Synergistic effect of Quercetin and Auxins on Somatic embryogenesis in Local Kutch Elite Date Palm (*Phoenix dactylifera* L.)

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Abstract

Date palm (*Phoenix dactylifera* L.), a vital fruit crop of arid regions is known for its agronomic and economic significance . This study investigates the synergistic effects of Quercetin and various auxins (NAA,2,4-D,and IAA) on the somatic embryogenesis in the Kutch elite date palm variety. Observation on callus texture, embryogenesis rates, and the responsiveness across 15 different media composition revealed that the combination of IAA (0.1mg/L) and Quercetin (0.1µM) was most effective ,achieving up to 70% embryogenesis . Statistical analysis (ANOVA and Tukey's HSD) confirmed that the IAA treatments had significantly greater effects on the embryogenesis than NAA and 2,4-D.These findings provide a robust framework for optimising the tissue culture protocols for the large -scale propagations of this economically important cultivar.

Keywords

Synergistic effect; Quercetin; Auxins; Somatic embryogenesis; Phoenix dactylifera.

Introduction

Date Palm is widely cultivated in the arid and semi-arid regions, with the major producers being the middle east,north Africa, and parts of USA . Although India is not a global leader in the date palm production, the Kutch regions in Gujarat has emerged as a significant centre for the cultivating of elite varieties due to its unique climatic and soil conditions. These varieties hold potential for the both commercial and research applications.

Conventional propagation methods in the date palm are hindered by slow growth rates and limited scalability ,making tissue culture approaches like somatic embryogenesis a preferred alternative. Auxins are the key

regulators of the cellular differentiation and division during the embryogenesis, while quercetin, a flavonoid with the significant antioxidant activity, aids in the mitigation of oxidative stress and enhances embryogenic efficiency in the in vitro condition .(1)

This study aims to assess the combined effects of quercetin and auxins on the somatic embryogenesis to establish efficient tissue culture protocols for the Kutch elite date palm.

Materials and Methods

Plant Materials

Explant samples were collected from the Kutch elite date palm cultivars maintained under the greenhouse conditions.

Culture Media Preparations:

Basal Medium:

Murashige and Skoog (MS) Medium was used as the base.

Auxins and Quercetin Concentrations: NAA, 2,4-D,and IAA were used at concentrations ranging from 0.1 to 2.0mg/L Quercetin was incorporated at the concentrations of 0.1 to 5.0 µM A total of 15 media compositions (Media Codes 1- 15) were tested (**Table 2**).

Experimental Design

Explants were cultured in the sterile conditions at 25±2º celsius under a 16hr photoperiod. Observations on the callus texture ,new callus formation and embryogenesis rates were recorded before and after 30 days of subculture.

Data analysis

ANOVA and Tukey's HSD post hoc test were performed to assess the statical significances of differences in the embryogenesis percentages across auxin treatments .Descriptive statistics were used for the data visualisations.

Results

Effect of Auxins and Quercetin on the embryogenesis (**Table 1**): Highest Embryogenesis rates:

1. Media code 11 (MS+ 0.1 mg/L IAA + 0.1 µM Quercetin) produced the highest embryogenesis rate of 70%,demonstrating the combined efficiency of low IAA concentrations and Quercetin in promoting cellular differentiation .(2)
2. Media code 14 (MS + 1.5 mg/L IAA + 2.0µM Quercetin) achieved a secondary peak (50%).

Media Code	**Observation before subculture**	**Observation after subculture (30 days)**			
		Callus Texture	New callus (%)	No response (%)	Embryogenesis (%)
1	C & P	C & P	60	40	0
2	B & P	N, L & P	0	100	0
3	D & P	D, L & P	40	60	20
4	L & P	L, P, N & E	70	20	30
5	C & P	L, C, P & N	20	80	0
6	C & P	N, C, P & E	40	60	20
7	B & P	L, N & P	50	50	0
8	D & P	L, N & P	40	60	0
9	L & P	D, P & E	10	90	30
10	C & P	L, P & N	70	30	10
11	C & B	L, C, N & E	100	0	70
12	L & P	L, P, N & E	60	40	40
13	C & B	L, P, N & E	40	60	20
14	C & B	L, P, N & E	100	0	50
15	C & B	D, L, P, N & E	20	80	30

