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REVIEW PAPER

Mature seed-derived nodal explants: A season-independent explant source for *in vitro* culture in maize

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Abstract Maize is an important industrial crop having multifarious uses including ethanol production and animal feed, and human food. To achieve fuel and food security there is a need to develop high-yielding and climate-resilient cultivars. Tissue culture and genetic transformation are viable approaches for developing crop plants with improved and novel traits. For maize tissue culture endeavours, immature embryos are the most widely used explant globally due to their very high regeneration potential. However, immature embryos as an explant source have a few major drawbacks including their availability in particular seasons, difficulty in storage, and excision of them is quite a laborious and time-consuming process. All these limitations can be easily addressed by utilizing mature seed-derived nodal explant as starting material for tissue culture experiments. These are season-independent (i.e., available throughout the year), and easy to handle and use. The present manuscript highlights the importance of mature seed-derived nodal explants for *in vitro* regeneration and transformation in maize. The various steps followed for obtaining embryogenic callus from nodal explants are also summarized here.

Keywords: Maize tissue culture · Embryogenic callus · Season independent explant · Nodal explants

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Introduction

Maize is one of the major cereal crops being utilized for poultry and animal feed, bioethanol production, staple food, and starch as well as other industries. About one-third of the people in sub-tropical and tropical regions are highly dependent on this crop for their living. Its demand is increasing day by day due to ever increasing population (Rakshit and Karjagi *et al*., 2018). It is ranked as the third most crucial cereal crop worldwide after wheat and rice. In India, this crop contributes to approximately 10 per cent of food grain production. Recently NITI Aayog, Government of India, has identified maize as one of the major feedstocks that can be used for bioethanol production (Pal *et al*., 2023). As a result, maize demand will rise to meet the government's goal of blending E20 and E30 by 2025-2026 and 2030 respectively under the Ethanol Blending Program (EBP).

In the past, conventional plant breeding methods have significantly contributed to maize genetic improvement. However, several limitations are associated with introducing desired traits via traditional breeding because of the lack of germplasm variation and species compatibility. To overcome such limitations, it's essential to use genetic engineering-based approaches (transgenesis, cisgenesis, intragenesis, and genome editing) to introduce novel traits into crop plants (Kumar *et al*., 2020).

To fully harness the potential of aforesaid genetic engineering approaches availability of highly efficient, reproducible, and easy transformation and regeneration protocol is a pre-requisite. In maize, to date, various explant sources have been utilized by different research groups globally for developing improved plants. For instance, immature embryos (Duncan *et al*., 1985; Ishida *et al*.,

1996; Rakshit *et al*., 2010; Malini *et al*., 2015), leaf segments (Conger *et al*., 1987), anthers (Barloy and Beckret *et al*., 1993), tassel and ear meristem (Pareddy *et al*., 1990), protoplasts (Sukhapinda *et al*., 1993) and shoot apices (Sairam *et al*., 2003), etc as explants have been used for tissue culture experiments (Table 1). Within the plant tissue culture, the explants are referred to as small sections of tissue which is utilized for growing and initiating cultures under *in vitro* laboratory conditions. Among all these aforementioned explants, immature embryos are the most widely used worldwide because of their higher regeneration potential under *in vitro* conditions as compared to other explant sources. The 1st case of maize regeneration via immature embryos was reported back in the year 1975 (Green and Philps *et al*., 1975). The successful *in vitro* culture practice in maize majorly depends on the choice of the tissue sample and explant. However, there were certain limitations associated with the use of all these explants including seasonal availability. For instance, leaf segments cannot produce consistent results because of their variability in physiological and genotypic state (Table 1). On the other hand, shoot apices are not easy to isolate and maintain in laboratory conditions (Kane *et al*., 2011). The immature embryos, one of the most commonly utilized plant materials, are not available throughout the year. The availability of immature embryos mainly depends on the breeding season of the maize plant. Therefore, Hi-tech and sophisticated glass houses or phytotron facilities are sometimes utilized to ensure the off-season availability of immature embryos. However, many times the quality of immature embryos obtained from plants grown in such advanced facilities is also insufficient for carrying out tissue culture experiments.

These limitations of the above-mentioned sources of explant in tissue culture result in the subsequent exploration of alternatives that provide better flexibility, year-round availability, and consistency. In response to these, nodal explants derived from mature seeds have emerged as potential candidates. The mature seed-derived nodal explants do not have seasonal constraints and offer continuous supply throughout the year (Tiwari *et al*., 2015; Kumar *et al*., 2022).

