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Studies on the effect of different surface sterilization agents under *in-vitro* culture of Strawberry (*Fragaria* × *ananassa* Duch.) variety "Chandler"

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Abstract
Present investigation Studies on the effect of different surface sterilization agents under *in-vitro* culture of Strawberry (*Fragaria* × *ananassa* Duch.) variety "Chandler" was carried out at the Tissue Culture Laboratory Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut during the year 2018-2019. Effect of two different surface sterilization agents' i.e., mercuric chloride and ethanol were tested on the contamination-free establishment of Strawberry cv. Chandler under *in vitro* conditions. All the sterilization agents performed better results when used individually for different time intervals. Minimum contamination percentage of explants after 10 days (32.72%) was noted under $HgCl_2$ 0.1 per cent treating for a period of 2 min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (68.71%) was noted under the treatment of $HgCl_2$ 0.1 per cent for a period of 2 min. Minimum contamination percentage of explants after 10 days (35.41%) was noted under ethanol 70 per cent treating for a period of 2 min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (70.78%) was noted under the treatment of ethanol 70 per cent for a period of 2 min. The combination of 0.1 per cent $Hgcl_2$ with 70 per cent ethanol was also found effective for sterilization of Strawberry explants. Best results with lower contamination 0.1 per cent with $HgCl_2$ for a period of 1 min. (21.76%). Whereas higher explant survival per cent (79.88%) was observed $HgCl_2$ 0.1 per cent with ethanol 70 per cent for a period of 1 min. The present study concludes that combination use of 0.1 per cent mercuric chloride + 70 percent ethanol for different time intervals was found to be best to generate contamination-free plants in Strawberry cv. Chandler.

Keywords: Sterilization, *in-vitro*, chandler, strawberry

Introduction
The strawberry (*Fragaria vesca* L.) was cultivated as early as 1300 in France. It was appreciated as much for its flowers as for the fruit. It was cultivated in England during tenth to fifteenth centuries. Fragaria x ananassa is a member of the Rosaceae family which also includes raspberry and blackberry. The Fragaria x ananassa is a perennial which arises from a crown of meristematic tissue or compressed stem tissue. Leaves, stems, runners, axillary crowns, inflorescences and roots all arise from the crown. The plant has trifoliate leaves which spiral around the crown, with buds in the leaf axils giving rise to the runners. It is one of the most popular fruits growing in the Northern hemisphere in temperate and sub temperate environment. Strawberry is one of the most important fruit plants which contain vitamin B complex, vitamin C, lots of antioxidant and mineral matters in this fruit plant. So, its cultivation in this region will be helpful for growers as well as consumers for energy as well as better health benefits.

There are many strawberry genotypes grown in tropical and subtropical environment but fruits of which are mostly unpalatable. Strawberry is traditionally propagated vegetatively by rooted runners. To improve the strawberry varieties this method was not suitable due to incidence of many diseases infection and environmental hazards and resulting in the gradual degeneration of cultivars performance. Moreover, the conventional way of production is not adequate to meet the commercial demand. Several improvements of the technology have been proposed by authors working with strawberry (Damiano, 1980; Swartz, 1987) [2, 6] but the highest genotypic, physiological and morphological quality of micro-propagated plants were produced by the method described by Boxus and coworkers (Boxus, 1974; Jemmali *et al*., 1995) [1, 3]. Micro propagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases; In order to increase yield potential, information about genetic variability is necessary, simultaneously micro propagation is the *in vitro* establishment of

contamination-free plantlets. This could be easily achieved by using effective chemical sterilization procedures. Therefore, the present study was designed to develop efficient sterilization procedure for *in vitro* clonal propagation of Strawberry with lower contamination and higher explant survival percentages.

Method and Material

The present study was carried out in the Tissue Culture Laboratory, Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh for developing efficient sterilization procedure for *in vitro* establishment of contamination free plantlets of Strawberry cv. Chandler. The runners and stem of Strawberry cv. Chandler were used as explants to investigate the effects of different surface sterilization agents the explants were collected from healthy and disease-free plants of 4-5 month of age and cleaned thoroughly by repeated washing under running tap water for minimum of 30 minutes, remove the soil and dust particles from the plants. After that explants were treated with 2% Extran (Merck) for 5 minutes with constant shaking and then thoroughly washed with sterilized distilled water to make the plant material free from superficial contamination. Rest of the sterilization procedure was carried out in laminar air flow. Explants were transferred to laminar air flow and rinsed the explants with 70% ethanol for about different time duration in minutes. Thereafter washed the explants with autoclaved distilled water 4 times to remove the ethanol. After that the explants were treated with 0.1% mercuric chloride ($Hgcl_2$) for different time durations, followed by washing with autoclaved distilled water to remove the particles of chemicals. The different concentrations of chemical treatments for ex-plants such as Mercuric Chloride ($Hgcl_2$) 0.l% were used for 2, 4, 6, 8, minutes, Ethanol (70%) 2, 4, 6, 8 minutes, and different combinations of chemical treatment concentrations for ex-plant Mercuric chloride ($Hgcl_2$) + Ethanol 70% for 1, 2, 3, 4, minutes were used. The culture vessels were washed with hot water containing 10% High Spark Cleaning Solution (Hi media) and rinsed with distilled water followed by sterilization in hot air oven at 150 ℃ for one hour. All needed glassware's, equipments and distilled water were autoclaved at a pressure of 15 psi at 121 ºC for 20 minutes. The inside surface of laminar flow was wiped by 70 per cent ethyl alcohol and was sterilized through Ultra Violet rays for 30 min prior to explant sterilization. Finally, the explants were inoculated in a culture room where the temperature was maintained 25 ± 2 ℃, humidity at 60% and either under continuous dark a photoperiod of 16 hr. per 8 hr. light / dark at light intensity of 25µ mole s-2 m-2. All the experiments were conducted in a complete randomized design. The effects of different treatments on various parameters were determined by ANOVA using software OP Stat.

