Research Article

Influence of Explant Sources on *in vitro* Callogenesis and Regeneration in Maize (*Zea mays* L.)

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Abstract | Maize is an important cereal crop that is widely consumed all around the world. Until now extensive work has been conducted to determine the efficiency of various explants sources for maize tissue culture, especially the immature embryos. However, the manipulation of immature embryos as explants is hampered due to its unavailability throughout the year and low regeneration response. The present study is aimed to investigate the effect of various explants sources for callogenesis and regeneration in maize (Pioner 3025). The main explants under consideration were: three parts of germinated seedlings, mature embryo and split seeds. Each of these explants was evaluated for its ability to form callus and to regenerate subsequently. For callogenesis from different parts of germinated seedlings, maximum frequency was shown by bulged inter node (45.60 %) along with the regeneration efficiency of 46.23 %. In case of split seed technique, the efficiency for callogenesis was 82% with the regeneration efficiency of 65.46%. For mature embryos the efficiency for callogenesis was 61% while for regeneration it was 78.06%. The results revealed that split seed technique is a preferable choice for explants for callogenesis, while mature embryos exhibit optimum frequency for regeneration in maize (Pioner 3025).

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Introduction

Maize (*Zea mays* L.) is one of the essential cereal crops produced all around the world. It is widely consumed as animal feed, human food, biofuel and is also utilized to synthesize a number of industrial products (Matazau *et al*., 2014). There is a continued rise in the demand for maize all across the world largely in Asia (Wada *et al*., 2008) In Pakistan, maize is one of the most profitable agricultural crops as it accounts for 0.4% of GDP and contributes 2.1% to the value added in agriculture. In fact, it is the fourth largest crop grown in Pakistan after wheat, rice and cotton, and holds 22nd position in world maize ranking. It is mainly cultivated in the rain-fed areas of Khyber Pakhtunkhwa and Punjab over 1,168,490 HA with estimated crop production of 4,944,210 tonnes (Ali *et al*., 2014). However, the yield and production of maize is usually hampered by certain biotic and abiotic stresses; including depletion of nutrients from the soil, water logging and salinity, aluminium toxicity, drought, diseases, pests and weeds. (Mushke *et al*., 2016). Therefore, according to the current data available the total area for maize cultivation in Pakistan is declined to 1,130 from 1,168 thousand hectares, while the overall production has been declined to 4,695 from 4,944 thousand tonnes (Ali *et al*., 2014).

To avoid such limitations effective and reliable genetic

transformation techniques are needed to be embraced (Akinyosoye *et al*., 2014). The introduction of desired characteristics into the maize genome will allow improvement in the nutritional quality, productivity and development of parental stock which in turn will lead to the development of genotypes tolerant to environmental stresses. Efficient *in vitro* regeneration techniques permitting complete plant development from various explants are of great importance to both clonal propagation and successful modification of plant genomes (Zang *et al*., 2016).

There are two promising methods used to introduce foreign genes in plants for transformation i.e. *Agrobacterium*-mediated gene transfer (Gelvin, 2003) and biolistic gene gun (Woods and Zito, 2008). However, the success of plant transformation is highly dependent on the regeneration efficiency of explants. As a matter of fact, an efficient plant tissue culture technique along with high regeneration frequency is the basis to tackle major issues like malnutrition currently prevailing in Pakistan (Aguado-Santacruz *et al*., 2007).

The availability of a good plant regeneration system is a prerequisite for clonal propagation. Regeneration from immature embryos of maize was first reported by Green and Philips (1975). Since then, maize regeneration has been stated from mature embryos, nodal regions, seedling segments, leaf tissues, anthers, tassels, protoplast and shoot apical meristems (Rakshit *et al*., 2010). Immature embryos were a popular choice to be used as explant for maize tissue culture. However, the challenge to maintain and produce immature embryo of maize makes them unfavorable to be used. Moreover, immature embryos are only seasonally available. Thus, the formation of immature seeds is time-consuming and requires well-equipped green house along with laborious artificial pollinated system (Mushke *et al*., 2016). On the contrary, surplus amounts of dry mature embryos are available throughout the year and are also amenable to tissue culture (Abebe *et al*., 2008).

Several studies have been reported in which mature embryos are used in tissue culture procedures. Xiang *et al*. (2007) investigated the regeneration efficiency of calli derived from the mature embryos of maize inbred lines SML295, CML304, and 18-599R to be 68.6%, 75.4% and 84.8% respectively. This indicates that regeneration is genotype dependent. Another study conducted by Akinyosoye *et al*. (2014) depicted that tissue culture response from mature embryos of maize, barley, wheat and rye depends on the size of the seed used. The present study is aimed to investigate the ability of different explants, derived from mature maize seeds, to form callus and then to determine the regeneration efficiency of various explants.