Extracting nodal explant from mature seeds and development of embryogenic calli

The procedure for obtaining nodal explant via mature seeds requires meticulous attention towards maize kernel sterilization. As mentioned in the previous research studies (Kumar *et al*., 2022), the various steps involved in obtaining nodal explant from mature seeds, callus regeneration, multiplying embryo, and regeneration are as follows:

Table 1. Details of various explants used for *in vitro* regeneration in maize

S.No.	Explant source	Merits	De-merits	References
1	Leaf segments	Easy to isolate andreadily available in a large amount	Usually, low regeneration efficiency	Conger *et al*., 1987; Ahmadabadi *et al*., 2007
2	Anthers	Potential for haploid & double haploid plant production	Limited tissue availability	Ting *et al*., 1981; Barloy and Beckret *et al*., 1993
3	Protoplasts	The ability for genetic transformation	Labor intensive, regeneration of complete plantlets is not easy	Sukhapinda *et al*., 1993
4	Tassel and Ear meristems	It takes only about 2 to 3 weeks to form callus	Lower transformation efficiency	Pared‘dy *et al*., 1990
5	Seedling-derived leaf and root tips	Easily obtained and root tip can generate a high frequency of primary embryogenic calli	Genotype- dependent	Wang *et al*., 2021
6	Shoot apical meristems	Rapid regeneration potential and can directly undergo organo-genesis without forming a callus.	Provide very little cellular material for growth as a very small number of meristem cells are present at the tip.	Sairam *et al*., 2003
7	Immature embryos	High regeneration potential, hence most preferred and widely used	Season-dependent, Time-consuming, Laborious	Ishida *et al*., 1996; Rakshit *et al*., 2010; Malini *et al*., 2015
8	Mature embryos	Season-independent and amenable to *in vitro* tissue culture	Recalcitrant for tissue culture and hence low regeneration frequency	Hung and Wei, 2004

1. Plant material and sterilisation of seeds

The very first step involves the use of a properly developed and bold mature seed (kernel) obtained from high-yielding elite genotypes. This step involved the surface sterilization of mature seeds which is carried out by a series of different treatments under laminar flow. The seeds are first washed with 1% bavistin for 20 minutes, followed by washing the seeds with double distilled water. Next, the seeds are immersed in 0.2% SDS solution and washed for 10 minutes. Lastly, seeds are treated with 0.1% $HgCl_2$ (Mercury chloride) for at least 5 minutes (for details, please see the "Methods" section in Kumar *et al*., 2022). All these steps are followed by rinsing the seeds with sterile double distilled water. Continuous and thorough shaking of seeds (rigorously) is a must to get rid of fungal contamination. Manual shaking is effective as compared to shaking using shakers.

2. Germination of seed and preparation of nodal explant

Once the seeds are sterilized and left for drying, they are placed on a germination medium which contains various components, including MS salts, plant growth hormones (L-proline and arginine), maltose, calcium chloride, and growth regulators such as BAP and picloram (Kumar *et al*., 2022). BAP and Picloram help to get a prominent bulge. After incubation for approximately 12 to 15 days, seedlings with a prominent bulge (node; which is usually present 3-5 cm above germinated seeds /shoot initiation point /hypocotyl-epicotyl axis) were selected and excised carefully by cutting just above and below the node under aseptic conditions (Figure 1).

3. Callus induction and multiplication

The excised part was further utilized for the process of callus induction. The excised part of the nodal region/node is split longitudinally into two halves with the help of sterilized sharps and subsequently placed onto a callus induction medium (Figure 1). The callus induction medium generally contains growth regulators (auxins and cytokinins; 2.2 mg/l picloram, 0.5 mg/l 2,4-D used in our study) and nutrient components essential for callus formation (for details, please see the "Methods" section in Kumar *et al*., 2022). The excised parts placed onto the callus media were properly sealed with the help of parafilm and subsequently, these petri plates were incubated for 4 to 5 weeks under dark light. After two weeks only embryogenic callus was selected and sub-cultured in fresh plates having the same media whereas non-embryonic ones were discarded.

