Impact of foliar spray of meta-Topolin and Glycine on Grapefruit Saplings cv. "Duncan" performance

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Abstract

An experiment was conducted in order to evaluate the effects of the foliar application of the growth regulator meta-Topolin (0, 5, 10, and 15 mg L^{-1}) and the amino acid glycine (0, 50 and 100 mg L^{-1}) on vegetative growth and chemical composition of grapefruit saplings (cv. Duncan) in a factorial experiment according to Randomized Complete Block Design (RCBD) with 3 replications. The experiment was carried out in lath house of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq during April to December, 2024. The results demonstrated a significant influence of meta-Topolin foliar application across all examined traits, particularly at the 10 mg L^{-1} concentration, which yielded the highest improvements. This concentration led to a statistically significant increase in the average number of secondary branches (8.05 branch $sapling^{-1}$), their length (24.95 cm), leaf number (71.17 leaf $sapling^{-1}$), leaves area (38.99 dm^2), dry weight of the vegetative parts (120.65 g), total carbohydrate content in branches (9.23%), leaf nitrogen (2.55%), phosphorus (0.28%), potassium (1.70%), and total chlorophyll content (1.49 mg g^{-1} FW). Similarly, foliar application of glycine, especially at 100 mg L^{-1}, significantly improved all measured parameters. This treatment achieved the highest values in secondary branch count (7.66 branch $sapling^{-1}$), branch length (24.96 cm), leaf number (66.29 leaf $sapling^{-1}$), leaves area (35.82 dm^2), and dry weight of the vegetative system (110.61 g). It also elevated the total carbohydrate content in the branches (8.96%), as well as the leaf nitrogen (2.53%), phosphorus (0.26%), and potassium (1.68%) percentages, while boosting the total chlorophyll content (1.46 mg g^{-1} FW.(

Keywords: meta-topolin, Glycine, Foliar application, Citrus, Grapefruit saplings.

Introduction

Grapefruit, or pomelo (Citrus paradisi Mac Fadyen), belongs to the grapefruit family, also called the Pummelo-Grapefruit group. It is native to the West Indies and is believed to have originated as a genetic mutation or hybrid between the shaddock and the orange [33,40]. The trees are large with a spreading, rounded canopy and dense vegetative growth. They have relatively few and small thorns. The leaves are glossy have distinct petiole wings, and the fruits are large-among the largest citrus fruits aside from shaddock-typically spherical with flattened ends. The rind is of medium thickness, and the spongy albedo layer contains naringin, a compound responsible for the characteristic bitter flavor of the fruit [32.[

In Iraq, the cultivar 'Duncan' has proven to be among the most successful under open-field cultivation. Its fruits have a pale yellowish-white pulp and contain polyembryonic seeds. The cultivar is early-maturing, possesses favorable traits, and is suitable for canning [8.[

Availability of healthy saplings is the basic necessity for orchardization and fruit production. Nevertheless, a slow growth rate and a long waiting period for the citrus saplings to reach the suitable size for budding or transplanting in the field are main difficulties for cultivation. This is also adding to the high cost of production. Hence, there is a need for alternative methods to improve sapling growth, such as foliar spray of plant growth regulators (PGRs), which is crucial in the improvement of plant growth and development.

The plant growth regulator meta-Topolin [6-(3-hydroxybenzylamino) purine] is a type of aromatic cytokinin. It contains an adenine base and a substituted benzyl group. This compound was first isolated from the leaves of poplar (Populus x Canadensis Moench, cv. Robusta) in 1960, although it was not named until the 1990s [37]. Its effectiveness surpasses that of Benzyladenine [22], as it metabolizes slowly due to its resistance to cytokinin oxidase (CKOx) enzyme activity [27]. Additionally, its primary metabolites are of the O-glucoside type [38], which are rapidly metabolized in all plant parts, contributing to its high efficacy [24]. Meta-tupolin contributes to plant growth and development by stimulating cell division, differentiation of the vascular system, apical meristem activity, chloroplast development, preventing rapid degradation of chlorophyll and protein molecules during aging, increasing photosynthetic efficiency, maintaining hormonal balance, regulating the transport and distribution of photosynthetic products and nutrients towards the plant's growth points, and resisting biotic and abiotic stresses and other physiological influences [39,11,4]. Numerous studies have highlighted the importance of foliar application of cytokinins in improving growth for various citrus saplings and trees, including Cleopatra Mandarin rootstock [5], Tangelo trees [1], Local Sweet Orange saplings [3], and Clementine Mandarin saplings [7.[