Materials and Methods

Seed sterilization

For surface disinfection, maize seeds, of variety Pioner 3025, were sterilized by using two approaches. In the first case (C1), the seeds were rinsed with autoclaved water for 1 minute, followed by immersion in 70% ethanol for about 2 minutes. The seeds were then washed with 50% Clorox bleach for 15-20 minutes. The final washing was done by autoclaved water for 8-10 times. In the second case (C2), 0.1% $HgCl_2$ was used instead of Clorox bleach while the remaining procedure remained same as in C1 (Mushke *et al*., 2016). Due to significant antimicrobial properties of mercuric chloride, 0.1% $HgCl_2$ method was used as it controls both bacterial and fungal contamination (Ramakrishna *et al*., 1991). All the steps for seed sterilization were performed in a laminar flow cabinet.

Seed germination

The sterilized seeds were germinated by using three different approaches. In the first approach, the seeds were placed between two sheets of autoclaved filter papers, initially moistened with 10ml of autoclaved distilled water. Throughout the germination period of seedling, water saturation was ensured by regularly moistening the filter paper with 1ml autoclaved water after every fourth day (Akinyosoye *et al*., 2014). The second approach involved the preparation of different germination media supplemented with varying concentrations of pant growth hormones (Table 1), along with 3% sucrose/maltose and 0.4% Gelzan. The final pH for each media was adjusted to 5.8 by using 1M NaOH and 1M HCl. The seeds were placed on sterile germination medium provided the axis side of the embryo in contact with the medium. In order to evaluate the effect of light on the rate of germination, half of the petri plates were kept in dark and half under light at 25±1 °C. In the third approach i.e. 'Split seed technique', the sterilized seeds were soaked in autoclaved media M_4 (Table 1) without any solidifying agent for 48 hours (Al-Abed *et al*., 2006). The soaked seeds were then placed on solidified M4

media (Table 1) after two days.

Table 1: *Different media used for germination.*

Symbols	Composition and concentration
M_1	MS-0 + 5mg/l 2,4-D + 3 mg/l BAP
M_2	MS-0 + 5mg/l 2,4-D
M_3	MS-0 without any growth hormone
F	Moistened Filter Paper
M_4	MS-0 + 9µM 2,4-D

Callogenesis from different explant sources

Sections of germinated seedlings: For callogenesis from parts of seedlings, two weeks old germinated seedlings were cut into three segments, i.e. T (tip of the germinated seedling), M (middle section of the germinated seedling) and E (bulged internode; part closest to the seed) as shown in Figure 1. Each of these parts was then further divided longitudinally and placed on CIM-1 (Table 2). After two weeks, the calli were then sub-cultured on the same medium (Pathi *et al.*, 2013).

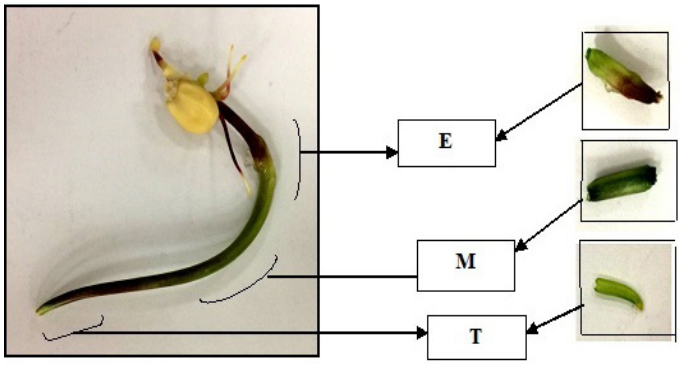

Figure 1: *Segments of germinated seedlingas explants for callus induction.*

Table 2: *Combination of plant growth hormones used for callogenesis and regeneration.*

Sr. No.	Name of media	Concentrations of plant growth hormones in MS-0 medium
1	CIM-1	MS-0 + 2mg/l 2,4-D + 1mg/l BAP
2	CIM-2	MS-0 + 9µmol 2,4-D
3	CIM-3	MS-0 + 1mg/ml 2,4-D
4	SIM	MS-0 + 2mg/l BAP + 1mg/l KIN
5	RIM-1	MS-0
6	RIM-2	MS-0 + 1mg/ml IBA

CIM: Callus Induction Medium; SIM: Shoot Induction Medium; RIM: Root Induction Medium.

Split seed technique: For callogenesis using 'Split seed technique', four days old germinated seedlings were split longitudinally into two identical halves and placed on CIM-2 (Table 2) for two weeks. The seed was removed from the callus as soon as callus formation was observed. The callus was then sub-cultured for further proliferation. After 15 days, the callus was sub-cultured for another one week.