Table 1: C: Creamy; P: Pasty; D: Dark brown; L: Light brown; E: Embryogenesis; N: New callus; B: Brown

Media code 1:	MS + 0.1 mg/l NAA + 0.1 µM Quercetins	Media code 8:	MS + 1.0 mg/l 2,4-D + 1.0 µM Quercetins
Media code 2:	MS + 0.5 mg/l NAA + 0.5 µM Quercetins	Media code 9:	MS + 1.5 mg/l 2,4-D + 2.0 µM Quercetins
Media code 3:	MS + 1.0 mg/l NAA + 1.0 µM Quercetins	Media code 10:	MS + 2.0 mg/l 2,4-D + 5.0 µM Quercetins
Media code 4:	MS + 1.5 mg/l NAA + 2.0 µM Quercetins	Media code 11:	MS + 0.1 mg/l IAA + 0.1 µM Quercetins
Media code 5:	MS + 2.0 mg/l NAA + 5.0 µM Quercetins	Media code 12:	MS + 0.5 mg/l IAA + 0.5 µM Quercetins

Media code 6:	MS + 0.1 mg/l 2,4-D + 0.1 µM Quercetins	Media code 13:	MS + 1.0 mg/l IAA + 1.0 µM Quercetins
Media code 7:	MS + 0.5 mg/l 2,4-D + 0.5 µM Quercetins	Media code 14:	MS + 1.5 mg/l IAA + 2.0 µM Quercetins
		Media code 15:	MS + 2.0 mg/l IAA + 5.0 µM Quercetins

Table 2: Media compositions

Suppressed Embryogenesis

High concentration of 2,4-D (e.g., Media code 10) resulted in moistly pasty callus with the minimal embryogenesis. Correlation between callus texture and embryogenesis: Light brown callus was consistently associated with the higher embryogenesis rates. Dark Brown and pasty texture correlated with the non- responsiveness.(**Figure 1**)