Foliar spraying of amino acids is regarded as an effective approach to supply plant-available building blocks for protein synthesis, a crucial to growth and development in plants. Among these amino acids, glycine is one of the simplest amino acids, with only one carbon atom attached to both an amino and carboxyl groups. It is a small, hydrophobic amino acid of low molecular weight [21]. In addition to other known functions such as chelating some mineral nutrients [35], participating in chlorophyll biosynthesis, improving photosynthetic efficiency and influencing stomatal movement [18,25]. Furthermore, it is engaged with the biosynthesis of various non-protein nitrogenous compounds such as pigments, vitamins and enzyme cofactors as well as being a component in the structure of nucleotides [15]. Glycine also mitigates various effects of environmental stresses in plants [29]. Several researchers have documented the positive effects of foliar glycine application in enhancing vegetative growth and chemical composition in various fruit saplings and trees. These include Washington Navel orange trees [26], mango trees [2], guava trees [10], and apricot trees [6.[

Accordingly, this study aims to evaluate the potential of foliar application of meta-topolin and glycine to accelerate the growth rate of grapefruit saplings, enabling them to reach a suitable stage for transplanting to their permanent location in the shortest time possible. The study also seeks to strengthen the saplings structurally for balanced growth and to investigate the interactive effects of both factors on vegetative and root development, as well as on the chemical composition of the saplings.

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Materials and Methods

Experimental Site and Sapling Management

The study was conducted from April to December 2024 on a total of 108 Duncan grapefruit (Citrus paradisi Mac Fadyen) saplings. These saplings, approximately one and a half years old, were carefully selected for uniformity in growth as much as possible, budded onto sour orange rootstock. The saplings were planted in plastic pots with a capacity of 15 kg and maintained under a lath house at the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Iraq. The saplings were obtained from a reputable private nursery in Al-Garraat, Baghdad on March 17, 2024, and were maintained under standard horticultural practices including regular weeding, pest control measures when necessary, and fertilization with NPK compound fertilizer (20:20:20) applied at 100 g per sapling in four split doses during April, May, September, and October [12]. Irrigation was performed using a drip system. Prior to initiating the experiment, soil samples were collected for physical and chemical analysis (Table 1.(

Table 1. The experimental soils' physical and chemical characteristics.

Physical properties (g kg^{-1} soil)				
Sand	Silt	Clay	Texture	
650.5	151.3	198.2	Sandy loam	
Chemical properties				
pH	EC (1:1) ds m^{-1}	N (mg kg^{-1} soil)	P (mg kg^{-1} soil)	K (mg kg^{-1} soil)
7.2	1.79	71.8	17.2	190.3

The first factor was foliar application of the growth regulator meta-Topolin, manufactured by Duchefa Biochemie B.V. / Netherlands, with a an active ingredient (A.I. %) of 99%. Four concentrations were used: 0, 5, 10, and 15 mg L-1, coded as mT0, mT5, mT10, and mT15, respectively. The second factor involved foliar spraying with the amino acid Glycine (99% A.I.), supplied by Himedia Co., Ltd. (India), at three concentrations: 0, 50, and 100 mg L-1, coded as G0, G50, and G100, respectively. Spraying for both factors was conducted early in the morning on April 1, May 1, September 1, and October 1, 2024.

A factorial experiment (4 × 3) was conducted using a Randomized Complete Block Design (RCBD) [9], with three replications per treatment. Each replicate consisted of three saplings per treatment. Data analysis was performed using GeneStat statistical software, and treatment means were compared using the Least Significant Difference (LSD) test at a 0.05 probability level.

