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# Response of different genotypes on *in vitro* regeneration of maize (*Zea mays* L.)

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**Abstract**
In the present experiment the regeneration of different genotypes of maize towards a specific media was studied. The response of the explants on the culture media is genotype dependent due to which the *in vitro* regeneration of the plant varies with respect to its genotype. Maize genotypes have profound differences for *in vitro* cultures (Armstrong and Green, 1985) and only small number of maize genotype posses' regeneration capacity. With the help of this, the genetic variability of the plant can be studied and the elite genotypes of maize can be manipulated with the desirable traits using the genetic engineering technology. In the present investigation three different genotypes namely, two hybrids K-25 and 4212; and a composite genotype BVM-2 were used. Mature embryo was used as the explants. Kernels of the three genotypes were surface sterilised and soaked in double distilled water and kept at 10-14°C for 4 days. Explants of all the genotypes were cultured in the N6 media. The data recorded on the three genotypes were subjected to the statistical analysis following Completely Randomised Design (CRD). Significant results were obtained which showed that the hybrid genotype K-25 gave the highest frequency of callusing with 66.66%.The result obtained suggest that the hybrid K-25 can be used for genetic engineering in furthering sustainable food requirement for the growing population and economic growth.

**Keywords:** Mature embryo, genetic engineering, regeneration

## 1. Introduction
The world-wide significance and the magnificent use of the maize crop have increased the demand to produce transgenic crop with desired trait. Mature embryos derived from dry seeds are easily available explants throughout the year irrespective of the seasons. Though in maize, the *in vitro* regeneration of plant is more efficient with the immature embryos but due to its seasonal availability its use becomes limited as compared to mature embryo. Several biotic and abiotic factors affect the yield of maize and thus interfere in its production. Plant biotechnology plays a vital role and provides techniques like genetic engineering to mitigate these problems within short period of time when compared to conventional breeding. The requirement of the time is to cater the basic needs of genetic engineering. The essential aspect of genetic engineering is to generate an efficient and reproducible method for the development of fertile plants using callus culture. Different explants can be used for the regeneration of fertile and genetically stable plants. The first tissue culture in maize was reported by Green and Philips in 1975 using the immature embryo as the explants. Since then different explants were used for the regeneration of the plant *viz*, mature embryo (Hodages *et al*.,1986; Huang and Wei, 2004; Al Abed *et al*.,2006), immature embryo (Duncan *et al*.,1985; Bohorova *et al*.,1995; Ishida *et al*.,1996; Rakshit *et al*.,2010), protoplast (Rhodes *et al*.,1988), shoot meristem (Sai Ram *et al*.,2003), tassel and ears (Preddy and Petolino,1990), leave tissue (Ahmadabadi *et al*.,2007). Genotypes of maize have tremendous genetic variability due to which it can survive in the tropical, subtropical and temperate conditions. The *in vitro* cultures are affected due to its variability (Pingali and Pandey, 2000) and only few genotypes shows better ability to regenerate. The formation of callus using endosperm tissue as the explants (Tabata *et al.*, 1965) and the somatic embryogenesis and regeneration in maize (Hodges *et al.,* 1986) was shown to be highly genotype specific. The genotype and nutrient composition are regarded to be the major sources of variation in *in vitro* culture (Khanna and Raina, 1998).Therefore, for successful genetic transformation it is important to identify those genotypes that respond well to the *in vitro* callus induction. The proposed objective of the present experiment was to see the response of different genotypes of maize upon *in vitro* regeneration.

## 2. Materials and method

### 1. Plant materials and surface sterilization

Kernels of the three genotypes used in the present study were collected from the Department of Genetics and Plant Breeding. Mature kernels were washed in running tap water for 15 minutes then a treatment of 70% ethanol for 5 minutes was given followed by 3 to 4 times washing thoroughly with distilled water. Treatment with $HgCl_2$ (0.1%) for 5 minutes was given to it followed by washing 3 to 4 times with double distilled water. Then it was immersed in double distilled water and kept at 10-14°C for 4 days (Fig 2.A).

