Optimization of Picloram and TDZ for callus formation and somatic embryogenesis in Date Palm inflorescences tissue culture

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Abstract: This study investigates the use of Picloram and Thidiazuron (TDZ) for optimizing the callus formation and somatic embryogenesis in the date palm (*Phoenix dactylifera* L.) using an inflorescences explant. The effects of different concentration of Picloram (1.0-5.0 mg/l) with or without TDZ supplementation were assessed on callus induction, browning rates, and embryogenic callus formation. Results showed that 5.0 mg/l Picloram significantly increased the callus formation (92.8%) and reduced the browning rates (20.3%). TDZ, particularly at 1.0 mg/further enhanced embryogenesis with the highest embryogenic rate (75.5%) observed in IF-85 medium. This study highlights the potential of inflorescences explant and optimized growth regulator combinations for large scale propagation of date palms through embryogenesis.

Keywords: Picloram, Thidiazuron, *Phoenix Dactylifera* L, Callus Formation, Inflorescences

Introduction: Date palm (*Phoenix dactylifera* L.) is a staple crop in the arid and semi- arid regions, providing critical economic and nutritional benefits. However, traditional propagation methods, such as offshoot propagation, are constrained by the low multiplication rates, genetic variability and susceptibility to biotic and abiotic stresses. Tissue culture, particularly somatic embryogenesis, offers an efficient alternative for mass propagation, genetic improvement, and conservation of elite date palm genotypes. (1)(2)

Among plant tissues, immature inflorescences are highly suitable explants for the tissue culture due to their active meristematic zones and high responsiveness to growth regulators. Picloram, a synthetic auxin, has been shown to induce somatic embryogenesis in monocots, while TDZ, a phenyl urea-derived cytokinin, enhances cell differentiation and proliferation. Despite the individual roles of these regulators, their synergistic effects on date palm inflorescence tissue remain underexplored.(3). This study evaluates the combined effects of picloram and TDZ on callus induction, browning rates, and somatic embryogenesis, with the aim of optimizing tissue culture protocols for the date palm.

Materials and Methods:

Plant Material and sterilization: Immature inflorescence of DP were collected form the healthy plants, inflorescences (IF) were sterilized with ethanol (70%) and mercuric chloride (0.1%) for 15minutes, followed by rinsing with sterile water. Explants were excised into 1-2 cm sections for culture.

Media Preparation: The MS medium was supplemented with the Picloram (0.5,1,2,5 and 5.0 mg/l), TDZ (0.5.1.0.2.0 mg/l) in selected treatments , additives such as sucrose (30g/l), activated charcoal (0.5g/l) and agar (5g/l). The pH was adjusted to 5.7 ± 0.1 before autoclaving. For the enhanced somatic embryo proliferation, TDZ (0.5mg/l) was added to the media.