Studied traits

The traits evaluated in this study included the increase in the number and length of secondary branches, as well as the increase in the number of leaves. These measurements were taken by recording the number and length of secondary branches and the total number of leaves per sapling before treatment application on March 26, 2024, and again at the end of the experiment on December 1, 2024. The rate of increase for each of these traits was then calculated. Additional parameters were measured at the conclusion of the experiment and included leaf area, determined using the method described by Chou [16]; dry weight of the vegetative system; total carbohydrate content in the branches following Joslyn [23]; and the percentage of nitrogen, phosphorus, and potassium in the leaves according to Bahargava and Raghupathi [13]. Total chlorophyll content in the leaves was also quantified using the method outlined by Gogoi and Basumatary [20.[

Results and Discussion

Vegetative Growth Traits

The data presented in Table (2) indicate a significant effect of foliar application of the plant growth regulator meta-topolin on all vegetative growth traits. The concentration of mT10 led to a statistically significant increase in the number and length of secondary branches, the number of leaves, leaves area, and vegetative parts dry weight, with values reaching 8.05 branch sapling-1, 24.95 cm, 71.17 leaf sapling-1, 38.99 dm², and 120.65 g, respectively. In contrast, the treatment mT0 recorded the lowest values for these traits: 4.11 branch sapling-1, 16.86 cm, 36.83 leaf sapling-1, 25.02 dm², and 74.02 g, respectively.

Similarly, Glycine application also had a significant impact on all traits, particularly at the G100 concentration. This concentration significantly outperformed other treatments in the average increase in secondary branch number (7.66 branch sapling-1), average increase in secondary branch length (24.96 cm), increase in leaf number (66.29 leaf sapling-1), leaves area (35.82 dm2), and vegetative dry weight (110.61 g). The lowest values for these traits were observed with the G0 concentration, measuring 4.28 branch sapling-1, 17.02 cm, 37.09 leaf sapling-1, 26.04 dm2, and 76.55 g, respectively.

The interaction between the two studied factors also showed a significant effect on several vegetative growth parameters, particularly in the increase in branch length, number of leaves, leaf area, and vegetative dry weight. The combined treatment mT10 G100 recorded the highest values for these traits: 27.37 cm, 88.17 leaf sapling-1, 44.88 dm², and 137.23 g, respectively, whereas the mT0 G0 combination resulted in the lowest values for these same traits.

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Table 2. Influence of spraying with meta-Topolin and glycine and their interaction on increase in number and length of secondary shoots, leaves number increment, leaves area, and vegetative system dry weight.

	Increment in number of secondary branches (branch sapling^{-1})	Increment in length of secondary branches (cm)	Leaves number increment (leaf sapling^{-1})	Leaves area (dm^2)	Vegetative system dry weight (g)
meta-Topolin (*m*T) g L^{-1}					
mT_0	4.11	16.86	36.83	25.02	74.02
mT_5	5.60	20.69	46.56	28.88	90.14
mT_{10}	8.05	24.95	71.17	38.99	120.65
mT_{15}	5.77	21.71	48.17	29.50	90.51
L.S.D.	0.94	1.84	7.88	1.93	3.11
Glycine (G) g L^{-1}					
G_0	4.28	17.02	37.09	26.04	76.55
G_{50}	5.70	21.18	48.67	29.92	94.34
G_{100}	7.66	24.96	66.29	35.82	110.61
L.S.D.	0.81	1.58	6.63	1.61	2.71
meta-Topolin x Glycine					
mT_0G_0	2.33	11.18	20.17	19.59	56.21
mT_0G_{50}	4.00	17.25	37.33	25.08	75.76
mT_0G_{100}	6.00	22.16	53.00	30.40	90.08
mT_5G_0	3.86	16.53	31.50	23.67	73.42
mT_5G_{50}	5.33	21.01	45.50	28.73	88.34
mT_5G_{100}	7.66	24.53	62.67	34.23	108.67
$mT_{10}G_0$	6.66	22.64	58.00	34.92	100.75
$mT_{10}G_{50}$	7.83	24.84	67.33	37.16	123.97
$mT_{10}G_{100}$	9.66	27.37	88.17	44.88	137.23
$mT_{15}G_0$	4.33	17.72	38.67	25.99	75.80
$mT_{15}G_{50}$	5.66	21.62	44.50	28.71	56.21
$mT_{15}G_{100}$	7.33	25.80	61.33	33.79	75.76
L.S.D.	N.S.	3.16	13.27	3.23	90.08

