. This may be due to the fact that gibberellic acid promotes germination by hydrolyzing food material and activate enzyme which helps in seed germination even when it is dormant (Salisbury and Ross, 8). MS media were also found effective for *in vitro* seed germination in tomato (Singh *et al*., 9) and vanilla (Pradeep Kumar *et al*., 5). Significant difference was observed among the cultivars

*Corresponding author's present address: Central Institute of Temperate Horticulture-Regional Station, Mukteshwar, Nainital, Uttarakhand 263138; E-mail: jkranjan2001@yahoo.co.in
**Division of Fruit and Horticultural Technology, IARI, New Delhi
***Division of Floriculture and Landscaping, IARI, New Delhi

Table 1. Effect of surface sterilants and duration of treatments on culture establishment in chilli.

Treatment	Duration (min.)	Culture establishment (%)				Mean
		ktPL-19	Pusa Sadabahar	ARCH-1	Salem	
$HgCl_2$ 0.1%	1	49.96	41.64	52.46	40.98	46.26
	2	90.17	88.90	90.03	84.37	88.36
	3	83.75	81.04	80.08	84.29	82.29
	Mean	74.62	70.52	74.19	69.88	
$HgCl_2$ 0.2%	1	41.07	37.59	29.27	34.88	35.70
	2	78.65	74.81	70.26	71.67	73.84
	3	76.34	67.12	60.53	71.49	68.87
	Mean	65.35	59.84	53.35	59.39	

$CD_{0.05}$: Treatment: 1.14; Duration: 1.40; Variety: 1.62; Treatment X Duration: 1.99;Treatment X Variety: 2.20; Duration X Variety: 2.81; Treatment X Duration X Variety: 3.98

Table 2. Germination of chilli seeds under *in vitro* conditions.

Medium	Treatment	Germination (%)				Mean
		ktPL-19	Pusa Sadabahar	ARCH-1	Salem	
M_1	T_1	25.99	24.29	67.04	56.75	43.51
	T_2	28.86	26.93	70.23	51.00	44.25
	T_3	30.94	30.80	73.95	61.44	49.17
	Mean	28.59	27.34	70.40	56.39	
M_2	T_1	70.19	62.15	65.78	60.16	64.57
	T_2	74.03	68.83	72.18	60.13	68.79
	T_3	80.33	74.48	76.58	64.14	73.88
	Mean	74.85	68.48	71.51	61.47	

$CD_{0.05}$: Media : 0.85; Treatment: 1.04; Variety: 1.20; Media X Treatment: 1.47; Media X Variety: 1.70; Treatment X Variety: 2.00; Media X Treatment X Variety: 2.95

Table 3. Days required for seed *in vitro* germination.

Medium	Treatment	Days				Mean
		ktPL-19	Pusa Sadabahar	ARCH-1	Salem	
M_1	T_1	14.66	12.76	15.02	13.59	14.00
	T_2	10.29	11.86	14.70	11.33	12.04
	T_3	8.54	10.29	13.06	11.34	10.80
	Mean	11.56	11.63	14.26	12.86	
M_2	T_1	12.51	12.52	11.35	11.37	11.93
	T_2	8.62	7.35	8.66	9.48	8.52
	T_3	6.96	7.70	7.38	9.04	7.77
	Mean	9.36	9.19	9.13	9.96	

$CD_{0.05}$: Media : 0.26; Treatment: 0.31; Variety: 0.36; Media X Treatment: 0.45; Media X Variety:0.52; Treatment X Variety: 0.63; Media X Treatment X Variety: 0.90

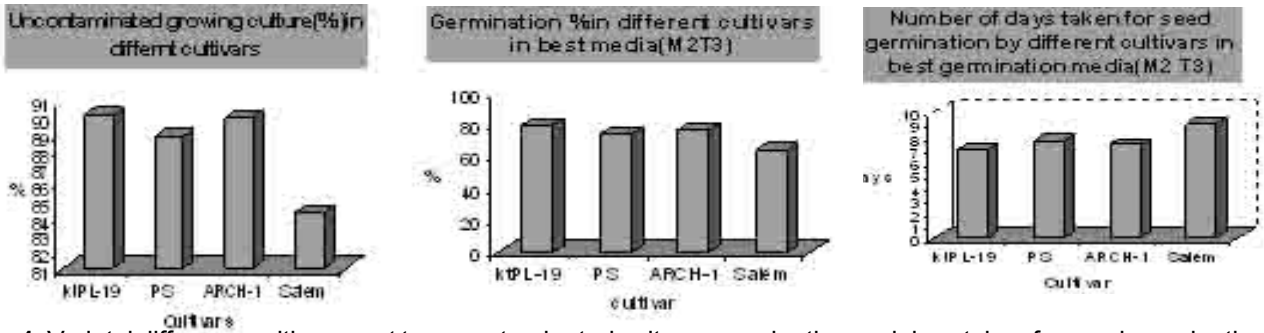

Fig. 1. Varietal difference with respect to uncontaminated culture, germination and days taken for seed germination

regarding per cent of seed germination, which may be due to the genotype of variety. Maximum germination was observed in cultivar KtPL-19 followed by ArCH-1. Days required for seed germination was also minimum in cultivar KtPL-19, when it was treated with 100 mg L^{-1} GA_3 and inoculated in a medium containing 2 mg L^{-1} GA_3.

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