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IN-VITRO OPTIMIZATION PROTOCOL OF WHEAT CULTIVARS IN NEWLY ESTABLISHED LAB OF PLANT TISSUE CULTURE, MUZAFFARABAD

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ABSTRACT

The experiment was conducted to check the sterilization effect of different concentrations of Clorox on six wheat varieties. This experiment was conducted in plant tissue culture lab of biotechnology, university of AJK. Seeds of selected wheat cultivars were used as an explant and surface sterilized with different (30%, 50% and &70%) concentrations of Clorox. After sterilization they were washed three times with double distilled water for 10 minutes. Different concentrations of Clorox effected differently the seeds. At 30% Clorox maximum germination was observed by the variety Galaxy-01 (16.6625%). Chakwal-50 (12.5%) and Borlog-14 (12.5%) showed the same germination at 30% Clorox. NARC-09 (8.25%) and Millet-11(8.25%) showed the same results and Pakistan-13 (0%) showed no germination at 30% Clorox. Maximum germination at 50% Clorox was shown by the variety Galaxy-01 (29.1625%) and Millet-11 (29.1625%). NARC-09, Pakistan-13 and Chakwal-50 showed 20.825%, 20.825% and 16.6625% germination respectively. Less germination was shown by the variety Borlog-14 (4.1625%). At the concentration of 70% Clorox the maximum germination was shown by the variety Borlog-14, which is 37.5%. Millet-11 showed 25% germination. NARC-09 and Pakistan-13 showed no germination at 70% Clorox. Less germination was recorded by the variety Galaxy-01(12.5%). The results indicated that all varieties behaved differently at different levels of concentrations. It is concluded that for sterilization of wheat seeds the 50% Clorox is better than other concentrations used.

KEYWORDS: Galaxy-01 (16.6625%), NARC-09 (8.25%) and Millet-11(8.25%).

INTRODUCTION

Wheat (Triticum aestivum L.) belongs to the family Poaceae and is growing all over the world. It is considered the main staple food grown all over the world and the most important cereal crop of the Pakistan. Among all the food crops, wheat is considered as one of the most abundant sources of energy and protein for the world population.[1] However wheat is subjected to various biotic (fungal, bacterial and viral diseases) and abiotic (drought, salt) stresses that cause major yield losses. In Pakistan the most common and destructive diseases of wheat are leaf blight, loose smut, stem rust and leaf rust that cause wheat losses up to 50% of yield.

The most important reason for losses is the contamination with microorganisms during in vitro plant tissue culture. Such type of microbes include bacteria, viruses, fungi and yeast etc. A critical stage for tissue culture of plants is to get cultures free of microbial contamination. Before culture for explants, surface sterilization is carried out, which doesn't affect the growth of micro-organisms inside the plant, especially when explants are taken from plants which are grown in fields and transferred to in vitro culture.[2,3]

The biological activity of the living materials should not be lose during the process of surface sterilization. And only the contaminants should be destructed; therefore explants are treated with disinfectant solutions for surface sterilization at suitable concentrations for a specific period of time. The widely using disinfectants are, calcium hypochlorite, sodium hypochlorite, mercuric chloride, ethanol, hydrogen peroxide, bromine water (Rai university lab-manual, 2003) and silver nitrate. As reported on Russcus hypoglossum and and Guizotia abyssinica, that Clorox and mercuric chloride can be used for sterilization of explant.[4,5] Highly efficient results for germ free seeds germination were provided by E. purpurea when its seeds were surface sterilized with 70% ethanol for 30 s and then immersion in 5.4% sodium hypochlorite for 18 min.[6] Actually the seeds are sterilized and germinated in vitro to provide clean

material. Prior to harvesting tissue for culture covering the growing shoots for several days or weeks may supply cleaner material.[7] Explants or material can be washed in soapy water and then placed for 1 to 2 hours under running water.