The effect of foliar spraying with meta-Topolin on increasing the number and length of secondary branches, the number of leaves, and leaves area, as well as the dry weight of the vegetative mass of grapefruit saplings., especially at a concentration of 10 mg L-1, may be due to the important role of the growth regulator in regulating plant growth and development. meta-Topolin stimulates lateral bud growth by breaking apical dominance while increasing apical meristem activity and promoting new cell formation [14,10]. Moreover, meta-Topolin plays a crucial role in enhancing the activity of enzymes involved in the synthesis of nucleic acids and proteins, which are fundamental to key physiological processes such as photosynthesis and carbohydrate biosynthesis [19,4]. These biochemical effects positively influence branch number and elongation .

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Regarding the increase in leaf number and area, meta-Topolin likely stimulates leaf primordia development and promotes chloroplast differentiation during leaf formation and expansion [37,31]. This leads to enhanced photosynthetic output and energy availability for vegetative growth. Furthermore, the improvement in branch characteristics (Table 2) from meta-Topolin application contributes to increased leaf number and area, ultimately resulting in greater dry matter accumulation. These findings align with previous research by Al-Janabi [5] and Abdulrhman & Al-Atrushy [3.[

As for the increase in vegetative growth characteristics resulting from spraying with the amino acid glycine, it may be due to the pivotal role it plays in the vital processes taking place inside the plant, as it is a basic source in building proteins and enzymes and providing energy that stimulates vegetative growth, in addition to its role in increasing the period and number of cell divisions and their elongation, in addition to its important role in the formation of chlorophyll pigment and increasing the efficiency of photosynthesis and its participation in many metabolic pathways and its entry as a raw material in the construction of many vital compounds such as nucleotides, secondary metabolites and non-protein nitrogenous compounds [15, 21,25]. These diverse biochemical functions collectively contribute to increased metabolic activity in grapefruit saplings. Manifesting as improved vegetative growth parameters - particularly in secondary branch number/length, leaf count, and leaves area, ultimately leading to greater vegetative dry matter accumulation. These findings corroborate previous research by Kheder and Abo-Elmaged [26] on citrus trees and Almutairi et al. [10] on guava, demonstrating glycine's consistent growth-promoting effects across different fruit species.

Chemical Traits

The results presented in Table (3) demonstrate that foliar application of meta-topolin significantly influenced the chemical composition of grapefruit saplings. The treatment mT10 was the treatment with the greatest effect, which evidenced the highest values in total carbohydrate content in branches (9.23%) and in leaf content of nitrogen (2.55%), phosphorus (0.28%), potassium (1.70%) and total chlorophyll (1.49 mg g^{-1} FW). mT0 treatment showed the lowest values: 8.25% for carbohydrates, 2.42% for nitrogen, 0.19% for phosphorus, 1.39% for potassium and 1.33 mg g^{-1} FW for total chlorophyll.

Glycine application also had a significant impact on these chemical traits. The highest values were observed at the G100 concentration, which led to an increase in total carbohydrates in the branches (8.96%), and in leaf content of nitrogen (2.53%), phosphorus (0.26%), potassium (1.68%), and total chlorophyll (1.46 mg g^{-1} FW). The lowest values were recorded in the control treatment G0, which showed 8.35% carbohydrates, 2.42% nitrogen, 0.19% phosphorus, 1.39% potassium, and 1.34 mg g^{-1} FW for chlorophyll.

The interaction between meta-topolin and glycine also exhibited a significant effect. The combined treatment mT10G100 yielded the highest values across several key traits: 9.49% total carbohydrates in the branches, 2.61% nitrogen, 1.89% potassium, and 1.57 mg g^{-1} FW total chlorophyll. Conversely, the lowest values for these parameters were observed in the control treatment mT0G0.