Mature embryos

For callus induction from mature embryos, the sterilized seeds were soaked in autoclaved distilled water. Four days old soaked seeds were then used for embryos excisions by using sharp sterilized forceps and scalpel. However, special precautions were taken into consideration not to cause any damage to embryo while removing the entire cotyledon. These excised embryos were then placed on CIM-3 (Table 2). The calli were sub-cultured after two weeks. All the calli were incubated in dark at 25 ±1 °C.

Table 3: *Seed germination rate on different media.*

Germination media	No. of days of germination	Rate of germination (%)
M_1	5	80.00%
M_2	8	78.10%
M_3	6	78.40%
F	9	22.20%

Regeneration

Shooting media: In order to investigate and compare the regeneration competency of calli derived from different explant sources, four weeks old calli were transferred to regeneration medium. For shoot induction, MS-0 medium supplemented with 2mg/l BAP and 1mg/l KIN was used. The culture tubes were kept in light (25 ± 2 °C) for 1-2 weeks.

Rooting media

As the shoots gained appropriate length, the regenerated seedlings were transferred to rooting media to initiate root development. Two rooting media were prepared during the course of this investigation: RIM-1 and RIM-2. For RIM-1, MS-0 was used without adding any growth hormone while for RIM-2 MS-0 was supplemented with IBA (1mg/ml) (Table 2).

Data analysis

The callus induction and regeneration data was subjected to one way Analysis of Variance (ANOVA) as shown in Table 4.

Table 4: *Analysis of variance of callus induction and regeneration from various explant sources.*

Source of variation	SS	Df	MS	F-value	P-value	F crit
Explant source	7262.05704	4	1815.514	24.74559**	0.001714	11.39193
Within groups	366.83585	5	73.36717			
Total	7628.89289	9				

***Significant at 1% level; Df: Degree of freedom; SS: sum of square; MSS: mean sum of square.*

Results and Discussion

The results of this study revealed that the use of mercuric chloride (C1) as a sterilizing agent shows similar results as obtained by using Clorox bleach (C2), since there was no significant difference between the frequencies of positive results for the two measures of seed sterilization. C1 method exhibited 86.4% seed germination while 74% seeds were germinated by C1 sterilization method (Figure 2).

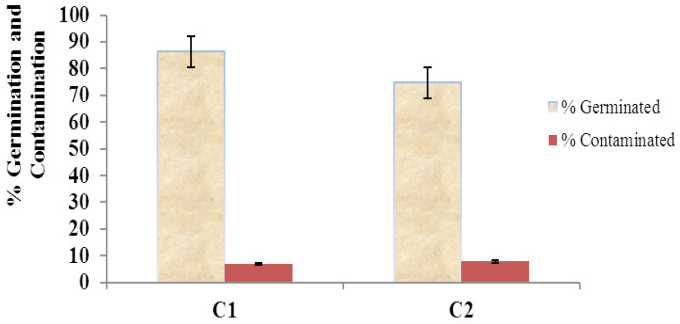

Figure 2: *Sterilization efficiency for seeds.*

To compare the effect of light on seed germination on different media used in the study (Table 1), the seeds were incubated in light as well as in dark. It was observed that the seedlings formed under light (25 ± 2 ˚C) on each medium were longer and green as compared to those grown in dark (Figure 3). The length of the shoots emerging in dark was slightly shorter and somewhat off-white in color, reducing their photosynthetic ability and thus making them less efficient for further proliferation. However, root length was found to be slightly longer when the seeds were allowed to germinate in dark.

Among different media used for seed germination, optimum germination was obtained on medium (M_1), as the seeds germinated after only 5 days on average, with the maximum efficiency of 80%. On the contrary, moistened filter paper (F), proved to be an undesirable method for germination as the shoots protruded out after an average of 9 days with the least germination efficiency of 22.2%. M2 and M3 also showed moderate germination results as the seeds germinated after an average of 8 and 6 days with the germination efficiency of 78.1% and 78.4% respectively (Figure 4, Table 3).

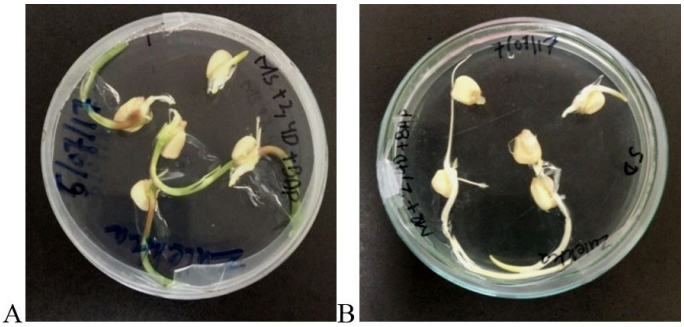

Figure 3: *Comparison of maize seed germination. (A) Seeds germinated in light; (B) Seeds germinated in dark.*

