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Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

Evaluating The Role of Meta-Topolin and Tryptophan Foliar Application in Reducing the Growth Period Before Budding in Flying Dragon [Poncirus Trifoliata (L.) Raf. Var. Monstrosa (T. Itô) Swingle] Rootstock

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Article info

Received: 2025-01-29
Accepted: 2025-05-06
Published: 2025-12-31

DOI-Crossref:
10.32649/ajas.2025.189162

Cite as:
Al-Janabi, A. M. I., and Aubied, I. A. (2025). Evaluating The Role of Meta-Topolin and Tryptophan Foliar Application in Reducing the Growth Period Before Budding in Flying Dragon [Poncirus Trifoliata (L.) Raf. Var. Monstrosa (T. Itô) Swingle] Rootstock. Anbar Journal of Agricultural Sciences, 23(2): 1154-1163.

Abstract

Foliar spraying using different levels of meta-topolin (0, 10, 20, and 30 mg L^{-1}) and tryptophan (0, 50, and 100 mg L^{-1}) was done to investigate their influence on the seedling growth and chemical composition of Flying Dragon rootstock. Additionally, a factorial experiment was conducted utilizing three replications following a randomized complete block design on the success of Washington navel orange scions. The study was conducted from September 2022 to June 2023 at a lath house at the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq. The results revealed that the two study factors significantly impacted all traits, particularly at higher concentrations. The 30 mg L^{-1} meta-topolin treatment significantly increased plant height, leaves number, leaves area, stem diameter, number of buddable seedlings, vegetative dry weight, stem total carbohydrate, leaves nitrogen content, and total chlorophyll content, and scion success. Moreover, 50 mg L^{-1} tryptophan foliar spraying significantly increased all the above traits, while the control treatment had the lowest values.

Keywords: Citrus, Flying Dragon rootstock, Foliar application, Meta-topolin, Tryptophan.

تقييم دور الرش الورقي بمنظم النمو meta–Topolin والتربتوفان في اختزال المدة اللازمة لأجراء التطعيم في اصل Flying Dragon [Poncirus trifoliata (L.) Raf. Var. monstrosa (T. Itô) Swingle]

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الخلاصة

لبيان تاثير الرش الورقي بمنظم النمو النباتي *meta*–Topolin (0، 10، 20 و30 ملغم لتر$^{-1}$) والتربتوفان (0، 50 و100) ملغم لتر$^{-1}$ في النمو الخضري والمحتوى الكيميائي لشتلات أصل الـ Flying Dragon فضلا عن نسبة نجاح طعوم البرتقال ابو سرة صنف واشنطن. اجريت تجربة عاملية بتصميم القطاعات الكاملة المعشاة وبثلاثة مكررات للفترة من ايلول 2022 الى حزيران 2023 في الظلة الخشبية التابعة لقسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة الانبار، العراق. وقد اظهرت النتائج التأثير المعنوي لعاملي الدراسة في كافة الصفات لاسيما التراكيز الاعلى منهما حيث اثرت المعاملة بالتركيز 30 ملغم لتر$^{-1}$ من الـ *meta*–Topolin معنويا في زيادة ارتفاع النبات، عدد الأوراق، المساحة الورقية، قطر الساق، نسبة الشتلات القابلة للتطعيم، الوزن الجاف للمجموع الخضري، النسبة المئوية للكربوهيدرات الكلية في الساق، محتوى الاوراق من النتروجين، محتوى الكلوروفيل الكلي، فضلا عن نسبة نجاح التطعيم. من جانب اخر ادى الرش الورقي بالتربتوفان بالمستوى 50 ملغم لتر$^{-1}$ الى زيادة جميع الصفات المدروسة اعلاه معنويا، فيما بلغت ادنى القيم للصفات المدروسة كافة عند معاملة المقارنة.

كلمات مفتاحية: الحمضيات، اصل Flying Dragon، الرش الورقي، meta–topolin، تربتوفان.

