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Research Article

MICROWAVE OVEN BASED STERILIZATION OF MEDIA FOR MICRO PROPAGATION OF BANANA

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ABSTRACT
Micropropagation of banana (*Musa paradisiaca* Linn.) var Grand naine was carried out with media sterilized by microwave radiation. Microwave oven was effectively used to sterilize the media for *in vitro* shoot-multiplication of banana against autoclave. Sterilization of media in microwave oven was successfully achieved at 900W/200 ml media (4 min). *In vitro* shoot-multiplication was higher in the microwave sterilized media as compared to the same in autoclave sterilized media.

Key Words: *Banana, Micropropagation, Media Sterilization, Microwave and Autoclave*

INTRODUCTION
Banana (*Musa paradisiaca* Linn.) is a highly valuable commercial crop belonging to monocot class. Banana is cultivated worldwide over an area of 4.8 million hectares, with an annual production of 99 million ton per annum (Anonymous, 2010). The whole plant is cut and replaced with fresh plantation. Being a monocarpic triploid in origin, underground rhizome and suckers are used for its propagation. The Cavendish variety, Grand naine, is a high yielding variety that produces bunches having 10 to12 hands with 175-225 numbers of fruits.
Plant tissue culture technology provides large number of plantlets within a short span of time irrespective of season and weather conditions. The demand for tissue cultured banana plantlets increases at a high rate of 25-30 % in India (Anonymous, 2005).
In present study, an alternative way of media sterilization was evaluated for multiplication of banana. This can reduce electricity cost remarkably as well as minimize time and labour required for sterilization in autoclave.

MATERIALS AND METHODS
Suckers of Grand naine variety of banana (*Musa paradisiaca* Linn.) were collected from Gujarat Green Revolution Company, Umareth, Gujarat in the month of June. They were washed under running tap-water (30 min) and trimmed to remove outer scales. The washed suckers were pretreated (20 min) with a mixture of 0.05 % of Carbendazim (Bavistin) and 0.1 % of activated charcoal on gyratory shaker (100 rpm). After thorough washing with distilled water (3 times) the apical shoots were isolated for inoculation (Vora and Jasrai, 2011). The multiplication was carried out in MS medium (Murashige and Skoog, 1962) containing 3 mg/l BA, 3 % sucrose and 0.8 % Agar-Agar (Cronauer and Krikorian, 1984) in culture bottles (10X5 cm). Shoot-clumps with 5 shoots each were transferred to MS medium containing 5 mg/l BA during subsequent shoot-multiplication cycles.
For media sterilization, microwave oven was utilized as an alternative to autoclave. The voltage and time period required for complete sterilization of media was standardized. For this, five culture bottles containing media (40 ml/bottle) for proliferation of banana were subjected to different wattages (180-900 W) in a microwave oven (Model CE1031LAT/XTL, Samsung Electronics) for different periods of time (10 s to 5 min). *In vitro* shoot-multiplication of banana was carried out in microwave oven sterilized media; while the media sterilized in autoclave at 121 ºC for 20 min was the control. The culture bottles were cleaned with soap solution (5 %, Laboratory detergent, Burgoyne Co) followed by a thorough wash with tap-water, rinsed with distilled water, dried and kept in hot-air oven at 160 ºC (2 h) prior to pouring media and sterilization.
All the cultures were kept in the culture room with 25$\pm$1 ºC temperature and 16 h photoperiod. For each treatment, 15 replicates were used during 7th subculture cycle. Data for each treatment was analyzed after 4 weeks in terms of increase in number of shoots, shoot-length and number of leaves.

Rooting of the regenerated shoots was carried out on half-strength MS medium with 3 mg/l IBA. Hardening of the rooted shoots was carried out after 4 weeks (Jasrai *et al*., 1999).

RESULTS AND DISCUSSION

Sterilization of media in microwave oven was successfully achieved at 900W/200 ml media (5 culture bottles with 40 ml each) for 4 min. *In vitro* shoot-multiplication of banana was higher in the media sterilized in microwave oven by 90 % as compared to the control (Figure 1). However, increase in length of shoot was the same in both control and microwave based sterilization. The number of leaves produced per largest shoot was higher in the microwave oven sterilized medium by 38.4 % over the control (Figure 1). Moreover, the medium sterilized in microwave oven showed better transparency as compared to that in autoclave (Fig. 2 A-C).