### 2. Inoculation

Kernels were taken out of water and their seed coat was removed. Mature embryo was carefully excised with the help of forceps and scalpels from the kernels and cultured in N6 medium (Chu et al., 1975). The pH of the media prepared was adjusted between 5.6-5.8. It was solidified with 0.7% (w/v) agar and autoclaved at 121°C temperature and 15lbs/sq inch pressure for 1 hour. The cultures were kept in the culture room where the temperature was maintained at 25±2°C with a photoperiod of 16hr of light (at 3000 lux approx) and 8 hours of dark period.

### 3. Data analysis

The formation of callus in different genotypes was recorded and the significant analysis of the data generated at various stages of the experiment was done statistically using CRD (Panse and Sukhatme, 1985).

## 3. Results and discussion

Mature embryo culture

Highest callus induction was reported in K-25 with 66.66% followed by 4212 (6.66%). BVM-2 did not show any response (Fig 1 and Fig 2. B, C, D). It may be due to the fact that the response is genotype dependent. Statistical analysis reveals that genotype K-25 was superior to 4212 and BVM-2 (table 1). Multiple reports consensus that mature embryo are relatively more resistant to respond *in vitro* micro propagation with respect to immature embryo as reported by Huang and Wei, 2004. Klein *et al.*, 1989 reported that embryo size with genotype, culture, media, composition and growth regulators are the factors influencing the expression of the totipotency in cell culture.

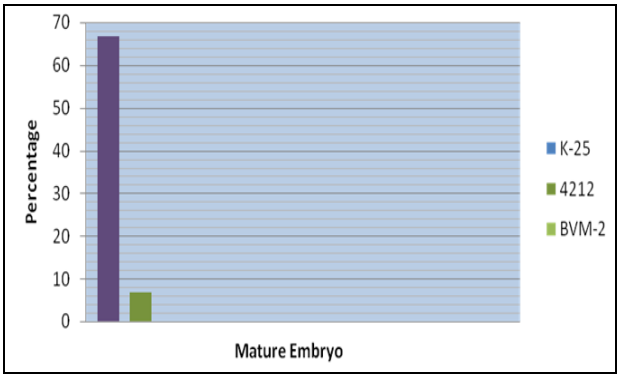


**Fig 1**: Callusing (%) of mature embryo of different maize genotypes in N6 media

**Table 1:** Mean square values for mature embryo in N6 media

| Source Of Variation | DF | MSS |
|---|---|---|
| Genotype | 2 | 6.66** |
| Error | 12 | 0.41 |

**Significant at $p<0.01$

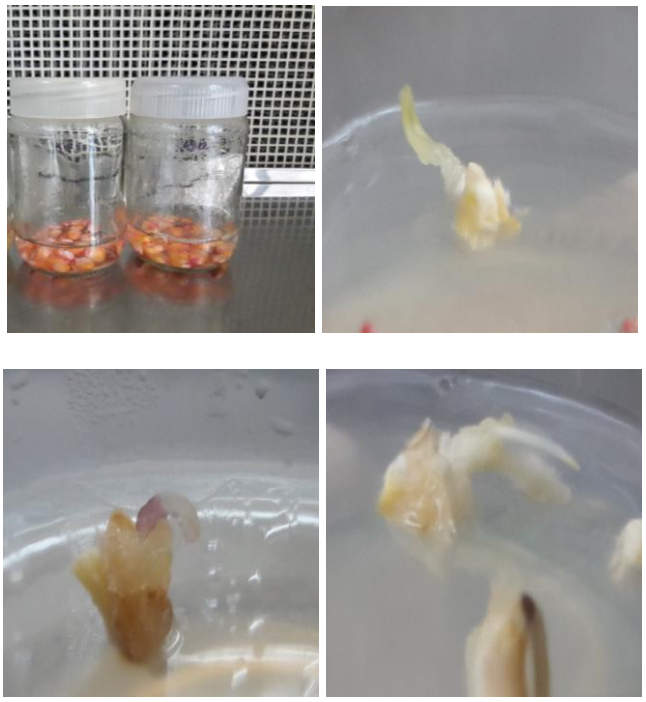

**Fig 2**: (A). Seeds soaked in distilled water for four days (K-25 and 4212), (B). Callusing in mature embryo of K-25, (C). Callusing and rooting in mature embryo of K-25, (D). Callusing and rooting in mature embryo of 4212.