Introduction

Citrus seedlings are commonly utilized as rootstocks for budding desired cultivars. Among the most popular dwarfing rootstocks is the Flying Dragon [*Poncirus trifoliata* (L.) Raf. var. monstrosa (T. Itô) Swingle], which can reduce the size of trees by approximately 75%. Trees budded onto this rootstock can reach a height of 2.5 m or less at maturity, making it suitable for high-density planting systems, thereby increasing yield. The Flying Dragon originated in Japan as a mutation of the trifoliate orange and was initially used as an ornamental plant in pots before being brought in 1915 to the United States (10, 20 and 31). It is characterized by its tolerance of the citrus tristeza virus (CTV), phytophthora spp., and citrus nematode resistance, as well as cold tolerance and suitability for heavy soils. Additionally, it is compatible with most citrus species, particularly sweet oranges (9 and 31).

The slow growth of citrus rootstocks, especially the Flying Dragon, during the nursery stage and the delay in reaching the appropriate stage for budding (1.5-2 years) have prompted nursery workers to adopt various methods for enhancing their growth towards reducing the time of nursery development. These methods include foliar spraying with plant growth regulators, amino acids, and other biostimulants.

The growth regulator meta-topolin (6-[3-hydroxy benzyl amino] purine) is a type of aromatic cytokinin that was extracted from the leaves of the Poplar (*Populus* x *Canadensis* Moench., cv. Robusta) in 1960, though it was not named until the 1990s (27). Its effectiveness surpasses benzyl adenine (19 and 25) due to its resistance to cytokinin oxidase (*CKOx*) activity. Additionally, its main metabolic products are of the O-glucoside type (29), which are rapidly metabolized throughout all parts of the plant, granting it high efficacy (17). Meta-topolin contributes to various developmental processes in plants by stimulating cellular division, differentiation of the vascular system, activation of the apical meristems, chloroplast development, preventing rapid degradation of chlorophyll and proteins during senescence, maintaining hormonal balance, facilitating the translocation of photosynthetic products and nutrients towards plant growth points, and other physiological effects (2, 8 and 30).

Plants require amino acids, especially during the early phases of their life cycle, and among these is tryptophan, an essential amino acid that can modify numerous vital processes owing to its capability to impact virtually all phases of plant growth. Tryptophan activates the enzymatic system, is involved in the biosynthesis of IAA, regulates the transport of ions and different nutrients, facilitates stomatal opening, activates chlorophyll molecules, decomposes dead cells and converts them into protein, increases root density, and enhances biochemical processes by regulating plant growth and differentiation. It improves the plant's ability to acquire nutrients and water and enhances resistance to abiotic stresses (15, 18 and 23).

Due to the lack of studies on Flying Dragon rootstock, this study evaluated the impact of spraying meta-topolin as well as tryptophan on improving its growth to achieve a suitable size for budding in the shortest possible time, as well as to assess the bud take success of Washington navel oranges.

Materials and Methods

This research was conducted on 720 Flying Dragon rootstock seedlings from a lath house belonging to the Horticulture and Landscape Gardening Department, Faculty of Agriculture, University of Anbar, Iraq, from September 2022 to June 2023. Five-month-old seedlings planted in 4-kg plastic containers were sourced from the certified citrus nursery (Saddat Al-Hindiya/Karbala) under the General Company for Horticulture and Forestry, Ministry of Agriculture, in mid-August 2022.

Service Operations: The seedlings were selected for maximum growth uniformity, with stem diameters of 15-20 cm ranging between 3-4 mm. All necessary care practices were performed, and NPK (20-20-20) chemical fertilizer was added at a rate of 80 g seedling over four doses in September, October, March, and April (28). Prior to the treatments, soil samples were taken for physical and chemical analyses (Table 1).

Table 1: Some physical and chemical properties of the soil.

Analysis	Average	Unit
EC	1.93	ds m^{-1}
pH	7.4	
Soil texture		
Sand	75	g kg^{-1}
Clay	11	g kg^{-1}
Silt	14	g kg^{-1}
Texture	Sandy loam	
Available N	71.2	mg kg^{-1} soil
Available P	13.8	mg kg^{-1} soil
Available K	162.8	mg kg^{-1} soil

Study Factors and Experimental Design: The seedlings were sprayed with the growth regulator meta-topolin, produced by Duchefa Biochemie B.V. Co., Netherlands (Assay 99.00%), at four concentrations of 0, 10, 20, and 30 mg L^{-1}, denoted M0, M10, M20, and M30, respectively. They were also sprayed with tryptophan, produced by Sigma-Aldrich Chemic Co., Germany (Assay 98.00%), at 0 (denoted T0), 50 (T50), and 100 (T100) mg L^{-1}. The spraying was conducted on September 5, and October 5, 2022, and March 5, 2023.