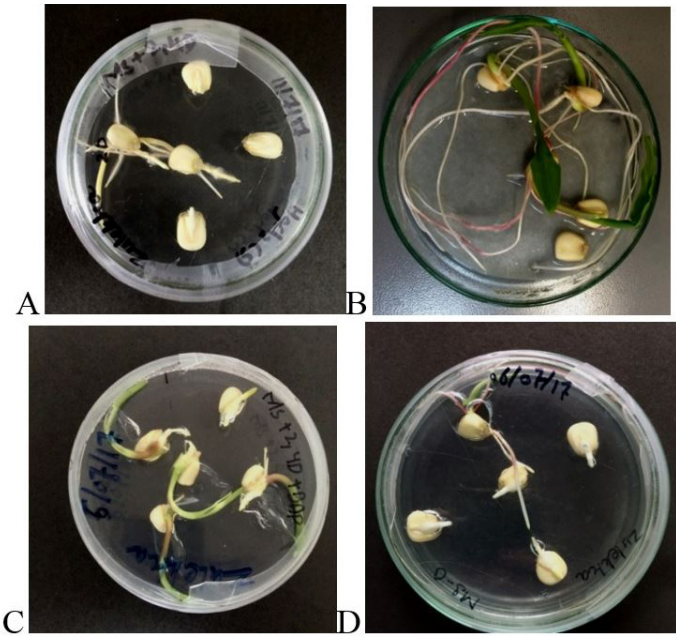

Figure 4: *Maize seeds germination. (A) Between two moistened filter papers; (B) On M_1 medium; (C) On M_2 medium; (D) On M_3 medium. The image for each germinating media was captured after 7 days.*

Mean callus induction percentage among different sections of germinated seedlings was also noticed (Figure 5). The maximum frequency for callogenesis was evident by swollen internodes (46.23%) as shown in Figure 10.

To investigate the efficiency of 'split seed technique' for callus induction, four days old germinated seeds

were longitudinally split and placed on CIM-2 (Table 2). Callogenesis was observed after an average of seven days (Figure 6). This protocol exhibited maximum callus induction efficiency with a mean percentage value of 82% (Figure 10). Moreover, the calli formed were larger in size and had the least false callus formation around it.

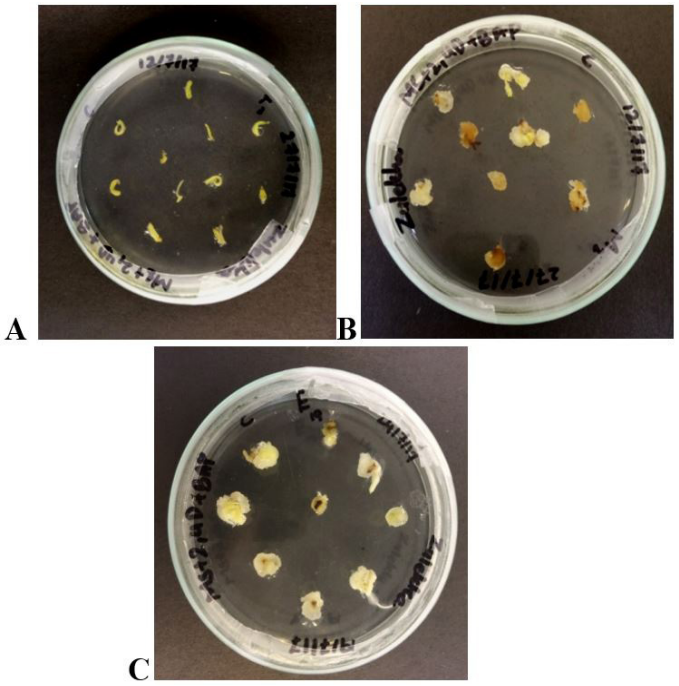

Figure 5: *Callogenesis from three parts of 22 days old germinated seedling. (A) Tip (T); (B) Middle part (M); (C) Bulged inter node (E).*