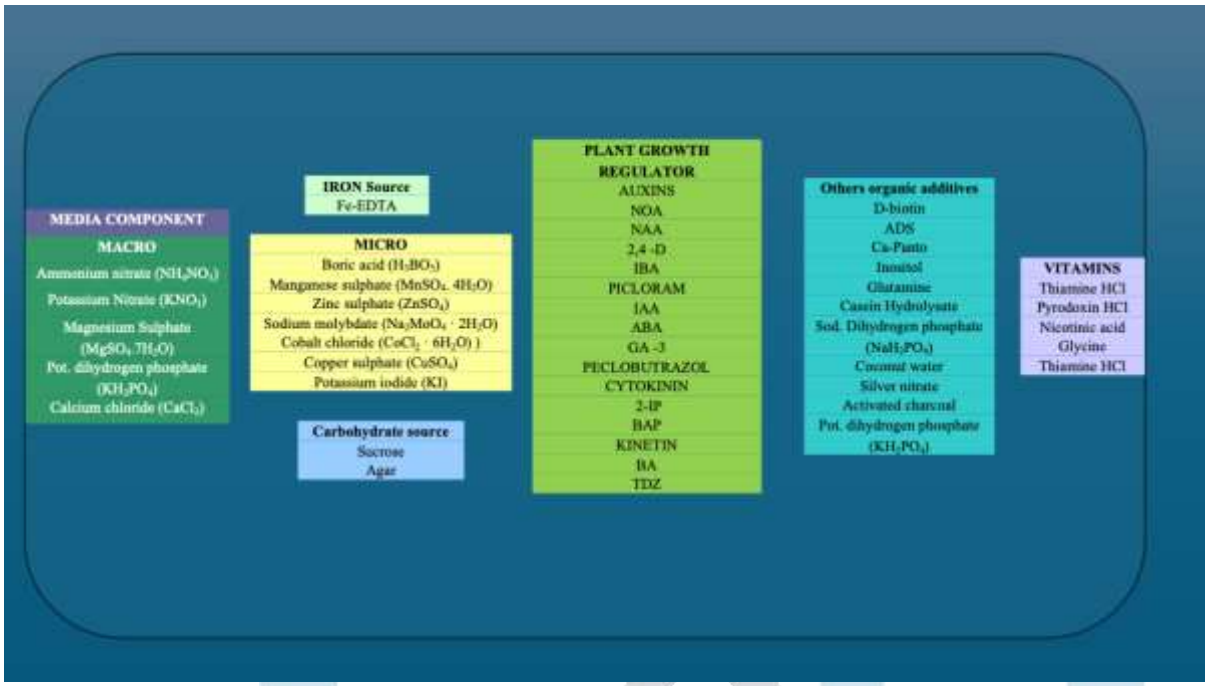

Table 1: Media composition for the inflorescence-based media for culturing

Culture Conditions: Cultures were maintained at 26 ± 2 º C in complete darkness. Explant were re-cultured every six weeks. Observations on callus texture, embryogenesis, and proliferation were recorded.

Data collection and Statistical Analysis:

Callus formation (%): Number of explants forming callus as a percentage of total explants. **Browning rate:** Proportion of explants exhibiting browning.
Embryogenic callus (%): Percentage of callus showing somatic embryogenesis. Data were analyzed using ANOVA with LSD post hoc tests ($p< 0.05$) .

Results

Callus Formation and browning rates: The effect of Picloram concentrations on callus formation and browning rates is shown in figure A. Callus formation increased with higher picloram concertation, peaking at 92.8% at 5.0mg.L. Concurrently, browning rates decreased ,with 5.0. mg/L Picloram producing the lowest browning (20.3%). This suggests that picloram not only promotes callus induction but also reduces oxidative stress in tissue culture.(Table 2). (4)(5)

Table 2: Callus Formation and Embryogenesis by Picloram and TDZ.

Picloram (mg/l)	TDZ (mg/l)	Callus formation (%)	Embryogenic Callus (%)	Embryo count per explant
0.5	-	45.3 ± 5.1	10.2 ± 2.3	2.1 ± 0.34
1.0	-	63.2 ± 4.2	20.4 ±4.0	5.3 ± 0.87
2.0	-	80.5 ± 3.7	40.1 ± 5.2	8.0 ± 1.12
5.0	-	92.8 ± 3.1	75.1 ±3.5	11.3 ± 1.24
5.0	0.5	92.8 ± 3.1	85.5 ± 2.1	11.5 ±1.74

Embryogenesis callus and Somatic embryogenesis: The addition of TDZ significantly enhanced embryogenesis, particularly in media containing 5.0 mg/l Picloram. Embryogenic callus formation reached its highest rate (75.5%) in IF 85 medium (5.0 mg/l Picloram + 1.0 mg/L TDZ). As illustrated in figure B.

Lower TDZ concentration (0.5mg/L) showed the moderate improvements while higher concentration (2.0 mg/L) had no additional benefits.

Combined effects: Heatmap Analysis: A heatmap (Fig. C) visualizes the combined effects of picloram, TDZ and media types on embryogenesis rates. Synergistic effects were most pronounced in media supplemented with 5.0 mg.; picloram and 1.0mg/l TDZ, emphasizing the importance of optimizing the growth regulator interactions.