A factorial experiment (4 x 3) was conducted utilizing three replications at 20 seedlings per experimental unit following a randomized complete block design (4). The data were analyzed with the GenStat statistical program, and the least significant difference at LSD 0.05 used to contrast the means.

Studied Traits: Plant height and stem diameter increments, and number of leaves were measured before the treatments were implemented (5/9/2022) and re-measured on 4/10/2023 to determine the rate of increase before the budding process. The other traits were measured before the budding, and included:

Leaf area (11 and 21).

Buddable seedlings percentage was calculated using the equation:

Buddable seedlings % = no. of seedlings with stem diameters of 5.4 mm or more at 15-20 cm height)/total no. of seedlings (14).

Dry weight of the vegetative system.

Stem carbohydrate content (%) and leaf nitrogen percentage (7).

Total leaf chlorophyll content (13).

Shield budding was performed at a height of 15-20 cm (16) using scions of Washington Navel oranges on April 10, 2023. The number of seedlings was standardized to 8 per experimental unit based on the lowest number of buddable seedlings in the control treatment. On May 20, 2023, the bud take success percentage was calculated utilizing the following equation:

Bud take success % = successful scion number/total number of budded seedlings.

Results and Discussion

Plant height, leaf number and area, stem diameter, and buddable percentage: The results in Table 2 indicate that the meta-topolin and tryptophan treatments had a significant influence on all the evaluated traits, especially at higher concentrations. Spraying with 30 mg L^{-1} of meta-topolin (M_{30}) significantly improved seedling height, leaf number, leaf area, stem diameter, and buddable seedling percentage at 16.81 cm, 16.55 leaf $seedling^{-1}$, 8.45 dm², 1.44 mm, and 76.66%, respectively. In contrast, the control treatment of 0 mg L^{-1} (M_0) recorded the lowest values at 10.51 cm, 10.89 leaf $seedling^{-1}$, 6.04 dm², 0.82 mm, and 53.33% for the same traits.

Similarly, tryptophan spraying led to significant increases in the traits. The 100 mg L^{-1} concentration (T_{100}) produced the greatest increase in seedling height, leaf number, leaf area, stem diameter, and buddable seedling percentage at 15.80 cm, 16.03 leaf $seedling^{-1}$, 8.00 dm², 1.31 mm, and 75.00%, respectively compared to the lowest values of 11.50 cm, 11.63 leaf $seedling^{-1}$, 6.41 dm², 0.93 mm, and 57.50% for the 0 mg L^{-1} treatment (T_0). Notably, there was no significant difference between the T_0 and T_{50} levels in the mentioned traits except for stem diameter and buddable seedlings.

The interaction between the growth regulator and amino acid spraying reached a significant level, particularly with the $M_{30}T_{100}$ treatment producing the highest values of 18.78 cm for seedling height, 18.22 leaf $seedling^{-1}$, 9.31 dm² leaf area, 1.66 mm stem diameter, and 85.00% buddable seedlings, respectively. In contrast, M_0T_0 gave the lowest results.

Table 2: Meta-topolin and tryptophan spraying and their interaction effects on vegetative growth and suitable bud seedlings of the flying dragon rootstock.