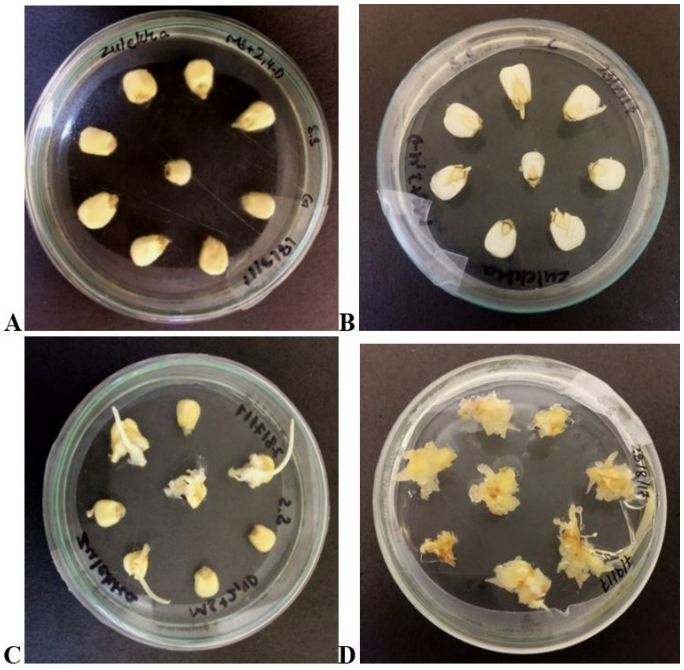

Figure 6: *Callogenesis by 'split seed technique'. (A) Sterilized seeds on germination medium; (B) Four days old germinated seeds split longitudinally and placed on callus induction medium; (C) Initial callus formation observed after 7 days in split seeds; (D) Final calli derived from split seed technique after 26 days.*

Callus induction in mature embryos as explants was investigated after an average of three days. Mature embryos exhibited remarkably higher callus formation with an efficiency of 61% (Figure 10) hence, making them a more preferable choice as explants in comparison with various sections of germinated shoots (Figure 7).

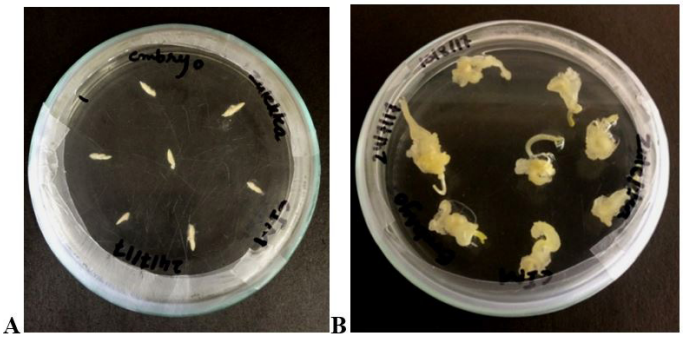

Figure 7: *Callogenesis from mature embryos on CIM-3. (A) Day 1; (B) Day 26.*

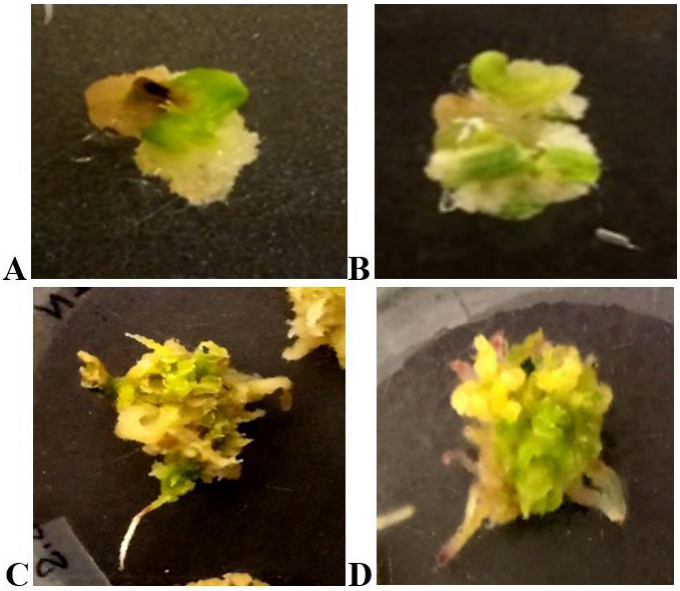

Figure 8: *Regeneration response of calli derived from different explants. (A) Middle part of shoot, M; (B) Bulged Internode, E; (C) Split Seed; (D) Mature Embryo.*

In the current research, in order to compare the regeneration response of various explants, the calli derived from these explants were monitored for their regeneration efficiency. Among different shoot sections, buldged internode (E) showed better regeneration efficiency of 46.23 % (Figure 10). In order to allow shoot and root elongation, they were transferred to regeneration media in culture tubes (Figures 8 and 9).

Mature embryos gave regeneration efficiency of 78.06% (Figure 10) through a pathway that involves callogenesis. Although regeneration efficiency of

calli derived directly from mature embryos is quite high, still the protocol involved is laborious. Split seed technique on the contrary, is a newly developed protocol which can regenerate higher number of shoots by callus formation in comparatively less time. The purpose behind this new regeneration technique was to expose three regeneration competent cells: the scutellum, the coleoptilar-ring, and the shoot apical meristems. Thus, increasing the regeneration efficiency and ultimately promoting novel gene engineering systems in maize. However, the present study depicts contradictory results (Figure 8). The regeneration efficiency of calli derived from mature embryos is higher (78.06%) as compared to the regeneration efficiency of calli derived from split seeds (65.46%) as shown in Figure 10.