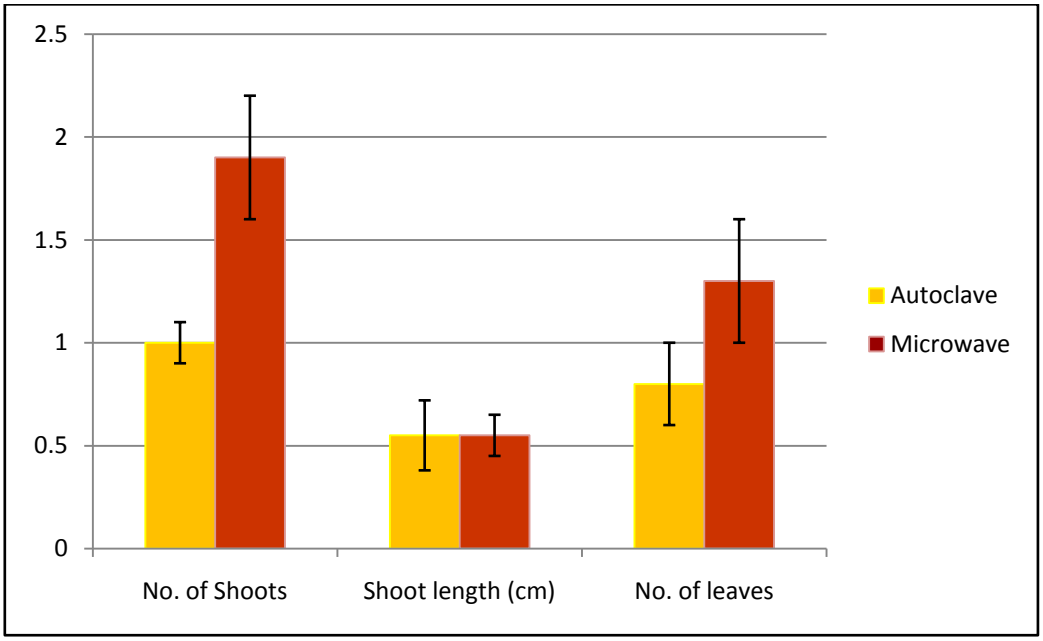

Figure 1: Effect of microwave oven based sterilization on *in vitro* shoot-multiplication of banana

During autoclave sterilization, breakdown of monosaccharides and precipitation of certain micronutrients, leading to the reduced quality of MS medium was noted by polarographic measurements (Schenk *et al.*, 1991). Autoclave sterilization caused sucrose hydrolysis by 56 % and 20 % in MS medium with and without activated charcoal respectively (Pan and Staden, 1999). The media sterilized in autoclave may have toxic and adverse effects on *in vitro* cultures (Buter *et al.*, 1993; Sawyer and Hsiao, 1992 and Wang and Hsiao, 1995). Disadvantages of autoclaved medium like pH changes, formation of precipitates and occurrence of trace metal contamination resulting from steam impurities are prevented in microwave sterilization (Keller *et al.*, 1988 and Tisserat *et al.*, 1992).

Sterilization of tissue culture media with home-type microwave oven for 5 min was earlier reported for strawberry culture (Wood and Lundergan, 1981). In addition, microwave treatment at 700W/ 1.5 l volume for 10 min was found effective for sterilization of phytoplankton culture media, eliminating bacterial, algal and fungal contamination (Keller *et al.*, 1988). Similarly, 700 W for 5 min and 50 min to sterilize 100 ml and 3000 ml media respectively was found necessary to culture strawberry, lemon and carrot with similar growth rates in autoclave (Tisserat *et al.*, 1992). Moreover, microwave sterilization of plastic tissue culture vessels (3 min) was found to be effective, rapid and relatively inexpensive to inactivate different test viruses, certain bacteria and fungi (Sanborn *et al.*, 1982). In addition to microwave radiation, gamma irradiation treatment was reported to be effective for sterilization of medical instruments, culture vessels (Prakash *et al.*, 2004), stored grains (Warchalewski *et al.*, 2000) and edible gelatin (Fu *et al.*, 2000) at low dosage. Microwave treatment to seeds was noted to enhance germination, plant height and fresh mass in ornamental perennial crops (Aladjadjiyan, 2002).