Treatment	Plant height increment (cm)	Leaf number increment (leaf $seedling^{-1}$)	Leaf area (dm^2)	Stem diameter increment (mm)	Buddable %
Meta-topolin (M) mg L^{-1}					
M_0	10.51	10.89	6.04	0.82	53.55
M_{10}	13.96	13.37	6.71	1.12	66.66
M_{20}	13.89	14.03	7.24	1.20	71.66
M_{30}	16.81	16.55	8.45	1.44	76.66
LSD	**4.61**	**2.43**	**0.82**	**0.04**	**8.31**
Tryptophan (T) mg L^{-1}					
T_0	11.50	11.63	6.41	0.93	57.50
T_{50}	14.08	13.47	6.91	1.19	68.75
T_{100}	15.80	16.03	8.00	1.31	75.00
LSD	**4.13**	**2.11**	**0.72**	**0.03**	**7.34**
Meta-topolin x Tryptophan					
M_0T_0	8.11	8.67	5.36	0.69	40.00
M_0T_{50}	11.00	10.44	5.94	0.84	55.00
M_0T_{100}	12.44	13.56	6.83	0.93	65.00
$M_{10}T_0$	11.67	11.33	6.17	0.96	65.00
$M_{10}T_{50}$	14.00	13.11	6.44	1.15	65.00
$M_{10}T_{100}$	16.22	15.67	7.52	1.26	70.00
$M_{20}T_0$	11.56	11.44	6.28	1.00	60.00
$M_{20}T_{50}$	14.33	14.00	7.09	1.21	75.00
$M_{20}T_{100}$	15.78	16.67	8.35	1.41	80.00
$M_{30}T_0$	14.67	15.11	7.84	1.09	65.00
$M_{30}T_{10}$	17.00	16.33	8.20	1.58	80.00
$M_{30}T_{20}$	18.78	18.22	9.31	1.66	85.00
LSD	**8.27**	**4.23**	**1.45**	**0.06**	**14.69**

Dry weight of vegetative parts, stem carbohydrate content, leaf nitrogen and chlorophyll content, and budding success: Table 3 shows significant differences from spraying levels of meta-topolin in the dry weight of aerial parts, stem carbohydrate content, leaf nitrogen, chlorophyll content, and the number of buddable seedlings. The M_{30} treatment showed significant superiority over the others producing the highest values of 11.60 g, 8.96%, 2.47%, 1.43 mg g^{-1} FW, and 79.16% for these traits, respectively compared to the lowest for the M_0 treatment at 9.12 g, 7.79%, 2.34%, 1.15 mg g^{-1} FW, and 54.16%.

Tryptophan foliar application also gave notable increases in all characteristics, particularly the T_{100} concentration, which significantly outperformed the others achieving the highest values of 11.07 g, 8.74%, 2.46%, 1.39 mg g^{-1} FW, and 75.00%, sequentially. Conversely, the lowest values were for the T_0 concentration at 9.40 g, 7.91%, 2.34%, 1.28 mg g^{-1} FW, and 59.37%, respectively.

The biostimulant interaction achieved a notable influence, with the $M_{30}T_{100}$ producing the highest dry weight of the vegetative system (12.14 g), carbohydrate content (9.11%), nitrogen (2.51%), chlorophyll (1.51 mg g_{-1} FW), and buddable seedlings (87.50%). In contrast, M_0T_0 showed the lowest results.

Table 3: Meta-topolin and tryptophan spraying and their interaction effects on chemical traits and percentage of budding success of the flying dragon rootstock seedlings.

Treatment	Vegetative parts DW (g)	Carbohydrates (%)	Nitrogen (%)	Chlorophyll content (mg g^{-1} FW)	Buddable %
Meta-topolin (M) mg L^{-1}					
M_0	9.12	7.79	2.34	1.15	54.16
M_{10}	10.32	8.32	2.39	1.31	62.50
M_{20}	10.35	8.36	2.41	1.40	66.66
M_{30}	11.60	8.96	2.47	1.43	79.16
LSD	**0.43**	**0.31**	**0.04**	**0.06**	**11.15**
Tryptophan (T) mg L^{-1}					
T_0	9.40	7.91	2.34	1.28	59.37
T_{50}	10.58	8.42	2.41	1.30	62.50
T_{100}	11.07	8.74	2.46	1.39	75.00
LSD	**0.37**	**0.28**	**0.03**	**0.05**	**9.52**
Meta-topolin x Tryptophan					
M_0T_0	8.12	7.31	2.28	1.09	50.00
M_0T_{50}	9.45	7.87	2.34	1.16	50.00
M_0T_{100}	9.80	8.20	2.41	1.21	62.50
$M_{10}T_0$	9.39	7.83	2.33	1.33	50.00
$M_{10}T_{50}$	10.48	8.36	2.40	1.20	62.50
$M_{10}T_{100}$	11.11	8.78	2.46	1.40	75.00
$M_{20}T_0$	9.42	7.90	2.34	1.34	62.50
$M_{20}T_{50}$	10.39	8.31	2.41	1.41	62.50
$M_{20}T_{100}$	11.24	8.89	2.48	1.46	75.00
$M_{30}T_0$	10.68	8.59	2.41	1.36	75.00
$M_{30}T_{10}$	12.00	9.17	2.49	1.42	75.00
$M_{30}T_{20}$	12.14	9.11	2.51	1.51	87.50
LSD	**0.75**	**0.57**	**0.06**	**0.11**	**19.05**