Hypochlorite is considered very effective and powerful killer of microorganisms particularly bacteria, even minimum concentrations of it are enough to reduce bacterial grow[8]. However, the actual mechanisms of microbial death by this disinfectant is not known. The hypochlorite salts [NaOCl, Ca (OCl) 2, LiOCl, and KOCl], when diluted in water, result to formation of HOCl, and bactericidal activity depends on its concentration.[9] HOCl itself is so reactive as it penetrates into the cells and reach the DNA; So killing of bacteria by HOCl may be due to lethal DNA damage[10]. It seems that HOCL (hypochlorous acid) is so reactive therefore, it reacts with a wide variety of cellular compounds e.g., membranes and proteins, etc. And the formation of these cellular compounds or secondary products is correlated with bactericidal activity.[11] When hypochlorous acid reacts with organic amines, there occurs formation of highly toxic compounds which are known as chloramines, and they are the strong chlorinating and oxidizing compounds that are known to be the actual killing agents. These chloramines have the diffusing property that enter the cells through the membranes and react with intracellular component of the cell, like DNA.[11,12] Sodium hypochlorite (NAOCL) most frequently using laundry bleach is considered the best for the process of surface sterilization. As compared to other sanitizers it can be diluted to suitable concentrations readily and easily available. As compared to sodium hypochlorite, Calcium hypochlorite Ca (OCL) is mostly used in Europe and considered less harmful to plant tissues. It is used in concentration of 3.25%. It is (https://www.msu.edu/course/css/451/).

Besides of strong and powerful sterilizing agent ethanol is considered as highly phyto-toxic agent. Therefore, the explants are exposed to it for a very short period of time i.e., only for a few seconds or minutes. Generally, Ethanol is used prior to treatment with other compounds. The two-step sterilization procedure has provided positive and beneficial impact on certain species. For effectiveness of the sterilization, ethanol is used in the combination with hypochlorite e.g. The use of sodium hypochlorite in 3.5% concentration for about 30 min and 90 or 70% ethanol for 3 min. Sometime, it is important to use monoxenic models in studies of plant-bacterium interactions, in which association occurs between a bacterial strain and surface-sterilized plant. The Most commonly using agents for the process of sterilization are the oxidative agents like household bleach that is composed of sodium hypochlorite. Before the process of bacterial inoculation, seeds are rinsed properly. The disinfectant should have the power of killing all contaminants strongly and lenient enough as it should not eliminate the microorganisms present inside the plant.[13,14]

MATERIALS AND METHODS

The experiment was conducted in plant tissue culture lab of department of biotechnology, university of A J K Muzaffarabad. Tissue culture protocols were adjusted for the first time in the biotechnology Lab University of AJK. Laboratory conditions were optimized as temperature was kept 25±2, humidity was maintained in between 60-80%. White light was 7000 lux. And before experiment UV light in the laminar flow was switched on for 20 minutes. Seeds were collected from NARC (National agriculture research centre) Islamabad. Seeds of six wheat varieties were surface sterilized with different (30% Clorox, 50% Clorox and 70%) concentrations of Clorox for 20 minutes each with continuous shaking under laminar air flow hood. Seeds were washed three times with autoclaved distilled water for 10 min to remove Clorox and then transferred on autoclaved filter papers in sterilized petri plates for drying. After drying they were placed in the autoclaved media for germination. The data was recorded after 3 days of culturing.

RESULTS AND DISCUSSION

Different concentrations of Clorox were used to determine its effect on the surface sterilization of wheat seeds. The results recorded during experiment showed that sterilization with 50% Clorox for 20 min proved the best one. The maximum percentage of seeds germination was recorded with 50% Clorox for 20 min was 29.1625%. However, sterilization with 30% and 70% recorded the lowest percentage of survival. Work reported on RAPD analysis and micro propagation of Echinacea angustifolia.[15] The results indicated that seeds sterilization with 5% Clorox for 20 min and 0.1% mercuric chloride (MC) for 1 min provided the best germination of seeds. The maximum percentage of seed germination and survival was recorded with 10 min (82.50%), and the lowest percentage of survival (42.50%) was recorded with sterilization for 20 minutes.

The work on sterilization of sunflower seeds revealed that combined use of 5% commercial bleach and dry heating at 45°C showed the best result.[16] The significant reduction in contamination and infection of seeds was observed with these sterilization methods, when compared with the sterilization of seeds by 14% commercial bleach and 5% commercial bleach + moist heating. Work was performed on optimization of explant sterilization condition in sugarcane cultivars.[17] The results of the experiment suggested that use of Clorox in different concentrations for different time intervals provided different results. Maximum growth (90%) was observed with 50% Clorox for 30 min. The reduction in the germination of the explant was observed with high concentration of Clorox (100%) with 30 minutes. It is concluded that use of Clorox with different concentrations had a great and clear impact on sterile seeds.[18]

CONCLUSION

The results indicated that all varieties behaved differently at different levels of concentrations. Hence, It was concluded that for sterilization of wheat seeds the 50% Clorox is better than other concentrations used.

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