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Tissue culture dependent regeneration of maize (*Zea mays* L.)

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Abstract
Maize (*Zea mays* L.) is the third most important cereal crop in the world. It is a major cereal crop for livestock feed, human nutrition and important raw material for several agro-based industries, there is great demand for maize of both quality and quantity. An efficient plant tissue culture procedure with high regeneration frequency is prerequisite for this approach. In light of the situation an experiment was conducted with an objective to optimize regeneration of plants using different explants of maize. The experiment was conducted using two hybrids (GS-802, 27P17) and one composite (BVM-2) for protocol development for *in vitro* regeneration of maize (*Zea mays* L.) using three different explants *viz.,* shoots, endosperm, ovules. Shoots of all the genotypes were inoculated in MS media supplemented with 1mg/l BAP, 2mg/l BAP, 3mg/l BAP, 4mg/l BAP and 5mg/l BAP along with a control. Endosperm of all the genotypes were cultured in callusing media containing MS basal salts supplemented with 1mg/l 2, 4-D+1mg/l NAA and 0.2mg/l 2, 4-D+ 2mg/l NAA. Ovules were inoculated in the N6 medium. Better response of kernels regeneration was observed when treated with $HgCl_2$for 10 minutes**.** The response was observed for shoot elongation with 2mg/l BAP in GS-802. Highest shoot survival was observed in GS-802 with 2mg/l BAP and 4mg/l BAP. In endosperm culture, callusing observed on MS medium with different concentration of 2, 4-D+NAA. Better response of GS-802 was recorded in the hormonal combination of 2, 4-D-0.5mg/l + NAA-2mg/l and for BVM-2 in the hormonal combination of 2, 4-D-1mg/l + NAA-1mg/l. In case of ovule culture 85.71% of sprouting was observed in GS-802 genotype followed by BVM-2 and 27P17 with 76.91% and 47.61% respectively.

Keywords: *In vitro* regeneration, Sprouting, Callusing, Genetic engineering

1. Introduction
Maize (*Zea mays* L. 2n=20) is the world's leading crop and is widely cultivated as cereal grain that was domesticated in Central America. It is also known as Queen of Cereals, because of its highest genetic yield potential. It is a C_4 crop with outstanding ability to maintain high rates of photosynthetic activity that is important for grain yield and biomass. Being a cross-pollinating species, it maintains broad morphological features, genetic variability and geographical adaptability. It is the only food cereal crop having wider adaptability that can be grown in different seasons, with equal success in temperate, subtropical and tropical regions of the world. Pathi *et al*. (2013) showed a new plant regeneration method for Indian maize cultivar (HQPM-1). This method is efficient, rapid, simple, genotype independent for obtaining shoots from mature seed-derived callus with successful rooting. This efficient regeneration system facilitates the application of plant tissue culture and genetic engineering approach in maize. MS medium supplemented with 2 mg/l BAP, 1 mg/l Kinetin and 0.5 mg/l NAA promoted the highest frequency of shoot induction. The highest frequency of root formation was observed when shoots were grown on MS medium. The regenerated plants were successfully hardened in earthen pots after adequate acclimatization. The important advantage of this improved method is shortening of regeneration time by providing an efficient and rapid regeneration tool for obtaining more stable transformants from mature seeds.

2. Materials and methods
1. Plant materials
Three tropical Indian maize line two hybrids and one composite namely: GS-802, 27P17 and BVM-2 were used in the present experiment. These genotypes were sown in the field of Birsa Agricultural University, Ranchi.

2. Surface sterilization
Kernels of the three genotypes were washed thoroughly in running tap water for 15 minutes

followed by washing 3 to 4 times with distilled water. Then it was treated with $HgCl_2$ for 10 min, 15min and 15 min inoculated with opposite orientation followed by washing vigorously with double distilled water for 3 to 4 times. Subsequently for ovules, the ears were covered with silk bag (fig4.A) before the emergence of silk. After 4 and 5 days it was collected between 7 and 8 am. Its leaves were removed and wiped with 70% ethanol followed by washing with $HgCl_2$ (0.05%) for 10 minutes.

