Seed Sterilization in Soybean for Establishment of Aseptic Cultures

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Abstract: The rise in world population has resulted in a substantial increase in food supply. So we have to develop and identify the plant crops which can be used and or help in combating with this ever-increasing demand of food products other than important cereals (wheat, maize and rice). *Glycine max* one of the most important crops of the world playing an important part in international trade is largely grown in United States of America, India, Argentina, Brazil and China. There is an urgent need to search for alternative non-conventional methods of propagation that can meet the desired objectives of conservation and the ever-increasing demand of food plant selected. In micropropagation aseptic culture establishment is the key to success of such methods and sterilization is first and farmost important step of micropropagation. Due to high percentage of losses during micropropagation because of contamination there is an urgent need of giving attention to sterilization of the explant.

Keywords: Soybean, sterilizing agent, explant, micropropagation, seeds.

Introduction

Soybean is an important oil seed crop of Rosaceae order, Leguminosae family and genus Glycine. *Glycine max* one of the most important crops of the world playing an important part in international trade is largely grown in United States of America, India, Argentina, Brazil and China. Human population is increasing worldwide with a great pace. The rise in world population has resulted in a substantial increase in food supply (Gilland, 2002). So we have to develop and identify the plant crops which can be used and or help in combating with this ever-

increasing demand of food products other than important cereals (wheat, maize and rice). The possible ways to achieve this is through species domestication and developing cultivation practises or *in situ/ex situ* conservation measures for sustainably exploiting them. There are some important factors to ensure first like botanical authentication, genetic potential and improvement, throughout supply during the year. This can be done by cultivating the wild varieties rather than wild collection. Further, elite genotypic selection by checking the variations at molecular and biochemical level can also be practised and exploited. This will help in selection of cultivars and reduce the chances of batch variations. Thus, there is an urgent need to search for alternative non-conventional methods of propagation that can meet the desired objectives of conservation and the ever-increasing demand of food plant. Therefore present study has been undertaken with reference to this immense plant soybean (*Glycine max*).

Biotechnology at present is having the potentiol in transforming human lives. Plant tissue culture is a recognized biotechnology area in the field of agricultural and biological sciences because of its potential use in regeneration of elite genotypes, conservation of commercially and economically valuable plant resources (Sharma et., 2013). The key process of in vitro secondary metabolite production is also a commercially viable sustainable alternative. One can achieve rapid mass multiplication of selected clone in a short period of time from the limited start stock and can achieve enhanced biomass quantities required for various purposes such as conservation of endangered species or to be used as source for phytoactive/bioactive extraction for industrial processes and product development ensuring availability throughout the year, without reducing the natural number and making them endangered (Sharma et al., 2018). In micropropagation aseptic culture establishment is the key to success of such methods and sterilization is first and farmost important step of micropropagation. In past many studies on micropropagation have been conducted and revealed the high percentage of losses during micropropagation due to contaminbation thus urging the need of giving attention to sterilization of the explant.

Material and method

Sample collection

Healthy seeds of soybeans were procured from local market or stores. Water soaking test was done to check viability of the seeds. Put the seeds into a beaker and add some distilled water or tap water in to the beaker. Cover the beaker with foil. Store the beaker at room temperature for overnight.

Material and Instruments required

1. Conical flask, Beaker, Measuring cylinder
2. Water (Distilled water, Tap water, Autoclave distilled water)
3. Petriplates, Forceps
4. Soybean Seeds
5. Autoclave, Laminar air flow, Weighing balance, Hot air oven
6. MS-media(liquid, semisolid)
7. Micropipette, Micropipette tips
8. Chemicals (sodium hypochlorite {NaOCl}, hydrogen peroxide{H_2O_2},Mercuric chloride{$HgCl_2$},Ethanol)
9. Detergent (tween-20)

Glassware and instruments used in the experimentation should be properly washed, cleaned and sterilized using autoclave at 15 psi for 40 minutes after drying them at 120ºC in oven.

Media preparation

Most popular medium in plant tissue culture is used i.e. MS medium (Murashige and Skoog, 1962). Firstly weigh the dry medium and then put the media in clean conical flask and add some distilled water. Then add 0.8%agar as solidifying agent for preparation of media. Mix thoroughly by mixing and heating and then set the pH. After that make final volume and cover the flask with the help of cotton plug. Then autoclave the media at 121℃ for 15 minutes. After autoclaving pour the media into petriplates. Then after 2-3 days seeds are inoculated in petriplates. After 4-5 days results were obtained (Fig.1).

Surface Sterilization

For surface sterilization, the seed explants were first washed properly in running water for atleast 15 minutes and then with liquid detergent (teepol) and Tween 20 for 10 min with shaking. Washed explants were then treated with distilled water to remove detergent for atleast 10 min with continuous shaking, the seed explants were placed in a laminar hood and left as such for atleast 10 minutes to remove the water and further sterilization. Freshly prepared solutions of mercuric chloride, sodium hypochlorite, ethanol, hydrogen peroxide were tested in different concentartions and times to evaluate the best treatment achieving the efficient sterlization (Table 1). After treatment with sterilizing agent, thorough washing of the treated seeds was done 3-4 times with sterile water. This was done to check the any possibility of sterilizing agent on the seed explant..