Result and Discussion

Percentage of explants contaminated and survived after 10 and 25 days ($HgCl_2$ 0.1%)

Table 1: Standardization of $Hgcl_2$ (0.1%) treatment period for surface sterilization of explants of strawberry cv. chandler

Treatment	Percentage of explants contaminated after 10 days	Percentage of explants survived after 25 days
2 Min.	32.72	68.71
4 Min.	40.06	62.37
6 Min.	50.27	50.06
8 Min.	70.31	35.64
SEm±	2.58	1.83
CD at 5%	8.57	6.07

Surface sterilization of runner of strawberry cv. Chandler through $HgCl_2$ treatments was significantly increased with increasing of duration from 2 to 8 minutes. Maximum contamination percentage of explants after 10 days (70.31%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 8 min. followed by 50.27 and 40.06 per cent with the duration of 6 and 4 min.; while the minimum (32.72%) was noted under $HgCl_2$ 0.1% treating for a period of 2 min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (68.71%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 2 min. followed by 62.37 and 50.06 per cent with the duration of 4 and 6 min.; while the minimum (35.64%) was noted under $HgC1_2$ 0.1% treating for a period of 8 min. whereas, mercuric chloride gave maximum survival of explants with minimum tissue injury when treated for 4 minutes in strawberry revealed by (Rattanpal *et al.*, 2011) [5].

Percentage of explants contaminated and survived after 10 and 25 days (Ethanol 70%)

Table 2: Standardization of ethanol (70%) treatment period for surface sterilization of runner of strawberry cv. chandler

Treatment	Percentage of explant contaminated after 10 days	Percentage of explants survived after 25 days
2 Min.	35.41	70.78
3 Min.	54.39	56.43
4 Min.	47.15	54.86
5 Min.	55.70	45.74
SEm±	1.92	1.93
CD at 5%	6.35	6.37

Surface sterilization of runner of strawberry cv. Chandler through $HgCl_2$ treatments was significantly increased with increasing of duration from 2 to 8 minutes. Maximum contamination percentage of explants after 10 days (70.31%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 8 min. followed by 50.27 and 40.06 per cent with the duration of 6 and 4 min.; while the minimum (32.72%) was noted under $HgCl_2$ 0.1% treating for a period of 2 min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (68.71%) was noted under the treatment of $HgCl_2$ 0.1% for a period of 2 min. followed by 62.37 and 50.06 per cent with the duration of 4

and 6 min.; while the minimum (35.64%) was noted under $HgC1_2$ 0.1% treating for a period of 8 min. whereas, mercuric chloride gave maximum survival of explants with minimum tissue injury when treated for 4 minutes in strawberry revealed by (Rattanpal *et al.*, 2011) [5].

Percentage of explants contaminated and survived after 10 and 25 days (Hgcl2 with Ethanol 70%)

Table 3: Standardization of mercuric chloride (0.1%) + ethanol (70%) treatment period for surface sterilization of explants for strawberry cv. chandler

Treatment	Percentage of explant contaminated after 10 days	Percentage of explants survived after 25 days
1 Min.	21.76	79.88
2 Min.	47.94	56.38
3 Min.	49.69	51.44
4 Min.	69.70	31.15
SEm±	2.62	1.86
CD at 5%	8.68	6.16

Surface sterilization of strawberry runner cv. Chandler through Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) significantly increased with increasing of duration from 1 to 4 minutes. Maximum contamination percentage explants after 10 days (69.70%) were noted under the treatment of Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) for a period of 4 min. followed by 49.69 and 47.94 percent with the duration of 3 and 2 min respectively; while the minimum (21.76%) was noted under Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) treating for a period of 1 min. A critical observation was recorded as the maximum survival percentage of explants after 25 days (79.88%) was noted under the treatment of Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) for a period of 1 min. followed by 56.38 and 51.44 per cent with the duration of 2 and 3 min.; while the minimum (31.15%) was noted Mercuric Chloride $HgCl_2$ (0.1%) + Ethanol (70%) treating for a period of 4 min. The same pattern was observed to reduce microorganism and sterilize the explant to clean material for *in-vitro* propagation, Ethanol with low concentration of $HgCl_2$ have been used by a number of research workers for disinfection purpose Jalil *et al.* (2003); achieved the contamination free Plantain culture (100%) in explants treated with $HgCl_2$ for 6 min.

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Conclusion
In this way concludes that combination use of 0.1 per cent mercuric chloride + 70 per cent ethanol for different time intervals was found to be best to generate contamination-free plants in Strawberry cv. Chandler.

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