3. Inoculation

Shoots were inoculated in the shooting medium (MS Basal+ 1mg/l BAP, 2mg/l BAP, 3mg/l BAP, 4mg/l BAP, 5mg/l BAP). Callusing medium (1mg/l 2, 4-D + 1mg/l NAA and 0.5mg/l 2, 4D + 2mg/l NAA) was used for the culturing of endosperm. Shoots and endosperm were excised from the *in vitro* grown seedlings. Ovule excised aseptically and cultured in N6 medium (Chu *et al*. 1975).

4. Data analysis

Data recorded was statistically analyses using CRD (Singh and Chaudhary, 1979).

3. Result and discussion

1. Shoot Culture

The analysis reveals the significant differences among the genotypes and hormonal concentration (BAP at 1mg/l, 2mg/l, 3mg/l, 4mg/l and 5mg/l). Genotype GS-802 is superior to 27P17 and BVM-2 whereas 27P17 and BVM-2 are at par in shoot elongation (fig1, fig4. A) In context of hormonal concentrations, MS medium supplemented with 2mg/l BAP and 4mg/l BAP are superior to 1mg/l BAP, 3mg/l BAP, 5mg/l BAP. In case of shoot (fig2) survival GS-802 genotype is superior to 27P17 and BVM-2; 27P17 and BVM-2 are at par. In context of the concentrations, MS medium supplemented with 2mg/l BAP are at par with 4mg/l BAP and superior to 1mg/l, 3mg/l and 5mg/l of BAP (table 1). Cytokinin affects *in vitro* plant cultures but when BAP was added to regeneration medium it had very little response on shoot induction as it responded in the shoot elongation rather than shoot multiplication. Similar result was proposed by (Bhaskaran and Smith, 1990).

2. Endosperm culture

The GS-802 responded in the hormonal combination of 2, 4-D-0.5mg/l + NAA-2mg/l (fig4.C). And BVM-2 responded in the hormonal combination of 2, 4-D-1mg/l +NAA-1mg/l. No statistical analysis was done for the experiment due to poor response of the genotypes. The varying levels of regeneration of genotypes in different media as observed in the present study indicates that somatic embryogenesis and regeneration in maize is genotype specific and the expression of the gene for regeneration ability is dependent upon media environment as reported by Hodges *et al*. (1986).

3. Ovule culture

GS-802 is more responsive with 85.71% of sprouting followed by BVM-2 and 27P17 (table 2) genotypes with 76.91% and 47.61% respectively, (fig 3) and it shows that GS-802 gives better result in short interval of time. From statistical analysis it was found that GS-802 and BVM-2 (fig4 D). Are at par and better than 27 P17.