Meta-topolin plays a direct role in influencing the physiological processes essential for plant growth and development. It stimulates cellular division, activates apical meristems, initiates and develops leaf primordia, promotes chlorophyll formation while delaying its degradation, and facilitates the translocation of photosynthate and minerals to the growth points of plants (2 and 30). This positive impact on growth, namely increased plant height, leaf numbers and area, is likely linked to improved photosynthetic efficiency and increased carbohydrate production. A portion of these carbohydrates is utilized for growth, and the excess stored in other plant organs, such as stems, leading to increased stem thickness. This, in turn, positively correlates with a higher percentage of buddable seedlings.

The impact of meta-topolin in increasing the budding success rate may be attributed to its role in stimulating callus initiation, cambium activity, and re-connection of vascular tissues in bud union (24 and 26). These outcomes align with the findings of (22), who reported that vegetative growth traits and the graft-able percentage increased noticeably with foliar application of benzyl adenine in mango seedlings. Similarly, the results align with (3), who found that treating with thidiazuron significantly improved the vegetative growth and the chemical content of the Clementine mandarin, as well as increasing the buddable seedlings and bud take success rates.

The overall improved growth features of flying dragon rootstock seedlings, particularly at the 100 mg L^{-1} tryptophan level, can be attributed to its vital role in providing the basic units for protein synthesis, and in activating the enzymatic system, opening stomata, and facilitating the transport of mineral ions and nutrients driven by transpiration. This leads to enhanced photosynthetic efficiency and increased output, as well as the biosynthesis of IAA, which, through its polar transport, stimulates vascular cambium activity and the differentiation of vascular tissues (1, 15 and 18). Consequently, this results in increased growth, along with a higher percentage of buddable saplings and bud take success. These results are consistent with (12) that vegetative growth and chemical content in mango seedlings significantly increased with tryptophan spraying, and with (5 and 6) who indicated that it led to increased growth and mineral elements percentage in peach trees and citrus rootstocks respectively.

Conclusions

The findings of this study underscore the significant impact of foliar spraying with meta-topolin and tryptophan on the growth and physiological attributes of flying dragon rootstock seedlings, as well as the scion success rates of Washington navel orange. The application of meta-topolin at 30 mg L^{-1} concentration proved to be the most effective, resulting in notable improvements in plant height, leaf number and area, stem diameter, dry weight of aerial parts, buddable seedling percentage, and key biochemical parameters of carbohydrate content, nitrogen levels, and total chlorophyll content. Similarly, tryptophan at 50 mg L^{-1} significantly enhanced the measured traits, though at a slightly lesser extent than the highest meta-topolin concentration.

These results highlight the potential of growth regulators and amino acids in optimizing citrus rootstock development and improving bud-take success rates. The study provides valuable insights for horticulturists and citrus growers, emphasizing the importance of precise hormonal treatments to maximize seedling vigor and budding success. Future research could further explore the interactive effects of these compounds across different environmental conditions to refine best practices for citrus propagation. Also, in light of the results of the study, it is possible to shorten the pre-budding growth period of flying dragon rootstock and Washington navel oranges to within 12 months, with a high percentage of budding success.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

A. M. I. Al-Janabi: Conceptualization, methodology, software, data curation, writing- original draft preparation, visualization, investigation, supervision; I. A. Aubied: Software, validation, writing- reviewing and editing.

Funding:

This work was not supported through any funding.

Institutional Review Board Statement:

The study was conducted following the protocol authorized by the Head of the Ethics Committee, University of Anbar, Iraq Republic.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

Data available upon request.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

The authors are extremely grateful for the academic support provided by the University of Anbar in conducting this study.

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