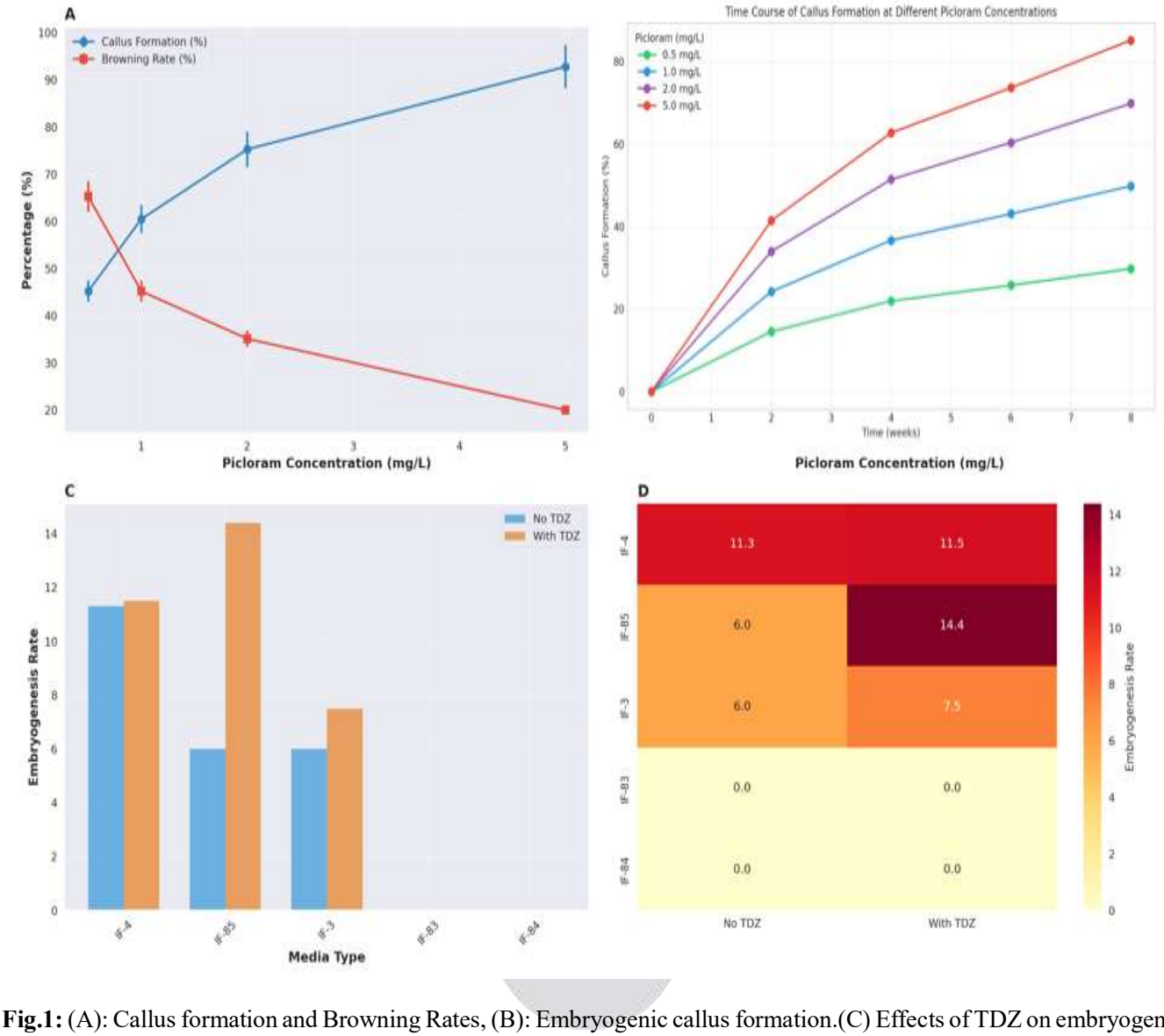

Fig.1: (A): Callus formation and Browning Rates, (B): Embryogenic callus formation.(C) Effects of TDZ on embryogenesis (D) Combined effects: Heatmap Analysis.

