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IN- VITRO OPTIMIZATION OF LOCAL MAIZE CULTIVAR OF MUZFFARABAD, AJK.

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ABSTRACT
This work was carried out to optimize different conditions for surface sterilization of maize (Zea mays L.) explants (seeds) of local variety against microbial contamination. Colorex was used as disinfectant to avoid contamination. Seeds were washed thoroughly with colorex (commercial bleach) with different concentrations like 30%, 50%, 70% and 100% for 30 minutes and then rinsed with distilled and autoclaved water for 20 minutes for three times. Concentration of colorex was the key factor in the sterilization process. The best growth rate was observed in the seeds washed with 30% colorex. Seeds with 100% colorex showed reduced growth.

KEYWORDS: Maize; microbial contamination; surface sterilization; colorex; concentration.

INTRODUCTION
Maize (*Zea mays* L.) ranks the third most significant staple cereal food crop position in the world after wheat and rice.[1] The area on which maize (*Zea mays* L.) is grown is nearly 100 million hectares in 125 developing countries and considered among the three most significantly grown crops in 75 of those countries.[2] By the year 2050 from now, the demand for maize in the developing world will increase by double, and in the 2025, maize production is expected to increase globally, especially in the developing countries. Yet, other factors are involved, but maize yields is being effected and reduced greatly by abiotic and biotic stresses.[3] Production may not be in accordance to the increasing demands without effective technological and policy interventions.[4] To inhibit bacterial and fungal contamination during tissue culture techniques, surface sterilization of explants is key step during tissue culture techniques. So we have to optimize the conditions to avoid this contamination which is prerequisite for in vitro culturing. Different chemicals which are used during the surface sterilization process e. g HgCl2 (mercuric chloride), NaOCl2 (sodium hypochlorite) etc.[5] Plant tissue culture is a collection of techniques used to maintain or grow in-vitro plant cells, tissue or organs through the explants taken from the mother plant in artificial medium under sterilized condition.[6] Though sterilized environment is provided, yet the in vitro plant cultures can be contaminated. Micro-organisms can be present in the explants or can be produced from when handling will not be proper, unclean conditions of the laboratory or from the unsterilized instruments used in the laboratory. Microbial contamination is a continuous problem, which often compromises artificial development cultures. The micro-organisms present in the culture try to absorb nutrients present in the medium of plant tissue culture, so their presence often causes increased culture death or causes irregular growth, deceased tissue growth, lessen shoot propagation and also results in reduced rooting observed by.[7] When explants are taken directly from field grown plants, the problem is worsen.[8]

In vitro propagation consists of various stages, selection of explants, aseptic culture establishment, multiplication, rooting and acclimatization of plants. The most important step for aseptic culture establishment is sterilization of explants. The successful tissue culture of all plant species the elimination of external and internal contaminating agents is necessary.[9,10] In vitro yeast, fungal and bacterial contamination is one of the most genuine issue of commercial and research plant tissue laboratories. Contaminated plants can reduce multiplication and rooting rates or may die. It is necessary to remove foreign contaminants including bacteria and fungi from explants and it is very difficult to obtain sterile plant material completely free from contamination. It becomes more problematic while dealing with woody plant material.[11]

The surfaces of living plant materials are naturally contaminated with microorganisms from the environment, so surface sterilization of explants by chemical solutions is a critical preparation step. Sodium hypochlorite, calcium hypochlorite, mercuric chloride, ethanol, hydrogen peroxide and silver nitrate are commonly used as disinfectants. Sodium or calcium hypochlorite or various commercial bleaches are used for surface sterilization of explants frequently by most of the laboratories.[12] Though the tissue culture usually comprises of growing small pieces of plants in manners that will reduce disease, treating the plant material with sterilizing chemicals and tools used for dissection are sterilized such as the vessels and media in which cultures are grown will wipe out surface microbes.[13]

MATERIALS AND METHODS

Experimental site

This study was carried out at Plant Tissue Culture Lab. of Department of Biotechnology, University of AJ & K. This was done first time here.

Plant Material

Seeds of local *Zea mays* L. variety were collected from Muzaffarabad. Healthy seeds were taken for in-vitro micro propagation.

Laboratory conditions

Temperature was adjusted at 25±2. White light will be 7000 lux. All the instruments were sterilized. Humidity was 60-80 percent. Environment was contamination free.

Autoclaving

All the instruments like petri dishes, forceps, cutter, filter papers and test tubes were used after autoclaving. These all were autoclaved at 20^0C for 15 minutes and the media was autoclaved at 15^0C for 20 minutes.