Table 1. Different sterilization agents used along with their respective concentrations and time of exposure

Sterilizing agent used	Concentration	Exposure time in minutes
Mercuric chloride ($HgCl_2$ w/v)	1. 0.05% 2. 0.10% 3. 0.15%	• 0,1,2,5,10 • 0,1,2,5,10 • 0,1,2,5,10
Hydrogen peroxide (H_2O_2 v/v)	1. 0.5% 2. 1.0% 3. 1.5%	• 0,1,2,5,10 • 0,1,2,5,10 • 0,1,2,,5,10
Sodium hypochlorite (NaOCl w/v)	1. 5.0% 2. 7.5% 3. 10.0%	• 0,1,2,5,10 • 0,1,2,5,10 • 0,1,2,5,10
Ethanol (EtOH)	1. 70% 2. 100%	• 0,1,2,5,10 • 0,1,2,5,10

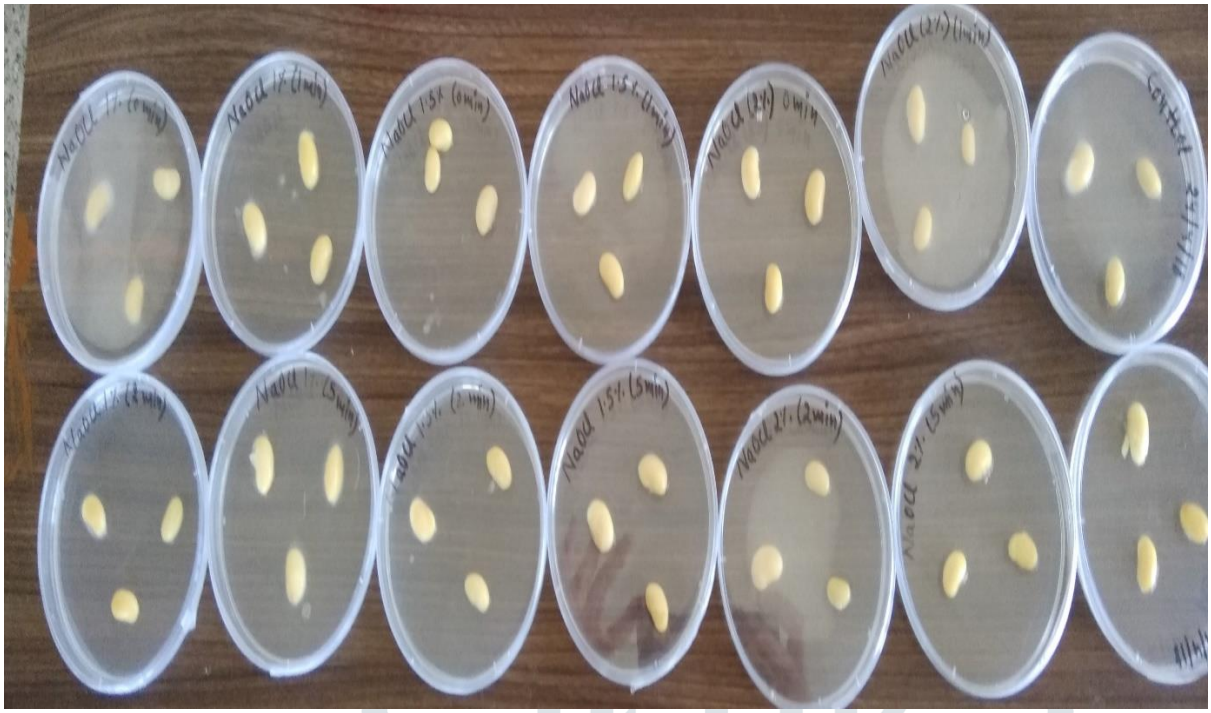

Fig1: Inoculation of soybean

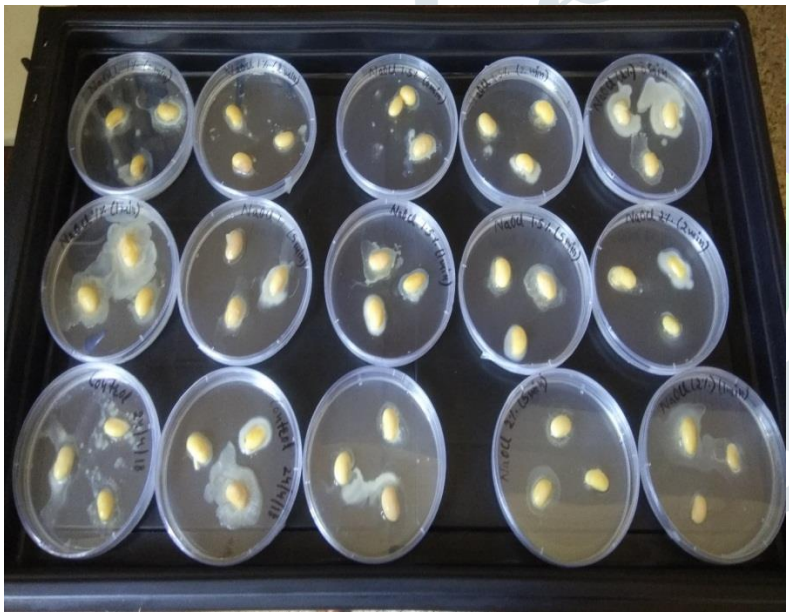

Fig. 2: contamination was observed in NaOCl

Results & Discussion

Seed steralization or explant steralization is a mandatory process for aseptic culture establishment (Srivastava et al., 2010). It should be properly done before inoculation. Various sterilizing agent with different concentration

and exposure times (Table 1) were tested. H_2O_2 gave the maximum germinated and healthy seedlings whem treated at a concentration of 1.5% for 10 minutes. Contamination incidences were more with NaOCl and hence the germination percentage. However, at 1.5% for 5 minutes was observed to be best treatment among all tested for NaOCl. Explant browning and thus poor germination was seen in treatments with $HgCl_2$. However, it checked the incidences of contamination at 0.1% for 10 minutes. Ethanol at both the percentages i.e. 70% and 95% were not effective in establishment of asepsis among the explant and resulted in contamination (Fig.2).

No doubt micrpropagation is an immense tool in the production of disease free propagules, it form the basis for all the in vitro breeding techniques for different plants, variety development using somaclona; variation and transgenics. But the technique suffers from different limitations. The success of micropropagation protocols is influenced by many factors and explant steralization is first and most important factor. As success of tissue culture is also measured through absolute degree of asepsis. Contamination in tissue culture in aseptic culture establishment is governed by many factors such as plant species, age of mother /stock plant, source of explant and prevailing weather conditions. Average losses ranges between 3-15% due to contamination under *in vitro* conditions during subculture in commercial and scientific plant tissue culture laboratories (Leifert *et al.*, 1989), majorily contaminating agents involved are fungal and bacterial (Leifert *et al.*, 1994).