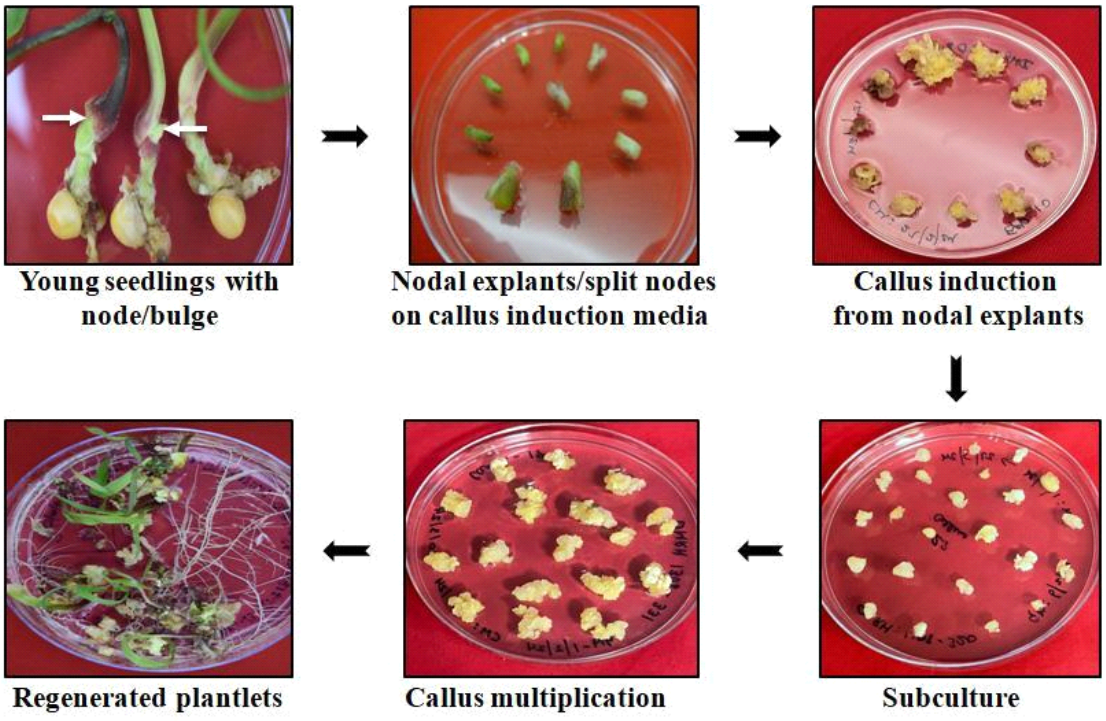

Figure 1. A pictorial representation of callus induction and regeneration from mature seed-derived nodal explants in maize

4. Plant regeneration

Embryogenic calli were further transferred to a different media known as regeneration media containing plant growth hormones such as cytokinins and auxins as per the requirement of the genotype. After 15 to 25 days, regenerated shoots were separated under aseptic conditions and were further utilized for root development by placing them onto the rooting medium in jam bottles. Generally, rooting media is MS salts without any hormones. However, 0.1 mg/l NAA may be used in case of lack of proper root formation. This step is followed by carefully transferring the rooted plantlets onto Coco peat and vermiculite mixture for acclimatization and hardening.

Nodal explants as a season independent explant source

The season-independent source of explant for tissue culture work can be utilized to perform genetic transformation experiments throughout the year. Among the various explants used in maize, explants derived from mature seeds are more suitable to obtain explant sources around the year. The most important benefit of using mature seed as the starting material is its availability throughout the year. Moreover, these seeds are easily produced and stored at room temperature for nearly 1 to 2 years; at 4°C for 2 to 3 years, and at -20°C for 4 to 5 years approximately. This offers a constantly reliable and available supply of quality material. Moreover, mature seeds are easy to transport which makes them best suited for utilization in tissue culture (Wang *et al*., 2012). Considering all these benefits of using mature seeds over immature seeds/ embryos as well as other explants, nodal explants (which were derived from young seedlings) emerge as an ideal explant to perform maize tissue culture and transformation work throughout the year (Kumar *et al*., 2022). Since callus culture from nodal explants solely depends on young seedlings which can be produced anytime in unlimited numbers from mature seeds, it facilitates the simple and rapid establishment of tissue cultures in maize. In this context nodal explant can be considered easy to handle, reproducible, user friendly, and thereby the most reliable explant source in maize.

Conclusion

In conclusion, mature seed-derived nodal explant is an ideal explant source for *in vitro* regeneration and transformation studies. It provides a reproducible, efficient, and season-independent source for generating transgenic and genome-edited maize with improved traits. However, these are genotype-dependent, and usually, some genotypes do not produce good quality and quantity of embryogenic calli from mature seed-based explants. Therefore, it necessitates identifying the best suitable maize genotype having good callus induction and regeneration efficiency from nodal explants by screening a set of germplasms. Once we have such a tissue culture-responsive genotype, then one can store the seed for years and perform tissue culture experiments throughout the year easily.