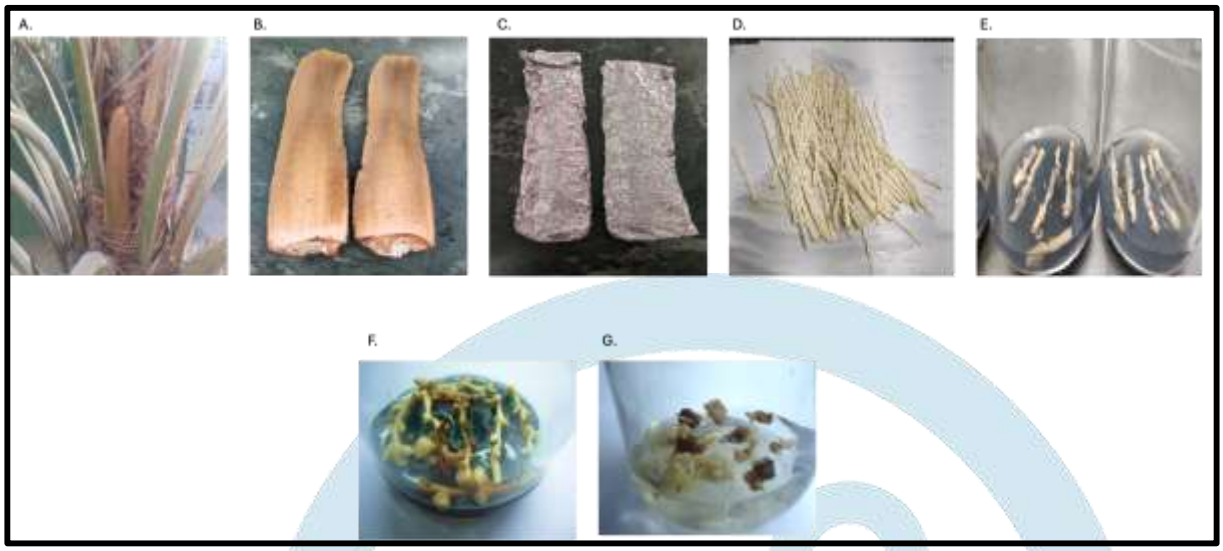

Fig. 2: Different stages for date palm inflorescences (IF) plant tissue culture. (A) date palm IF (B) Collected IF (C)IF wrapped with aluminum foil, (D) IF spikes (E) explant, inoculation, (F) 12 week explant (G) explant with callus.

Discussion: This study highlights the combined effects of Picloram and Thidiazuron (TDZ) on callus formation and somatic embryogenesis using immature inflorescences explants of *phoenix dactylifera*. L. The findings underscore the pivotal role of these growth regulators in overcoming the key challenges in the date palm tissue culture, such as browning and low embryogenic potential.

Role of Picloram in callus formation and browning reduction: Picloram emerged as a critical auxin for inducing callus formation, with a concentration of 5.0mg/l yielding the highest callus formation rate (92.8%) and the lowest browning rate (20.3%). Auxins, including picloram, promote cellular de-differentiation and the proliferation by stimulating the expression of genes involved in the cell division .The observed reduction in the browning at higher picloram concentration could be attributed to its antioxidative properties, which mitigates the effects of phenolic oxidation which is a major cause of tissue browning in vitro (6).These results align with the studies in other monocot species, such as oil palm and banana, where picloram effectively reduced the browning while enhancing the callus vigor.(7)(8)(9)

Synergistic effects of the picloram and TDZ on the somatic embryogenesis: The study demonstrates that TDZ significantly enhances the embryogenic potential of callus when combined with picloram. The highest embryogenic rate (75.5%) was observed in IF-85 Medium containing 5.0 mg/L picloram and 1.0mg/L TDZ, a cytokinin-like growth regulator, is known to stimulate the secondary metabolite production and enhance the cellular differentiation. Its action was likely to complements the picloram by modulating hormonal crosstalk between auxin and cytokinin pathways creating the favorable environment for embryogenesis. Previous studies have reported similar synergistic effects in *Cocos nucifera* and *Zea mays*, where TDZ enhanced auxin-induced somatic embryogenesis (10)(11)

Media composition and nutrient -driven interactions: The heatmap analysis revealed the critical interactions between media composition, Picloram and TDZ. Media such as IF-85 supported the high embryogenic rates due to a balanced nutrient profile that likely complemented the action of growth regulators. Conversely, IF-83 and IF-84 showed no embryogenesis, suggesting the nutrient deficiencies or imbalance that inhibited growth. These findings emphasize the importance of optimizing both hormonal and nutritional components to achieve the consistent and high embryogenic outcomes.(12)(13).(14) (15)

Practical Implications and Future research direction: Our results of this study offer practical insights for large scale propagation of date palms. The optimized combination of 5.0mg/l picloram and 1.0mg/l TDZ can be integrated into commercial tissue culture protocols to enhance the efficiency and reduce the production costs. Future studies should focus on

- Exploring the molecular mechanism underlying the picloram-TDZ synergy, including gene expression analysis of auxin and cytokinin responsive pathways.
- Evaluating the genetic stability and field performance of plants regenerated through this protocol
- Assessing the scalability of this protocol for other date palm cultivars and monocot species.