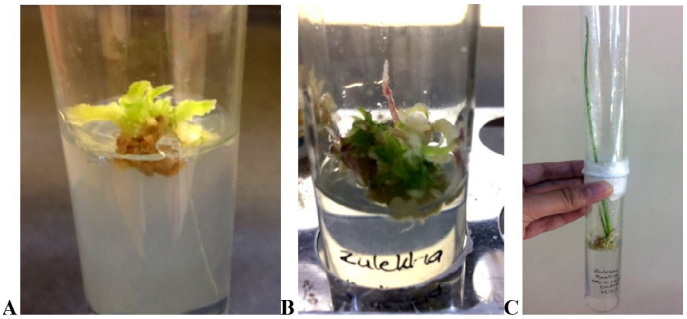

Figure 9: *Shoot and root elongation. (A) Buldged internode, E; (B) Split Seed; (C) Mature Embryo.*

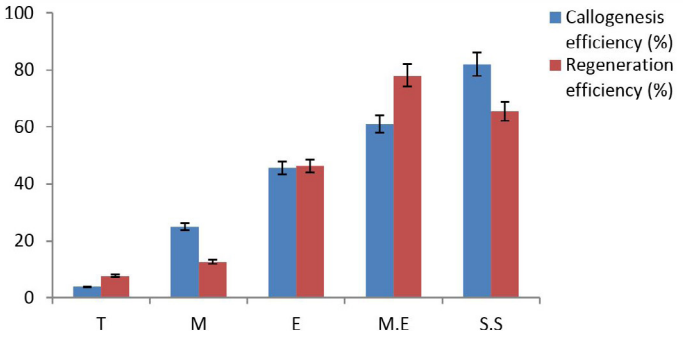

Figure 10: *Callogenesis efficiency (%) and regeneration efficiency (%) of different explant sources.*

Analysis of variance for percent callus induction and regeneration revealed explant sources varying significantly (Table 4). The study conducted showed significant difference ($P \leq 0.01$) in callogenesis and regeneration response to various explant.

One of the major issues in tissue culture is the poor regeneration response of various explants. Therefore, an appropriate choice of explant is a pre-requisite to initiate tissue culture for any crop plant as the selection of an effective explant increases the likelihood for the cells to become more regeneration competent. Until recently, the production of transgenic maize usually relied upon immature embryos as regeneration explants. However, it is extremely difficult to maintain the availability of immature embryos throughout the year, as it requires special care during winter along with a high quality greenhouse space (Frame *et al.*, 2002). Therefore, the present study is aimed to investigate the potential of different explants for maize cultivar (Pioner 3025) for their callogenesis and regeneration efficiency.

In order to establish tissue culture technique, proper seed sterilization is a crucial step. In the present investigation, mercuric chloride was used in one of the sterilization methods due to its significant antimicrobial activities. At low concentrations, i.e. up to 0.1%, it works effectively against both fungi and bacteria (Ramakrishna *et al.*, 1991). However, its toxicity and volatile property reduces its significance to be used as a sterilizing agent. The current study revealed its implication as a sterilizing agent (method 2), however, the rate of germination and contamination was found to be similar as that of method 1 where Clorox bleach was used instead of mercuric chloride. It has been observed that lower concentrations of mercuric chloride are more preferable (Sameer and Nabeel, 2016).

In the present study, a remarkable influence of various explant sources used was found on callogenesis and regeneration (Table 4). As far as callogenesis from segments of two week old seedling is concerned, swollen internodes showed the optimum frequency (45.60%). In a comparable study done by Pathi *et al.* (2013) the maximum frequency attained for callus proliferation from internodes was 85%. This deviation might be the result of variation in genotype as regeneration is genotype dependent. However, this rapid and efficient method claims to be a promising regeneration tool for providing more stable trans formants from mature viable seeds for a number of maize varieties, especially HQPM-1 (Pathi *et al.*, 2013).

Our study depicted that callogenesis efficiency from split seed technique (82%) was remarkably greater as compared to callus induction efficiency obtained from mature embryos (61 %) as explants. In order to conduct a successful transgenic research on any crop plant, higher regeneration response is inevitable. In our investigation, though the callus induction response