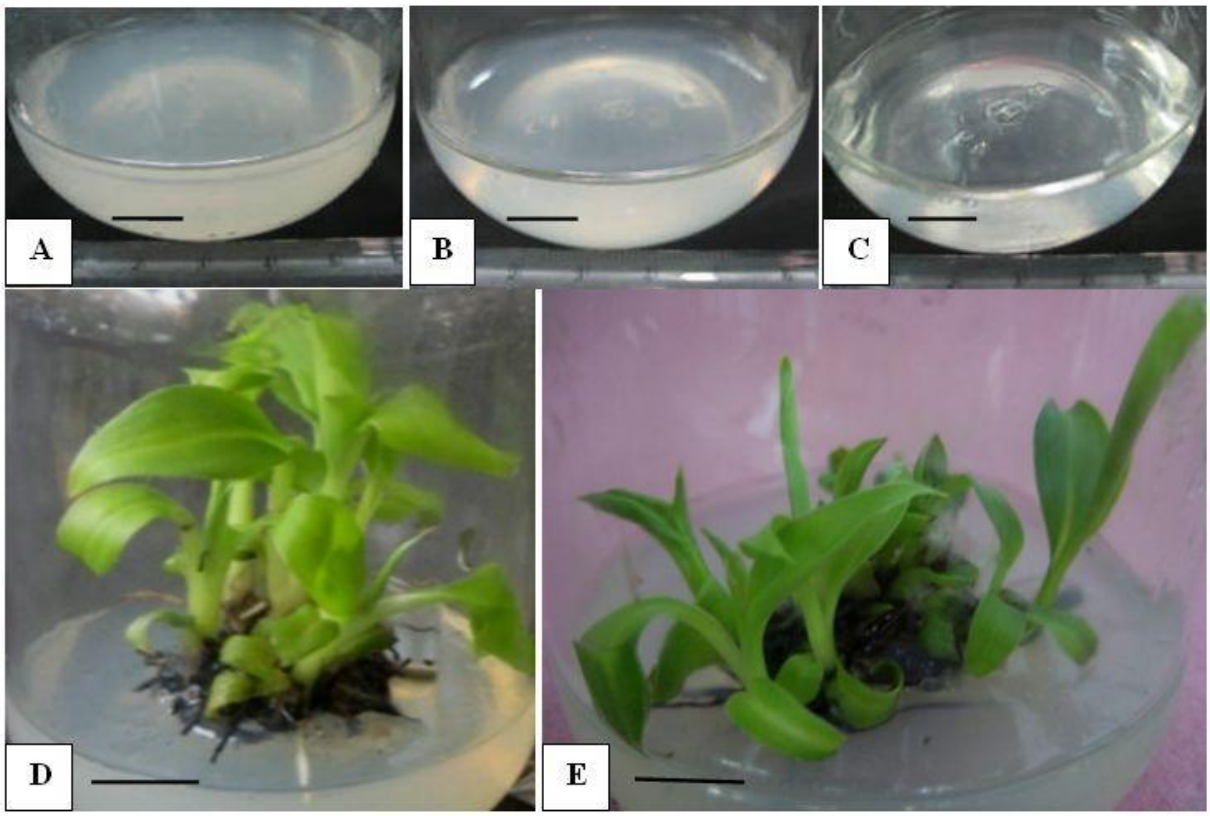

Figure 2: A - Medium gelled with Agar-agar and sterilized in autoclave; B - Medium gelled with Agar-agar and sterilized in microwave oven, showing better transparency of the gel; C - Medium gelled with Gelrite and sterilized in autoclave for the comparision of the gel transparency; D – *In vitro* shoot-multiplication on microwave sterilized medium; E - *In vitro* shoot-multiplication on autoclave sterilized medium (Horizontal bar = 1cm).

Banana is the most important commercial crop, which is being multiplied through micropropagation technique. For commercial purpose, high rate of proliferation as well as cost-effective micropropagation protocols (Ganapati *et al.*, 2002; Vora and Jasrai 2012; Vora, 2011 and Das and Gupta, 2009) are always favoured by the tissue culture units. In conclusion, Microwave sterilization provided higher multiplication rate and was found easier, quicker, consuming lesser time and electricity than autoclave sterilization.

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