EFFECT OF VARIOUS STERILIZATION TECHNIQUES ON

Nodes and Infections

The dissected explants for concentration studies were collected from various sterilization plants in the field of the Institute of Horticulture University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania. Explants were placed under running tap water with detergent for 30 min to remove any foreign contaminants. After washing, explants were disinfested using a laminar air flow hood with rinsed with ethanol 70% for 2.5 min then were washed with distilled water three times for 2.5 min each. For peach tissue culture initiation, four sterilization agents were tested in 18 different variants. Sodium hypochlorite (NaOCl) in three concentrations: 5%, 10%, and 100%, and for 5 and 10 min; Hydrogen peroxide (H₂O₂), in two concentrations: 5% and 10%, for 10 and 20 min; Captan (50%) and WBP fungicide, in four concentrations: 1%, 2%, 3%, and 4%, for 5 and 10 min and for 5 and 10 min. The explants (shoots and nodes) were cultured in MS (Murashige and Skoog, 1962) basal medium supplemented with 30 g sucrose, as carbon source, and 7 g agar. pH was adjusted between 5.7 and 5.8 by using either 1N HCl or 1N NaOH before the agar was added. Media was then heated on a hot plate with continuous stirring using a magnetic stirrer until agar is dissolved and media put in the culture tubes. The culture tubes were covered with lids and put in trays and autoclaved. Autoclave was adjusted at a temperature of 121°C for 15 min. The growth chamber for the all vitro cultures had 22±2°C temperature and 80-85% relative humidity, with a photoperiod of 16 h day light and 8h dark (Shukla et al., 2002). In this study, ten replicates (one explants in one tube culture) were used for each treatment and the experiment was repeated twice.

Results and Discussion

EFFECT OF STERILIZATION FACTORS

The results obtained for various concentrations and time exposure on mean shoots length were presented in Table 3. The results showed that there was a correlation between the dipping period and the concentration of the substance used in the sterilization of explants. In the percentage of mean shoots length reduction the highest number of loss explants resulted. The influence of sterilizing chemical on shoot production functions of microbe's enzymatic system (Rashid et al., 2012). But the increasing concentration and duration of sterilization affects above certain optimum time interval the amount of explants because of the antioxidant chemical ingredient run the plant tissue as well (Danso et al., 2011). These findings are similar to the negative effects of Sodium hypochlorite on high concentration of explants length and leaves number formed when using concentrations less. These results are similar with (Razadost et al., 2013) who confirmed that the least effect of sterilants is observed with explants and plant species.

EFFECT OF EXPLANTS

The study showed there are differences in the extent of the introduction of explants, different embryos micropropagation procedures, and length and leaves number, and the different concentration and rate survival of explants (table 2 and 3). Also, the shoots had been registered the lowest rate of infection and the highest increase the concentration culture. The results showed that there were different depend on the sterilization factors, exposure time and explants for the sterilization protocol. The protocol is recommended for this study to be used among the different sterilization variants, sodium hypochlorite was the most effective treatment with 50% survival rate at V5 (15% for 5 min) and 65% survival rate at V4 (10% for 10 min). Recommended also the sodium peroxide (H₂O₂) at 10% for 20 min, Captan at 5% for 5 min; Boric acid (B(OH)₃) at 2% for 10 min recommended to use in the initial sterilization.

Conclusion

Among the different sterilization protocols tested for the surface sterilization and micropropagation protocols, the protocol is recommended. Our results showed that during the sterilization were different depend on the sterilization factors, exposure time and explants for the sterilization protocol. The protocol is recommended for this study to be used among the different sterilization variants, sodium hypochlorite was the most effective treatment with 50% survival rate at V5 (15% for 5 min) and 65% survival rate at V4 (10% for 10 min). Recommended also the sodium peroxide (H₂O₂) at 10% for 20 min, Captan at 5% for 5 min; Boric acid (B(OH)₃) at 2% for 10 min recommended to use in the initial sterilization.

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References