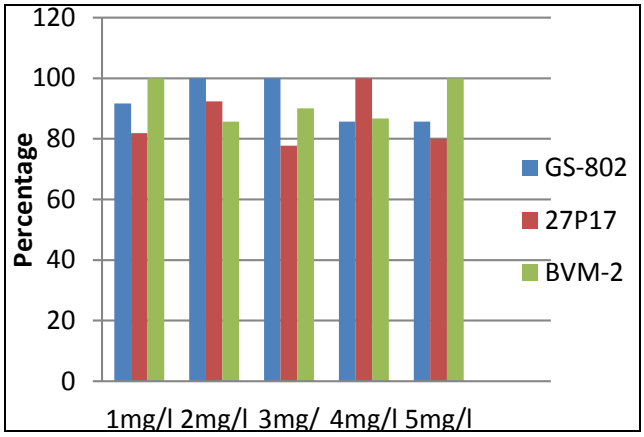


Fig 1: Shoot elongation (%) at different concentrations Of BAP in MS media

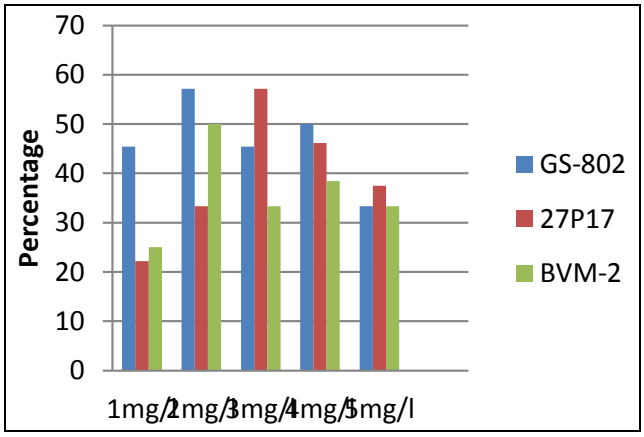


Fig2. Shoot survival (%) at different concentrations of BAP in MS media

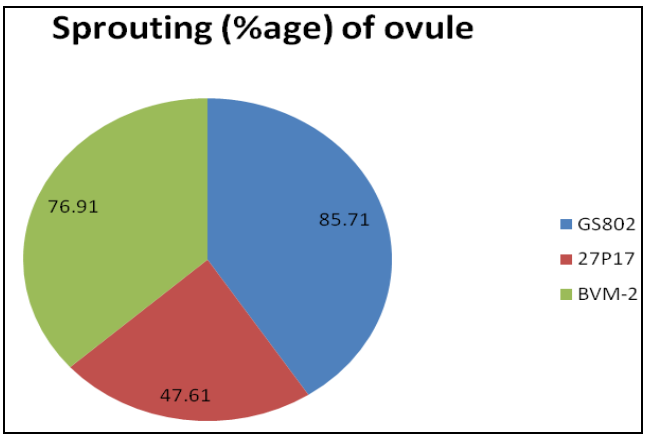


Fig 3: Sprouting (%age) of ovule of maize genotype in N6 media

Table1: Mean square values for shoot elongation and shoot survival in MS media with different concentration of BAP

Character	Source of Variation	Df	Mss
Shoot Elongation	Bap	5	18.05**
	Genotype	2	0.88*
	Bap *Genotype	10	4.45**
	Error	54	0.19
Shoot Survival	Bap	5	3.56**
	Genotype	2	0.54
	Bap *Genotype	10	1.20**
	Error	54	0.35

*Significant at $p<0.05$,
**Significant at $p<0.01$

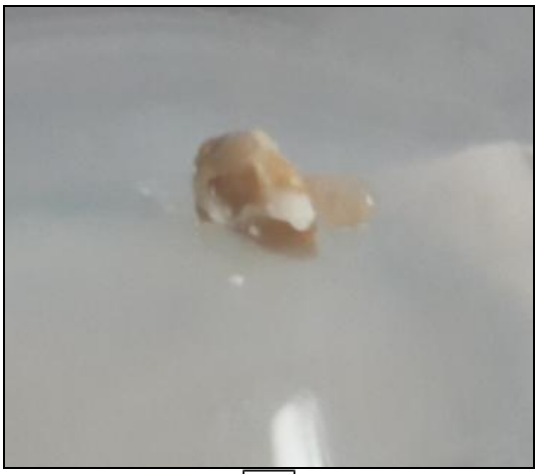
Table 2: Mean square values of sprouting of ovule in N6 media

Source of Variation	DF	MSS
Treatment	2	3.47**
Error	12	0.33

*Significant at $p<0.05$,
**Significant at $p<0.01$

A

B



C

D

Fig 4: (A) Ears covered with silk bag before the emergence of silk for ovule culture (BVM-2), (B). Shoot elongation in MS+BAP of BVM-2, (C). Callusing in endosperm in MS+2, 4-D+NAA of GS-802, (D). Sprouting in ovule of BVM-2.

4. Conclusion

The genotype GS-802 was found to give the best response in terms of shoot elongation. In case of shoot survival GS-802 response was highest 27P17 and BVM-were equally effective. In case of endosperm callusing was observed in GS-802 and BVM-2. Ovule culture followed by 27P17 and BVM-2. Sprouting of ovule was observed in GS-802 genotype followed by BVM-2 and 27P17. From the present study it can be concluded that the response of maize is genotype specific and depend on various factors like temperate, nature of genotype.

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