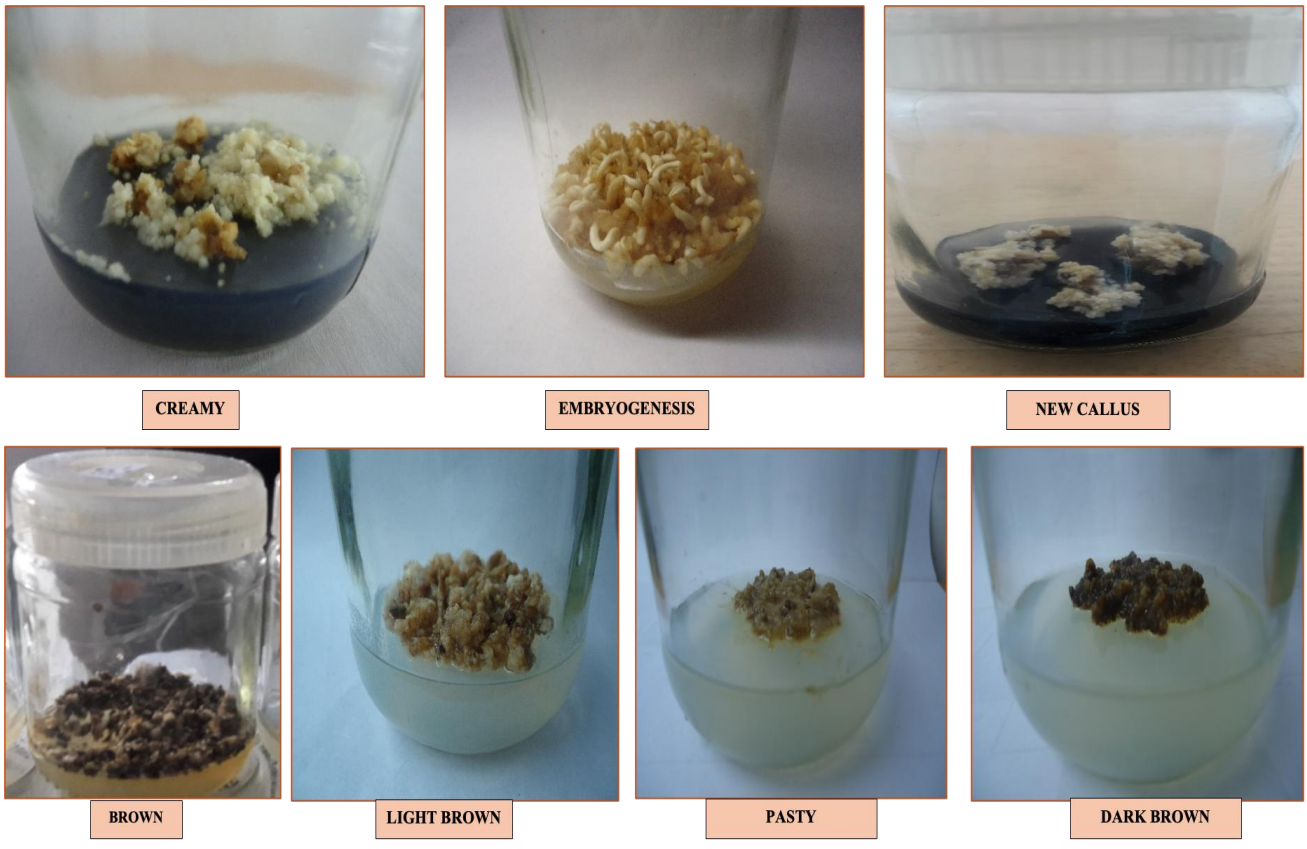

Figure 1: Effect of different concentration of quercetin and auxins on the date plant.

Statistical Validation

ANOVA Results: A statically significant differences in the embryogenesis percentage was found among the auxin treatments (F-statistic = 6.51,p =0.012).

Tukey's HSD Post Hoc Test: IAA treatments produces significantly higher embryogenesis rates compared to NAA and 2,4-D.No significant differences was observed between NAA and 2,4-D treatments.

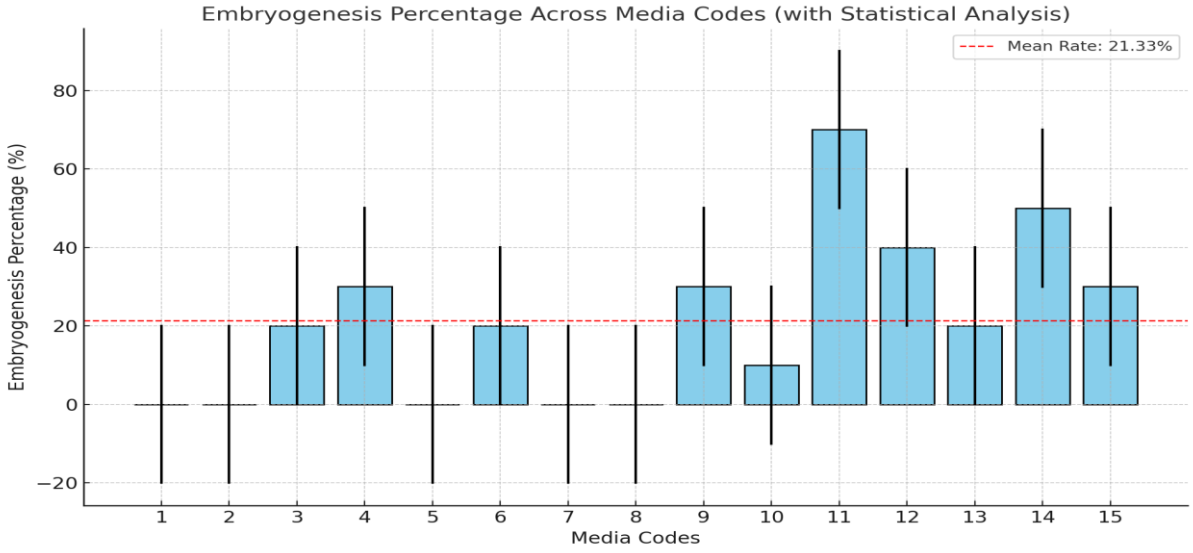

Figure 2:

Embryogenesis percentage across media codes, displays embryogenesis percentages across media codes, with error bars added to represent variability.

