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Callus Induction from Seeds of *Zea mays* Var. EV-2097

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Abstract: The present study was conducted on maize, with the aim to work out a suitable surface disinfecting procedure for seeds and to know the best concentration of 2,4-dichlorophenoxyacetic acid (2,4 -D) for callus induction in N6 medium using var. EV-2097. Better disinfection of seeds was achieved with 30 minutes Clorox treatment (86 %). Maximum callus induction (47 %) was recorded with 2 mg l^{-1} followed by 3 mg l^{-1} (18 %) and 1 mg l^{-1} 2,4 -D (16 %).

Key words: *Zea mays*, callus, 2,4 -D., cereals, seeds disinfection

Introduction

Maize belongs to family Gramineae and ranks as one of the four principal cereal crops of the world (Leonard and Martin, 1963). It is used primarily as food for human in most areas of the world and also used as feed for animals (Jugenheimer, 1976). Pakistan has an agricultural economy and maize along with other major crops such as wheat, rice and cotton continues to account for nearly ninety percent of the value added in the agricultural crop sector (Anonymous, 2000).

Maize is especially well suited for research and development due to its high genetic diversity as it is a cross-pollinated crop (Walden, 1978). In order to obtain best varieties having desired agronomic characters different non-conventional techniques should be applied. Tissue culture technique is being used for a long time in this respect. Many cereals have been brought into culture and complete plantlet redifferentiated from their respective calli, following sequential application of plant hormones. Callus is a rapidly proliferating undifferentiated mass of cells, which can be obtained by culturing explants on nutrient media containing specific growth hormones. Callus culture maintained by subculture may consist solely of cells that are tetraploid or octaploid with few diploid cells, hence show genetic heterogeneity. Organization can be brought about in callus by the controlled initiation of organ primordia through manipulation of nutrients and hormones in culture media (Narayanaswamy, 1990).

Calli of different species may vary in the texture, friability and colouration. They may be pale yellow or albino, chlorophyll or anthocyanin pigmented (Narayanaswamy, 1990). As trigger compounds for callus induction auxins are essential for most plant tissues. The most effective auxins are synthetic, e.g. 2,4 - dichlorophenoxy acetic acid (2,4-D) and naphthalene acetic acid (NAA) (Yamada, 1990). Other auxin-like plant growth regulators, particularly analogs of 2,4-D such as p-chlorophenoxy acetic acid (MCPA) and 2,4,5-trichlophenoxy acetic acid (2,4,5-T) have also been used with limited success (Close and Ludeman, 1987).

Since, the first successful callus induction of cereal plants from the young endosperm of maize (Straus and LaRue, 1954), a large number of inbreds and hybrids have been studied and regenerated from tissue cultures. Chu (1978) pointed out that N6 medium is a suitable synthetic medium for anther culture of cereal crops. Piralov and Abraimova (1999) studied callus culture of maize inbred DK 675. Best results were obtained when 2,4-D was lower than 1 mg l^{-1} and when calli were cultured at high densities. Lowe *et al.* (1985) reported that embryogenic callus could be distinguished by its friability, rapid growth rate and mucilaginous texture. Sawahel and Ali (1994) cultured maize kernels for callus induction. They observed that mostly callus grew from coleoptile and seedling shoot. Li *et al.* (1999) reported highest frequency of embryogenic calli using immature embryos. Dicamba and 2,4-D found to have similar effect on callus initiation.

The maize variety, which was used in present study, is an experimental variety. It has stay green character, shows early maturity and is comparatively drought tolerant. It has not been tested for *in vitro* manipulation via tissue culture. The objectives of the study were to develop a suitable procedure for surface sterilization of seeds and to investigate a suitable concentration of 2,4-D for callusing in N6 medium.

Materials and Methods

The present study was carried out in Agricultural Biotechnology Institute at National Agricultural Research Centre. Seeds of *Z. mays* var. EV-2097 were used as explant source, obtained from Crop Science Institute of the same centre.

Seed disinfection: Clean, healthy and nearly equal sized seeds were sorted out, washed thoroughly with distilled water and a dilute detergent solution. For disinfection seeds were divided into two groups. One group was dipped in concentrated Clorox for 15 minutes and the other for 30 minutes. Seeds were then washed three times under aseptic conditions with autoclaved water.

Media preparation: N6 basal media (Chu *et al.*, 1975) was used for disinfection experiment. For callus induction N6 basal medium with different concentrations (1,2 and 3 mg l^{-1}) of 2,4-D was used. After addition of all components, pH of the media was adjusted at 5.8. Media were then solidified with 0.6% agar and poured into clean and dried test tubes followed by plugging with sterilized cotton. For sterilization, media were autoclaved at 121 °C and 15 psi for twenty minutes.

Inoculation: For determining efficiency of disinfection procedure, both seed groups were cultured on N6 basal medium. All cultures were incubated at 25 ± 3 °C for sixteen hours photoperiod (2000 lux). Calli were transferred for maintenance on similar medium after four weeks. There were two replicates for all treatments. Two parameters i.e. percentage of contamination and callus induction frequency were monitored. Callus appearance and colour was also noted. The data so collected was statistically analyzed by applying test for two proportions in case of disinfection experiment. ANOVA was applied for callus induction experiment, followed by LSD to determine significant difference among the treatment means.