## Conclusion

Highest response of callusing was observed in genotype K-25 in the N6 media followed by 4212. The composite genotype does not show any response. The result clearly shows the differential response of the genotype observed in the N6 media. These suggest that though the explants and the media are same but the difference that arises may be due to the genetic variability of the crop and thus the expression of gene varies according to the genotype.

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## References

1. Ahmadabadi M, Ruf S, Bock R. A leaf-based regeneration and transformation system for maize (*Zea mays* L.). Transgenic Research. 2007; 16(4):437-448.
2. Al-Abed D, Rudrabhatla S, Talla R, Goldman S. Split-seed: A new tool for maize researchers. *Planta.* 2006; 223(6):1355-1360.
3. Armstrong C, Green CE. Establishment and maintenance of frible, embryogenic maize callus and involvement of L-proline. Planta. 1985; 164(2):207-214.
4. Bohorova NE, Luna B, Brito RM, Huerta LD, Hoisington DA. Regeneration potential of tropical, sub tropical, mid altitude and highland maize inbreds. Maydica. 1995; 40:275-281.
5. Duncan DR, Williams ME, Zehr BE, Widholm JM. The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* (L.) genotypes. *Planta*. 1985; 165:322-332.
6. Green CE, Philips RL. Plant regeneration from tissue culture of maize. *Crop science society of maize.* 1975; 3:417-421.
7. Hodages TK, Kamo KK, Imbrie CW, Becwar MW.

Genotype specificity of somatic embryogenesis and regeneration in maize. Biotechnology. 1986; 4:219-233.
8. Huang XQ, Wei ZM. High frequency plant regeneration through callus initiation from mature embryos of maize. *Plant Cell Rep*. 2004; 22:793-800.
9. Ishida Y, Saito H, Ohta S, Hiei Y, Komari T, Kumashiro T. High efficiency transformation of maize (*Zea mays* L.) mediated by Agrobacterium tumefaciens. Nature Biotechnology. 1996; 14:745-750.
10. Khanna HK, Raina SK. Genotype and culture media interaction effects on regeneration response of three *indica* rice cultivars. *Plant Cell, Tissue and Organ Culture*. 1998; 52(3):145-153.
11. Klein TM, Kornstein L, Sanford JC, Fromm ME. Genetic transformation of maize cells by particle bombardment. Plant Physiol. 1989; 91:440-444.
12. Panse VG, Sukhatme PV. Statistical method for agricultural workers, Indian Council of Agricultural Research, New Delhi, 1985.
13. Pareddy DR, Petolino JF. Somatic embryogenesis and plant regeneration from immature inflorescences of several elite inbreds of maize. Plant Sci. 1990; 46:225-232.
14. Pingali PL, Pandey S. Meeting world maize needs: technological opportunities and priorities for public sector. In CIMMYT 1999-2000 World Maize Facts and Trends. 2000, 1-22.
15. Rakshit S, Rashid Z, Sekhar JC, Fatma T, Dass S. Callus induction and whole plant regeneration in elite Indian maize (*Zea mays* L.) inbreds. Plant Cell Tissue Organ Culture. 2010; **100**:31-37.
16. Rhodes CA, Lowe KS, Ruby KL. Plant regeneration from protoplasts isolated from embryogenic maize cell cultures. Biotechnology. 1988; 6:56-60.
17. Sairam RV, Paran M, Franklin G, Lifeng Z, Smith B, MacDougall J et al. Shoot meristem an ideal explants for *Zea mays* L. transformation. Genome. 2003; 46:323-329.
18. Tabata M, Motoyoshi F. Heriditary control of callus formation in maize endosperm cultured *in vitro*. J Genetics. 1965; 40:343-355.