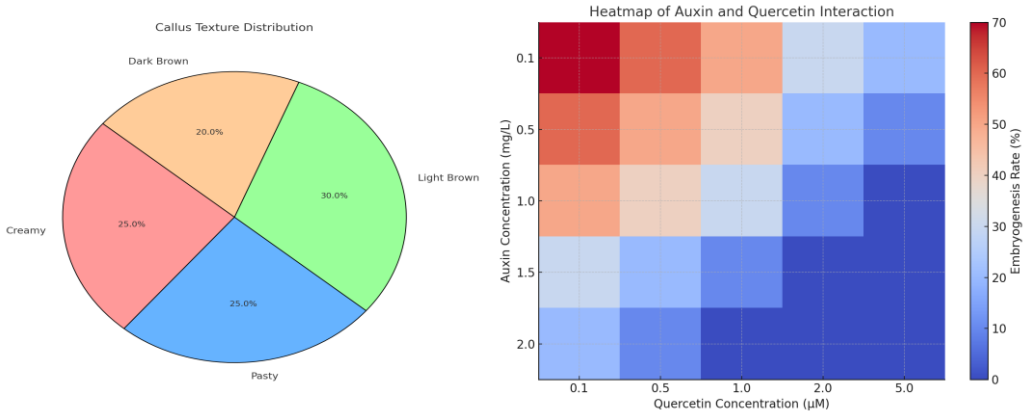

Figure 3: Callus texture distribution (*Pie Chart*) Shows the callus texture distribution, using distinct colours for improved readability. (**Figure. 4)** Heatmap of Auxin and Quercetin interaction (*Heatmap*) illustrates the interaction between auxin and quercetin concentrations, highlighting embryogenesis rate.

Discussion:

Statistical analysis validated these findings, with ANOVA revealing significant differences among auxin treatments (F= statistic= 6.51,p=0.012).Tukey's HSD further confirmed that IAA significantly outperformed NAA and 2,4-D, establishing its superior role in the somatic embryogenesis .(3)

The findings underscore the importance of precise media optimization in tissue culture protocols for date palms, especially for challenging genotypes like the Kutch elute variety.

This study systematically evaluated the synergistic effects of Quercetins and auxins (NAA,2,4-D and IAA) on somatic embryogenesis in Kutch elite date palm. A cultivar of local importance with the significant commercial potential. The results revealed that low concentrations of IAA (0.1mg/L) in combination with quercetin (0.1µM) consistently outperformed other treatments achieving the highest embryogenesis rate of 70%. This is a significant outcomes as it demonstrates the potential of IAA as the preferred auxin for promoting somatic embryogenesis in the date palm when paired with Quercetins (Figure 4).

Furthermore, the study highlighted the critical role of Quercetins an antioxidant in mitigating the oxidative stress in explants, herby enhancing the embryogenesis efficiency. This findings underscores the importance of incorporating the antioxidants into tissue culture protocols, particularly for species and genotype prone to oxidative stress.

The statistical validation using ANOVA and Tukey's HSD provided strong evidence of the superiority of IAA treatments over NAA and 2,4-D ,establishing IAA as the most effective auxin for the date palm tissue culture. The correlation between callus textures and embryogenesis outcomes further supports the use of qualitative indicators such as the presence of light brown callus, as markers for embryogenic potential (Figure 2). The findings of this study hold significant implications for both research and commercial propagation:

a. Optimised protocols: The demonstrated efficacy of IAA and Quercetins can be directly applied to refine tissue culture protocols for large scale propagation of elite date palm cultivars (4).

b. Scalability and cost effectiveness: By reducing reliance on higher auxin concentration and optimising antioxidant usage ,these protocols can be made more cost effective and scalable for commercial nurseries.(5)(6)

c. Conservation Efforts: The optimised protocols can aid in the existing conservation of rare and economically valuable date palm varieties especially in the arid regions.

The study opens avenues for further exploration ,including the followings

d. Testing the synergistic efforts of Quercetins with other phytohormones like cytokinin to enhance regeneration efficiency.(7)(8)

e. Investigating the molecular mechanism underlying the role of antioxidants in promoting the somatic embryogenesis.(9) (10) (11)

f. Expanding the study to include other economically important varieties of the date palm to validate the universality of the findings.

Conclusion

The integration of IAA at 0.1mg/L and Quercetin at 0.1uM offers a strategic advantages for effect somatic embryogenesis protocols, showcasing the significant potential for enhancing the propagation of elite date palm cultivars .(12)

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