Results and Discussion

Sterilization of explants is a prerequisite for inoculation in a tissue culture experiment. Historically different sterilizing agents have been used for this purpose, which include different alcohols, mercuric chloride, hypochlorites of Na^+, K^+ and aldehydes etc. Among these, Sodium hypochlorite alone or in combination with ethanol has proved not only to be very effective but also safe and economical (Russell *et al.*, 1992). In this study sodium hypochlorite, available as commercial bleach was used for surface sterilization of maize seeds. Undiluted bleach was used for 15 and

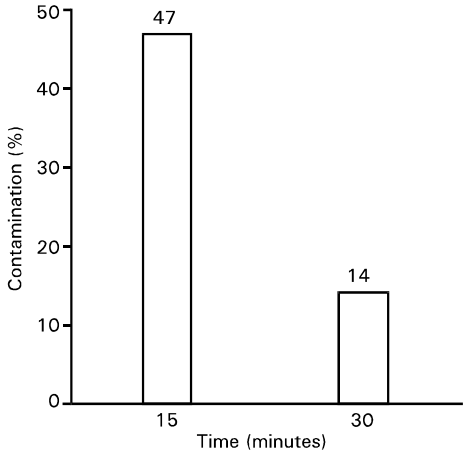

Fig. 1: Effect of duration of exposure in 100% clorox on maize seed disinfection

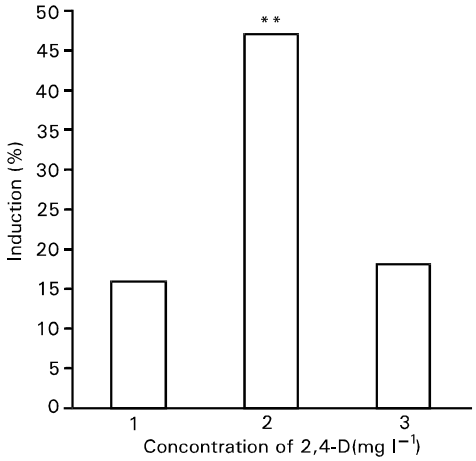

Fig. 2: Seeds of maize variety EV-2097 were inoculated in N media containing 1,2 or 3 mg l^{-1} 2,4-dichlorophenoxy acetic acid. Percent callus induction was recorded and data was analyzed by ANOVA. ** indicates highly significant difference

30 minutes resulting in 53 and 86 % surface sterilization respectively. The data collected from disinfection experiment showed that percentage of contamination was significantly lower with 30 minutes treatment of concentrated Clorox (14%) while higher with 15 minutes exposure i.e. 47% (Fig.1). With 15 minutes treatment, germination frequency was higher and browning of seeds was not observed. On contrary in 30 minutes treatment less contamination was observed, however germination frequency was lower and occasional browning was also observed (Data not shown). For callus induction three treatments (1, 2 and 3 mg l^{-1}) of 2,4-D were used. Calli were induced on all three concentrations. Callus induction in case of 1 and 3 mg l^{-1} of 2,4-D was not significantly different. For 2 mg l^{-1} of 2,4-D it was significantly different from other two treatments. Callus induction frequency was highest when 2 mg l^{-1} of 2,4-D was used (47%) while it was 16% in 1 mg l^{-1} and 18% in 3 mg l^{-1} treatments (Fig. 2).

2,4–D is a synthetic auxin and is very commonly used in tissue culture experiments for callus induction. It is comparatively stable (Wernike *et al.*, 1986) and is very slowly metabolized by cells (Ashton and Crafts, 1981). Maximum callus induction was obtained when concentration of 2, 4–D was 2 mg l^{-1}. Callus induction started after a week of culturing the seeds. Colour of calli changed from off-white to lemon yellow during one month time period. Calli were soft and slimy in texture, but not very compact. After four weeks these were subcultured on similar media. Following subculture calli grew in size. Small globular structures also appeared on the surface of some calli.

Microscopic examination of one and two weeks old calli revealed the presence of small cells indicating rapid division, while one month old calli were composed of large elongated cells indicating slow dividing activity. These results are consistent with the findings of Ashton and Crafts (1981), who reported that low levels of 2,4–D induce cell enlargement by increasing the activity of autolytic and synthetic enzymes responsible for cell wall loosening and synthesis of new cell wall materials. Turgor pressure *per se* causes the actual cell enlargement.

The maximum induction frequency obtained in the present study with 2 mg l^{-1} of 2,4-D is consistent with the results of Pareddy and Petolino (1990), using MS medium and immature inflorescence of maize. Some other researchers (Lowe *et al.,* 1985; Piralov and Abraimova, 1999) have reported maximum callus induction with 2,4-D less than 2 mg l^{-1}. This difference may be attributed to genotypic differences as different genotypes have been reported to show different responses on same media (Naqvi *et al., 1989;* Pareddy and Petolino, 1990).

It was observed that although maximum callus induction among 1, 2 and 3 mg l^{-1} was achieved with 2 mg l^{-1} of 2,4–D, the induction efficiency was low (47 %). Many seeds cultured on the same media did not induce callus rather grew into shoot and root. It is concluded from this study that efficiency of callus induction of maize var. EV-2097 is not very high with N6 medium and that too laid within a close range. Some other media recipes however, of 2,4-D may yield better results.

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