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# Callus Induction and In Vitro Plant Regeneration of Indian Maize Inbreds by Embryo Culture.

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## Abstract
Regeneration ability and callus induction of three Indian maize inbred lines HKI335 and HKI1105 and LM5 were evaluated. Immature embryos were used as explants. Genotype, medium type of auxin and their concentrations influenced callus induction MS medium supplemented with different concentrations of 2, 4 D (0.5 – 2 mg/L) were used for callus induction. MS media supplemented with 2mg/L of 2, 4D has shown highest percentage of callus induction 80%. Among the three genotypes tested HKI335 and HKI1105 both have shown higher regeneration percentage.

## Key Words
Maize, Invitro culture, regeneration.

## Introduction
Maize (zea mays L.) is the most important crop in the world in terms of global annual production (food and agricultural organization 2009). Maize is top ranking cereal in terms of productivity and has significance as human food, animal feed and fodder and industrial products (raw material as maize) like corn starch and in fermentation and distillation industries. The demand for maize is increasing across the world, and more predominantly in Asia (Wada et.al 2008) due to uses of uses of maize and its based products. Genetic transformation of maize with genes conferring resistance to biotic and abiotic stress conditions, obtained using tissue culture compared to plants produced using conventional plant breeding methods. Immature embryos are predominantly used for establishing regeneration competent cells or callus cultures for genetic transformation, Green and Philips (1975) first reported regeneration of maize from immature embryos. Golden Kamm et.al (1990) first developed transgenic maize for biolophas resistance. Koziel et.al (1993) developed insect – resistant transgenic maize with Cryl 1 Ab for the first time. Monsanto has actively involved in transgenic research for drought tolerance is maize variety. Wang (1981) successfully regenerated plants from mature embryos of two maize inbreds B73 and MO17. With the rapid development of tissue culture techniques, many types of explants, including genetic embryos and leaf tissue has been successfully regenerated into plants by tissue culture (Aulinger et.al 2003, Huang and Wei 2004, Ahamadabad et.al 2007). Screening of genotypes for invitro plant regeneration is important. Tissue culture technique is vital to many areas of plant science and crop improvement. The objective in the present investigation was to establish a reproducible, regeneration protocol for maize inbred lines and to compare the efficiency of different sources of auxins on callus induction and effect of cytokinin-auxin combination on regeneration in the inbred lines.

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## Materials and Methods
Three tropical Indian maize inbred lines namely, HKI335 and HKI1105 and LM5 were used in the study. Immature kernels were extracted and washed with tween-20 (1-2 drops) followed by surface sterilization with sodium hypo chloride (0.6%) for 20 min. Subsequently immature kernels were washed with 70% ethanol for 30 sec and revised five times with sterile water. Immature embryos of 1.0 – 2mm size were aseptically excised from surface sterilized kernels under laminar flow and placed with scutellar side up and flat surface down on the callus induction medium solidified with 0.8% agar. Callus induction media and regeneration medium included MS (1962) medium supplemented with various concentrations of 2.4D (0.5 to 2mg/L) IAA, IBA, BAP (0.5 to 2mg/L) and sucrose (2%). pH the different media was adjusted to 5.6 prior to autoclaving at $121^0$C for 20 min. Explants were

inoculated and incubated in the dark for 24 h at $25^0$C ± and 16 h photoperiod.

## Results and Discussion

Maize genotypes have profound differences for in vitro culture (Armstrong and Green 1985) and only a few numbers of maize genotypes possess regeneration in embryos culture method. Hence it is important to standardize an efficient protocol which is done in present study. All genotypes responded at various concentrations of 2, 4D (0.5-2mg/L) alone for callus induction. Callus indication was observed within 1 week of culture with swelling of the scutellum. The frequency of callus induction varied at different concentration of 2, 4D as shown in (Table 1) callus was friable and organogenic in nature which is obtained from immature embryos. The highest frequency of callus induction was observed on the MS medium supplemented will 2, 4D 2mg/L for all three genotypes but the response of or callusing reduced as the level of 2, 4D further increased in the medium (plate 1). These findings were similar to those reported by Al Abed et. al (2006). Where by elevated levels of 2, 4D decreased maize callus induction and resulted in browning of calli. After 2 weeks organogenic calli were transferred to regeneration medium (MS medium supplemented with different combination of cytokinin and auxins BAP, IBA IAA at different concentration (0.5 to 2mg/L). Sheet initiation was observed after 3 weeks of culturing on MS medium supplemented with BAP 1mg/L + IAA (0.5mg/L) gave good response and standardized for shoot development (Plate1). IBA did not initiate shoot development. No separate rooting medium was used for shooting. Regenerated plantlets were transferred into sand and soil 1.2 ratio containing plastic cups, later into pots maintained in green house. HKI335 and HKI1105 showed a maximum of 45% of regeneration followed by LM5 (30%). Analysis of variance revealed genotypic difference which was highly significant for regeneration. This implies differential genetic material for regeneration in tested genotypes (Carvalho et. al.1997, Binnat et.al. 2008) reported that not all tropical genotypes that initiated embryogenic calli could regenerate plants and also some genotypes classified as non embryogenic. This shows that plant regeneration is achievable in both embryogenic and non embryogenic genotypes under appropriate tissue culture conditions.

## Discussion

Genotypes are reported to play an important role in callusing response in various crop plants including maize (Bohorova et.al 1995) reported genotype dependent regeneration response among tropical and sub tropical maize lines. Genotype dependent regeneration response has also been reported by various authors (Wenbin. et.al 2002). Genotype differences are terms of regeneration response might to relate to variations in endogenous harmonic levels (Bhaskaran and Smith 1990). The percentage of callus obtained was more in auxin 2. 4 D alone showed maximum frequency of callusing 80% in HKI335, 75% in HKI1105 and LM5 is 50%. There results can be substantiated by the findings of Laxmi Sita et.al (2000) in Gymmena. Medium composition is one of the most important factors affecting maize tissue culture (Frare et al.2006). Regeneration of plantlets was successful on medium with cytokinin and auxin combination (BAP and IAA) used in present study also earlier reported by Robert et.al (1987) power and Beckhams (1989).

## Conclusion

HKI1105, HKI335 are inbred liner gave good response in regeneration of plantlets than LM5 variety. LM5 is one of the parent of India's first released single cross hybrid. Thus, the established regeneration protocol for there lines night possibly be useful in developing Indian Maize (Inbred line).

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**Fig. 1**

**Fig. 2**

**Fig. 3**

**Fig. 4**

**Fig. 1-4:** Callus culture.

**Table 1:** Effect of hormones on callus induction in three varieties of Maize.

| **Genotypes** | **Callus induction** | **Mean ± S.D.** |
|---|---|---|
| HKI335 | 80% | 79.3 ± 0.94 |
| HKI1105 | 75% | 74 ± 0.81 |
| LM5 | 50% | 51 ± 0.81 |

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