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Table 3. Influence of spraying with meta-Topolin and glycine and their interaction on the shoots content of total carbohydrates, percentage of nitrogen, phosphorus, potassium in leaves, as well as their chlorophyll content.

	Total carbohydrates (%)	N (%)	P (%)	K (%)	Total chlorophyll (mg g^{-1} F.W.)
meta-Topolin (*m*T) g L^{-1}					
mT_0	8.25	2.42	0.19	1.39	1.33
mT_5	8.51	2.46	0.21	1.49	1.38
mT_{10}	9.23	2.55	0.28	1.70	1.49
mT_{15}	8.53	2.47	0.23	1.56	1.40
L.S.D.	0.15	0.04	0.039	0.03	0.08
Glycine (G) g L^{-1}					
G_0	8.35	2.42	0.19	1.39	1.34
G_{50}	8.57	2.47	0.23	1.53	1.40
G_{100}	8.96	2.53	0.26	1.68	1.46
L.S.D.	0.12	0.03	0.034	0.02	0.07
meta-Topolin x Glycine					
mT_0G_0	8.05	2.36	0.16	1.24	1.26
mT_0G_{50}	8.19	2.43	0.20	1.38	1.34
mT_0G_{100}	8.51	2.48	0.23	1.55	1.40
mT_5G_0	8.17	2.40	0.18	1.36	1.32
mT_5G_{50}	8.42	2.46	0.21	1.48	1.38
mT_5G_{100}	8.94	2.52	0.25	1.64	1.45
$mT_{10}G_0$	8.97	2.50	0.24	1.52	1.42
$mT_{10}G_{50}$	9.24	2.55	0.28	1.69	1.49
$mT_{10}G_{100}$	9.49	2.61	0.32	1.89	1.57
$mT_{15}G_0$	8.24	2.44	0.21	1.45	1.37
$mT_{15}G_{50}$	8.45	2.47	0.24	1.60	1.41
$mT_{15}G_{100}$	8.92	2.51	0.26	1.65	1.44
L.S.D.	0.25	0.06	N.S.	0.05	0.14

The reason for the increase in the nitrogen, phosphorus and potassium content of the leaves due to the two factors studied may be due to the role of meta-tupolin in stimulating the absorption, movement and transport of mineral elements towards the treated tissues and increasing their concentration in the leaves [30,31]. This growth regulator appears to modify source-sink relationships, promoting nutrient accumulation in leaves. Similarly, glycine treatment improved ion flux and mineral accumulation in plant tissues [34,18], potentially through its role as a chelating agent and its influence on membrane transport processes .

The increase in the content of total chlorophyll in foliar spray with the growth regulant and amino acid may be the effect of stimulating the role of the compound meta-Topolin in the biosynthesis of this pigment by increasing the activity of the enzyme NADH-protochlorophyllid reductase, and the suppression of chlorophyllase, the enzyme responsible for degradation of this pigment [41,19]. As for Glycine, it plays a fundamental

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role in chlorophyll biosynthesis by contributing to the synthesis of proteins that are integral to the chlorophyll molecule structure [17,25]. It is also likely that the increase in the chlorophyll content is indirectly related to the correction of the nutritional status of the saplings. following the enhanced leaf nitrogen content (Table 3). Nitrogen is an important component in the porphyrin ring of chlorophyll; thus, its supply has a direct impact on chlorophyll formation and photosynthetic performance [28,36.[

Conclusion

The foliar application of meta-Topolin and Glycine significantly enhanced the vegetative growth and chemical composition of "Duncan" grapefruit saplings. The application of meta-Topolin at 10 mg L-1 combined with glycine at 100 mg L-1 resulted in the greatest improvement in the vegetative growth traits, e.g., branch length, number of leaves, leaves area, dry mass, N content, K content, as well as total chlorophyll concentration. The positive effects of meta-Topolin on cell division, nutrient translocation, and preventing chlorophyll degradation and glycine on protein synthesis, enzymatic activity, and N use were synergistic. These findings suggest a promising strategy for accelerating grapefruit sapling. development, preparing them for transplanting in a shorter timeframe, and fostering a robust plant structure.

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