Conclusion: Picloram at an optimal concertation of 5mg/significantly enhances callus formation and somatic embryogenesis in the date palm tissue cultures, particularly when combined with TDZ. This study provides a robust framework for the scaling up the micropropagation systems, contributing to the sustainable cultivation of this vital crop.

Reference:

1. Hadrami, I.E. and Hadrami, A.E., 2009. Breeding date palm. In *Breeding plantation tree crops: tropical species* (pp. 191-216). New York, NY: Springer New York.
2. Jaradat, A.A., 2016. Genetic erosion of Phoenix dactylifera L.: perceptible, probable, or possible. *Genetic Diversity and Erosion in Plants: Case Histories*, pp.131-213.
3. Custódio, L., Charles, G., Magné, C., Barba-Espín, G., Piqueras, A., Hernández, J.A., Ben Hamed, K., Castañeda-Loaiza, V., Fernandes, E. and Rodrigues, M.J., 2022. Application of in vitro plant tissue culture techniques to halophyte species: A review. *Plants*, *12*(1), p.126.
4. Dai, L., Zhou, Q., Li, R., Du, Y., He, J., Wang, D., Cheng, S., Zhang, J. and Wang, Y., 2015. Establishment of a picloram-induced somatic embryogenesis system in Vitis vinifera cv. chardonnay and genetic transformation of a stilbene synthase gene from wild-growing Vitis species. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *121*, pp.397-412.
5. Phillips, G.C. and Luteyn, K.J., 1983. Effects of picloram and other auxins on onion tissue cultures. *Journal of the American Society for Horticultural Science*, *108*(6), pp.948-953.
6. Baskaran, P., Kumari, A., Naidoo, D. and Van Staden, J., 2015. In vitro propagation and biochemical changes in Aloe pruinosa. *Industrial Crops and Products*, *77*, pp.51-58.
7. Rao, I.S. and Madhulety, T.Y., 2005. Role of herbicides in improving crop yields. *Developments in Physiology, Biochemistry and Molecular Biology of Plants*, *1*, pp.203-287.
8. Kumar, S., Mishra, S. and Mishra, A.P., 2016. *Plant tissue culture: theory and techniques*. Scientific Publishers.
9. Bhattacharya, A., 2022. Plant growth hormones in plants under low-temperature stress: a review. *Physiological processes in plants under low temperature stress*, pp.517-627.
10. Khatri, P. and Joshee, N., 2024. Effect of Picloram and Desiccation on the Somatic Embryogenesis of Lycium barbarum L. *Plants*, *13*(2), p.151.
11. Thomas, T.D., 2008. The role of activated charcoal in plant tissue culture. *Biotechnology advances*, *26*(6), pp.618-631.
12. Thatcher, W.W., Moreira, F., Santos, J.E.P., Mattos, R.C., Lopes, F.L., Pancarci, S.M. and Risco, C.A., 2001. Effects of hormonal treatments on reproductive performance and embryo production. *Theriogenology*, *55*(1), pp.75-89.
13. Deo, P.C., Tyagi, A.P., Taylor, M., Harding, R. and Becker, D., 2010. Factors affecting somatic embryogenesis and transformation in modern plant breeding. *The South Pacific Journal of Natural and Applied Sciences*, *28*(1), pp.27-40.

14. Gardner, D.K., Pool, T.B. and Lane, M., 2000. Embryo nutrition and energy metabolism and its relationship to embryo growth, differentiation, and viability. In *Seminars in reproductive medicine* (Vol. 18, No. 02, pp. 205-218). Copyright© 2000 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA. Tel.:+ 1 (212) 584-4662.

15. Swain, J.E., Carrell, D., Cobo, A., Meseguer, M., Rubio, C. and Smith, G.D., 2016. Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential. *Fertility and sterility*, *105*(3), pp.571-587.