Author's contribution

KK has conceptualized the theme of this review. GS and MT have written the draft of the manuscript. AKJ, Neha, and PP have helped in tissue culture work and the preparation of schematic diagrams/figures. KK, BK, HSJ, and NS edited the manuscript. All authors finally read and approved the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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References

Ahmadabadi, M., Ruf, S. & Bock, R. (2007). A leaf-based regeneration and transformation system for maize (*Zea mays* L.). *Transgenic Research*, **16**: 437-448.

Barloy, D. & Beckert, M. (1993). Improvement of regeneration ability of androgenetic embryos by early anther transfer in maize. *Plant Cell, Tissue and Organ Culture*, **33**: 45-50.

Conger, B. V., Novak, F. J., Afza, R. & Erdelsky, K. (1987). Somatic embryogenesis from cultured leaf segments of *Zea mays*. *Plant Cell Reports*, **6**: 345-347.

Duncan, D. R., Williams, M. E., Zehr, B. E. & Widholm, J. M. (1985). The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. *Planta*, **165**: 322-332.

Green, C. E. & Phillips, R. L. (1975). Plant regeneration from tissue culture of maize. *Crop Science,* **15**: 417–421.

Ishida, Y., Saito, H., Ohta, S. H., Hiei, Y., Komari, T. & Kumashiro, T. (1996). High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. *Nature Biotechnology,* **14**: 745–750.

Kane, M. (2011). Propagation by shoot culture. *Plant Tissue Culture, Development and Biotechnology. CRC Press, Boca Raton, FL*, 181-191.

Kumar, K., Gambhir, G., Dass, A., Tripathi, A. K., Singh, A., Jha, A. K. & Rakshit, S. (2020). Genetically modified crops: current status and future prospects. *Planta*, **251**(4): 91.

Kumar, K., Jha, A. K., Kumar, B., Karjagi, C. G., Abhishek, A., Gambhir, G. & Rakshit, S. (2022). Development of an efficient and reproducible in vitro regeneration and transformation protocol for tropical maize (*Zea mays* L.) using mature seed-derived nodal explants. *Plant Cell, Tissue and Organ Culture (PCTOC)*, **148**(3): 557-571.

Malini, N., Ananadakumar, C. R. & Ramakrishnan, S. H. (2015). Regeneration of Indian maize genotypes (*Zea mays* L.) from immature embryo culture through callus induction. *Journal of Applied and Natural Science,* **7**(1): 131–137.

Pal, B. (2023). Utilization of corn for ethanol production: An analysis of production, economy, and grain type requirements. *Maize Journal*, **12**(1): 58-60.

Pareddy, D. R. & Petolino, J. F. (1990). Somatic embryogenesis and plant regeneration from immature inflorescences of several elite inbreds of maize. *Plant Science*, **67**(2): 211-219.

Rakshit, S. & Karjagi, C. G. (2018). Perspective of maize scenario in India: way forward. *Maize Journal,* **7**(2): 49–55.

Rakshit, S., Rashid, Z., Sekhar, J. C., Fatma, T. & Dass, S. (2010). Callus induction and whole plant regeneration in elite Indian maize (*Zea mays* L.) inbreds. *Plant Cell, Tissue and Organ Culture,* **100**: 31–37.

Sairam, R. V., Parani, M., Franklin, G., Lifeng, Z., Smith, B., MacDougall, J. & Goldman, S. L. (2003). Shoot meristem: an ideal explant for *Zea mays* L. transformation. *Genome*, **46**(2): 323-329.

Sukhapinda, K., Kozuch, M. E., Rubin-Wilson, B., Ainley, W. M. & Merlo, D. J. (1993). Transformation of maize (*Zea mays* L.) protoplasts and regeneration of haploid transgenic plants. *Plant Cell Reports*, **13**(2): 63-68.

Ting, Y. C., Yu, M. & Zheng, W. Z. (1981). Improved anther culture of maize (*Zea mays*). *Plant Science Letters*, **23**(2): 139-145.

Tiwari, S., Agrawal, P. K., Pande, V. & Gupta, H. S. (2015). Callus induction and whole plant regeneration in sub-tropical maize (*Zea mays* L.) using mature embryos as explants. *Indian Society of Genetics and Plant Breeding,* **75**(3): 330–335. https://doi.org/10.5958/0975-6906.2015.00052.8

Wang, C., Ma, H., Zhu, W., Zhang, J., Zhao, X. & Li, X. (2021). Seedling derived leaf and root tip as alternative explants for callus induction and plant regeneration in maize. *Physiologia Plantarum*, **172**(3): 1570-1581.

Wang, H., Cheng, J., Cheng, Y. & Zhou, X. (2012). Study progress on tissue culture of maize mature embryo. *Physics Procedia*, **25**: 2225-2227.