Surface sterilization with colorex

To avoid bacterial and fungal contamination surface sterilization is necessary, otherwise results will be spoiled**.** Whole work was performed under laminar flow in the presence of uv light, flame and white light. For the purpose of sterilization different concentration of colorex was used as a disinfectant. Different concentrations of colorex used were 30%, 50%, 70% and 100% for the three replications. Explants were washed with these concentrations of colorex respectively for about 30 minutes and this is repeated for three times. After that for the removal of colorex penetration, explants were rinsed with autoclaved water. Again three washings were performed, each for 20 minutes. After that seeds were dried on the autoclaved filter papers.

Use of spirit

During working hands are the main source through which microbes can contaminate the explants. So hands were washed with spirit after some interval or while different instruments like forcep, beakers etc were carried out. This greatly reduced the chance of microbial entrance into the laminar flow.

Culturing of explants

The dried seeds were cultured with the help of autoclaved forceps in the test tubes containing media which were autoclaved and then aseptically covered. Then these were kept in growth chamber for approximately three weeks for in vitro micropropagation.

RESULTS AND DISCUSSION

Concentration of colorex used for the washing of explants was the important factor in sterilization, because explants were effected by a number of contaminants. This was obvious by comparing treated and non- treated seeds, as treated seeds were looking more fresh and clean than those of non-treated. Percentage of colorex effected the growth of the explants for the three replications R1, R2, R3. The plants which were treated with 100% colorex had showed the least and reduced growth as the greater rate of 58.3%. Secondly the explants treated with 50% colorex showed more growth than that of previous one which was 75%. After that the explants ranked which were treated with 70%. The percentage was 79.1%. The percentage of colorex at which the growth rate of explants was highest is 30% which was 87.5%. I had given the same time of exposure to the explants in the washing with colorex. I gave 30 minutes when explants were washed with colorex for three times and 20 minutes were given for the washing with distilled and autoclaved water. So concentration of colorex is more important factor as compared to time of exposure. Work performed on optimization of explants sterilization conditions in sugarcane. They also used different concentrations of colorex with cefotaxime (antibiotic) in three cultivar of sugarcane HSF-240, CP-77-400 and CPF-237[5]. When 50% colorex for 30 minutes with cefotaxime (500 mg/l) was used in HSF-240 and CPF-237 cultivar respectively, 70% and 90% growth percentage was observed. While in CP-77-400 cultivar growth percentage was 85% using 50% colorex for 20 minutes. The percentage of browning was 60% when 100% colorex for 30 minutes was used for CPF-237. The result was that the high percentage of colorex reduced the growth of explants. When washed the seeds of *Echinacea ingustifolia* with 20% colorex for 20 min obtained the results which were showing (76.25%), the highest percentage of free contamination while when treated with colorex for 10 min, they observed (47.50%).[13] While the highest percentage of survived and germinated seeds were recorded (82.5) for 10 min sterilization and sterilization for 20 min showed the lowest percentage of survival (42.50%).

Sterilization with UV

Before starting the experiment uv was on for 15 min to remove any contaminating agent. This also made contamination free environment due to which experiment will be more successful.

Rinsing with distilled water

After treating with colorex explants were rinsed with distilled water three times for 20 mins. The reason was that it removed the colorex which was penetrated in the seed because colorex penetration is dangerous for viability of cells.

Autoclave Sterilization

Another contributing factor was the autoclave. If the media used for the micropropagation was not well autoclaved than explants were more prone to contamination. Media was autoclaved at 15^0C for 20 minutes. Then the result observed satisfactory. In one of the experiments when media was not well autoclaved then all the explants were contaminated with fungus immediately. So for the best surface sterilization of the explants 30% colorex washing for 30 minutes was the best to get contamination free germination of explants.

Optimization of explants sterilization

Table 1. Effects of different colorex concentration on the growth and contamination in explants of local maize cultivar.

Variety	Time of exposure	%tage of colorex	Total seeds	R1	R2	R3	Mean	S.D	%age	Remarks
Local maize	30 mins	30%	8	6	7	8	7		87.5	
		50%	8	5	6	7	6		75	
		70%	8	5	7	7	6.333		79.1	
		100%	8	4	5	5	4.666		58.3	

CONCLUSION

Concentration of colorex was the key factor in the sterilization process. The best growth rate was observed in the seeds washed with 30% colorex. Seeds with 100% colorex showed reduced growth.

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