In the present case the use of H_2O_2 as a sterilizing agent for soybean seed as resulted in a good number of aspetic seeds and also influenced the germination potential comparatively. The ethanol was not at all successful in achieving the sterilization. For *in vitro* mass multiplication production of clean and aseptic *in vitro* plantlets is essential that results in contaminant reduction and ensures higher survival rates of the explants (Sharma et al., 2014). The morphological features like softness /hardness of the tissue are very important and play important role therefore requirement of sterilizing agent may differ accordingly for different plant parts or different types of plants. The present work besides concluding its importance in plant biotechnological aspects of propagation also accounts for the successful asceptic establishment and out of various sterilizing agents. This study also opens wide horizons in the field of explant sterilizations and support for the establishment of new sterilizing agents and methods to achieve more higher rates of success.

References

- Gilland B. 2002. World population and food supply: can food production keep pace with population growth in the next half-century,Food Policy 27: 47-63.
- Leifert, C., Morris, C. and Waites, W.M. (1994) Ecology of microbial saprophytes and Pathogens in fieldgrown and tissue cultured plants. *CRC Critical reviews plant science.*13:139-183.
- Leifert, C., Waites, W.M. and Nicholas, J.R. (1989). Bacterial contaminants of micropropagated plant cultures. *Journal of Applied* Bacteriology. 67: 353– 361.
- Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with Tobacco tissue culture. Physiol. Plant. 15:473-479
- Sharma V, Kamal B, Srivastava N, Dobriyal AK, Jadon V. Effects of Additives in Shoot Multiplication and Genetic Validation in Swertia chirayita revealed through RAPD Analysis. Plant Tiss Cult Biotech. 2013; 23(1):11-19.
- Sharma V, Kumari K, Chaudhary P, Sharma S (2018) Elicitor Mediated Enhanced Caulogenesis and Reduced Contamination During Micropropagation in Ocimum Sanctum L. Research J Biotechnology. 13(10):92-97.
- Sharma V, Srivastava N, Kamal B, Dobriyal AK & Jadon VS (2014). Effecient Sterilization Protocol for Different explants of endangered medicinal herb *Swertia chirayita*. Trends in Life sciences. 3(1):5-9.
- Srivastava N, Kamal B, Sharma V, Negi YK, Dobriyal AK, Gupta S & Jadon VS (2010). Standardization of Sterilization Protocol for Micropropagation of *Aconitum heterophyllum*- An Endangered Medicinal Herb. *Academia Arena*. 2 (6):37-42.