using 'split seed technique' is higher, the results showed inconsistency in relation to regeneration response. The regeneration efficiency of calli derived from mature embryos was higher (78.06%) than the regeneration efficiency of calli derived from split seeds (65.46%) as shown in Figure 10. As a matter of fact, an appropriate regeneration system tends to provide strong basis for genetic transformations. A number of reports have been published suggesting efficacious regeneration in maize from mature embryos (Huang and Wei, 2004), split seeds (Al-Abed *et al*., 2006), nodal culture (Vladimir *et al*., 2006) other than immature embryos to be used as explants. Currently, maize immature embryos are extensively used as explants for maize tissue culture as compared to other plant parts (Liang *et al*., 2010). However, there are limitations of growth period, geographical condition and development season while using immature embryos as explants. Immature embryos must be utilized within 9-12 days following pollination. In contrast, mature embryos are easily stored and can be conveniently obtained without time and quantity constraint. In the current investigation, in order to compare the regeneration response of various explants for mature maize seeds, the calli derived from these explants were monitored for their regeneration response. Mature embryos gave regeneration efficiency of 78.06% (Figure 10) through a pathway involving callus induction. A comparative study conducted by Ali *et al*. (2014) showed less significant difference between regeneration frequency using immature embryos (55-80%) and mature embryos (40-75%). Although regeneration from calli derived directly from mature embryos is quite high, still the protocol involved is laborious and tiresome. Split-seed technique, on the other hand, is a newly developed protocol for maize regeneration which can regenerate higher number of shoots by callus formation within short time period. The notion behind this technique was to expose three different tissues simultaneously, namely the scutellum, the coleoptilar-ring and the shoot apical meristem. A successful plant regeneration from calli induced from anthers, immature tassels, immature inflorescences, seedling segments, leaf segments, shoot apical meristems, shoot tips and protoplasts were also reported (Mushke *et al*., 2016). The purpose to introduce this novel explant source was to successfully regenerate maize plants at a higher frequency within a significantly reduced amount of time. The study conducted by Al-Abed *et al*. (2006) using split seeds as an explants revealed significant results. The callus induction frequency and regeneration efficiency from split seeds was reported to be higher as compared to mature embryos as explant source.

Various growth hormones were used according during the entire course of this investigation. Media enriched with 2, 4-D and BAP is widely used for the formation of embryogenic callus (Pathi *et al*., 2013). The role of 2,4-D in inducing the formation of regenerable calli from mature embryo was justified by Huang and Wei (2004) which stated that auxins, especially 2.4-D are essential in the range of 1- 3mg l^{-1} for the formation of embryogenic callus. In the light of this study, 2 mg l^{-1} of 2,4-D was supplemented in MS-0 media along with 1 mg l^{-1} BAP for callus induction in the present investigation. The study conducted by Sompornpailin and Khunchuay (2016) manifested that MS media enriched with cytonkinins (BAP and KIN), along with low concentrations of auxins are required for regeneration. The present investigation justifies the significance of appropriate cytokinin (BAP and KIN) and auxin (2, 4-D) ratio, in order to enhance regeneration response in maize.

Conclusions and Recommendations

Various explant sources have a significant impact on callus induction and regeneration responses. The present investigation suggested 'Split seed technique' an imperative method to be opted for callogenesis and regeneration in maize plants since it gives better results in short period of time. Moreover, an appropriate cytokinin (BAP and KIN) and auxin (2, 4-D) ratio is crucial to enhance regeneration response in maize.

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Novelty Statement

Higher callogenesis efficiency was observed using split seed technique. However, mature embryos prove to be more promising for better regeneration efficiency.

Author's Contribution

Zulekha Zameer: Conducted the experimental work.

Samreen Mohsin: Designed the experiment.
Ammarah Hasnain: Performed the statistical analysis of work.
Asma Maqbool: Did the interpretation and analysis of results.
Kauser Abdulla Malik: Involved in discussion.

Conflict of interest

The authors have declared no conflict of interest.

References

Abebe, D., W. Teffera and J.S. Machuka. 2008. Regeneration of tropical maize lines (*Zea mays* L.) from mature zygotic embryo through callus initiation. Afr. J. Biotechnol., 7(13): 2181-2186.

Aguado-Santacruz, G.A., E. Garcia-Moya, J.L. Aguilar-Acuna and B. Moreno-Gomez. 2007. *In vitro* plant regeneration from quality maize protein (QPM). *In Vitro* Cell. Dev. Biol. Plant, 43(3): 215-224. https://doi.org/10.1007/s11627-007-9042-9

Al-Abed, D., S. Rudrabhtlaa, R. Talla and S. Goldman. 2006. Split-Seed: A new tool for maize researchers. Planta, 223(6): 1355-1360. https://doi.org/10.1007/s00425-006-0237-9

Akinyosoye, S.T., J.A. Adetumbi, O.D. Amusa, M.O. Olowolafe and J.O. Olasoji. 2014. Effect of seed size on *in vitro* seed germination, seedling growth, embryogenic callus induction and plantlet regeneration from embryo of maize (*Zea mays* L.) seed. Niger. J. Genet., 28(2): 1-7. https://doi.org/10.1016/j.nigjg.2015.06.001

Ali, F., M. Ahsan, N.A. Saeed, M. Ahmed, Q. Ali and N. Kanwal. 2014. Establishment and Optimization of callus-to-plant regeneration system using mature and immature embryos of maize (*Zea mays* L). Int. J. Agric. Biol., 16: 111-117.

Frame, B.R., H. Shou, R.K. Chikwamba, Z. Zhang and C. Xiang. 2002. *Agrobacterium tumifaciens*-mediated transformation of maize embryos using a standard binary vector system. Plant Physiol., 129(1): 13-22. https://doi.org/10.1104/pp.000653

Gelvin, S.B., 2003. *Agrobacterium*-Mediated Plant Transformation: The Biology behind the "Gene-Jockeying" Tool. Microbiol. Mol. Biol. Rev., 67(1): 16-37. https://doi.org/10.1128/MMBR.67.1.16-37.2003

Green, C.E. and R.L. Phillips. 1975. Plant regeneration from tissue cultures of maize. Crop Sci., 15: 417-420. https://doi.org/10.2135/cropsci1975.0011183X001500030040x

Huang, X.Q. and Z.M. Wei. 2004. High-frequency plant regeneration through callus initiation from mature embryos of maize (*Zea Mays* L.). Plant Cell Rep., 22(11): 793-800. https://doi.org/10.1007/s00299-003-0748-9

Liang, G., D.I. Hong, L.U. Cuihua, L. Zhang, L. Dong, Z. Wang, L. Jiang and Y. Zhou. 2010. Study on immature embryos regeneration of maize inbred lines. J. Northeast Agric. Univ., 41(2): 11-14.

Matazau, N.U., N.A. Shaharuddin, M.R. Ismail, I.K. Matazu and M. Mahmood. 2014. Indirect organogenesis and multiple shoots formation from (*Zea mays* L.) mature embryo. International Conference on Food, Biological and Medical Sciences.

Mushke, R., R. Yarra and M. Bulle. 2016. Efficient *in vitro* direct shoot organogenesis from seedling derived split node explants of maize (*Zea mays* L.). J. Genet. Eng. Biotechnol., 14(1): 49-63. https://doi.org/10.1016/j.jgeb.2016.03.001

Pathi, K.M., S. Tula, K.M. Huda, V.K. Srivastava and N. Tuteja. 2013. An efficient and rapid regeneration via multiple shoot induction from mature seed derived embryogenic and organogenic callus of Indian maize (*Zea mays* L.). Plant Signal. Behav., 8(10): e25891. https://doi.org/10.4161/psb.25891

Rakshit, S., Z. Rashid, J.C. Sekhar, T. Fatima and S. Dass. 2010. Callus induction and whole plant regeneration in elite Indian maize (*Zea mays* L.) inbred. Plant Cell Tiss. Organ. Cult., 100(1): 31-37. https://doi.org/10.1007/s11240-009-9613-z

Ramakrishna, N., J. Lacey and J. Smith. 1991. Effect of surface sterilization, fumigation and gamma irradiation in the microflora and germination of barley seeds. Int. J. Food Microbial., 13(1): 47-57. https://doi.org/10.1016/0168-1605(91)90135-C

Sameer, N.M. and K.A. Nabeel. 2016. Effect of different sterilization methods on contamination and viability of nodal segments of *Cestrum nocturnum* L. Int. J. Res. Stud. Biosci., 4(1): 4-9. https://doi.org/10.20431/2349-0365.0401002

Sompornpailin, K. and C. Khunchuay. 2016. Synergistic effects of BAP and kinetin media additives on regeneration of vetiver grass

(*Vetiveria zizanioides* L. Nash). Aust. J. Crop Sci., 10(5): 726-731. https://doi.org/10.21475/ajcs.2016.10.05.p7439

Vladimir, S., L. Gilbertson, P. Adae and D. Duncan. 2006. *Agrobacterium* mediated transformation of seedlings-derived maize callus. Plant Cell Rep., 25: 320-328.

Wada N., C. Feng and A. Gulati. 2008. Introduction and overview in Maize in Asia changing markets and incentives. A. Gulati and J. Dixon (eds.). Academic Foundation, New Delhi. pp. 27-75.

Woods, G. and K. Zito. 2008. Preparation of gene gun bullets and biolistic transfection of neurons in slice culture. J. Vis. Exp., 12: 675. https://doi.org/10.3791/675

Xiang, Y., J. Hai-yangb and Z. Su-wenb. 2007. Establishment of plant regeneration system from mature embryos of maize elite inbred lines. Acta Laser Biol., 16(5): 649-654.

Zang, Q., L. Zhou, F. Zhuge, H. Yang, X. Wang and X. Lin. 2016. Callus induction and regeneration via shoot tips of dendrocalamus hamiltonii. Springerplus, 5(1): 1799. https://doi.org/10.1186/s40064-016-3520-7