RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 58 Issue 4: 664-669 (August 2024)

Softwood Grafting of Clementine Mandarin as Affected by Thidiazuron Treatment and Rootstock Type

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10.18805/IJARe.AF-853

ABSTRACT

Background: Citrus are propagated on a commercial scale by budding on different rootstocks species; nevertheless, one of the main problems nurserymen encounter is the slow growth of these rootstocks in the early phases of their growth, which drives up production costs. Therefore, the process of softwood grafting is one of the promising methods for quick propagating species of citrus.

Methods: The present study on softwood grafting in clementine mandarin was conducted under lath house conditions during 2022-2023, to illustrate the impact of dipping the scion shoots in Thidiazuron at a concentration of 0, 10 and 20 mg L^{-1} and types of rootstocks (Sour orange, Rough lemon, Rangpur lime and Swingle citrumelo) on the percentage of grafting success and some vegetative characteristics of resultant saplings. A factorial experiment was executed out utilizing a randomized complete block design (RCBD) with three replications.

Result: The two factors of study, particularly the treatment with 20 mg L^{-1} of Thidiazuron and Rough lemon rootstock, showed significant superiority in all traits, such as earliness in the initiation and completion of sprouting, graft success per cent, graft survival per cent, plant height, scion and rootstock diameter, as well as the number of branches and leaves $plant^{-1}$. On the other hand, the un-treated with growth regulator and Swingle citrumelo rootstock recorded the lowest values for these traits.

Key words: Citrus rootstocks, Mandarin, Soft wood grafting, Thidiazuron, Vegetative growth.

INTRODUCTION

Mandarin (*Citrus reticulata* Blanco) is one of the smallest species in the citrus genus in height and size, characterized by a dense, spreading canopy with thin, flexible branches, giving it a drooping appearance (Saunt, 2000; Memarne *et al.*, 2021). Clementine mandarin, which is popular and successful cultivars in Iraq, is distinguished by medium growth, small, elongated leaves without petioles and early-maturing fruits that are juicy and of medium size, with peel adhering to the pulp and a small navel can be observed on it (Al-Khafaji *et al.*, 1990). In Iraq, total number of mandarin trees during 2021 was 241.549 and annual production of 4.494 tons and average productivity was 18.6 kg $fruits\ tree^{-1}$ (FAOSTAT, 2021). Citrus propagated commercially through shield budding method. However, the rootstock's slow in growth and the length of time needed to reach the proper budding stage (1-1.5 years), along with the persistence of dormant scions after successful the bud union (Hartmann *et al.*, 2011), it is one of the major issues causing saplings to remain in nurseries for long periods of time and raising production expenses. Therefore, the method of propagation by softwood grafting is a promising method for rapid multiplication of citrus, in addition to being efficient, inexpensive, with high success per cent and lower likelihood grafts mortality. Furthermore, saplings are ready within a year and exhibit uniform growth (Ram and Pathak, 2006).

Cytokinins are widely used to stimulate the union of scion and rootstock by promoting callus production, cambium activity and differentiation of vascular tissues (Immanen *et al.*, 2016; Nanda and Melnyk, 2018; Sharma and Zheng, 2019). Thidiazuron (TDZ), a derivative of phenylurea, stands out for its high effectiveness compared to adenine-based cytokinins, as it is not degraded by CKO_x enzyme (Mok *et al.*, 1982; Ahmed and Faisal, 2018; Nisler *et al.*, 2021). Thidiazuron has numerous physiological impacts, comprising promoting cell division and differentiation, lateral branching, delayed chlorophyll breakdown, transports photo-assimilates and mineral elements, *etc.* (Gou *et al.*, 2011; Nisler, 2018).

The sour orange is a common rootstock in Iraq for propagating various citrus species and cultivars, characterized by many favorable traits. However, it is criticized for its high sensitivity to quick decline disease. Therefore, the selection of a suitable rootstock is crucial to obtain trees that are resistant to various environmental conditions, as well as to influence the strength of the trees growing, quantitative and qualitative of fruit traits and diseases resistance (Castle, 2010; Lacey *et al.*, 2012; Bowman and Joubert, 2020; Aubied *et al.*, 2023; Qureshi *et al.*, 2023).

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How to cite this article: Al-Janabi, A.M.I. and Abd, N.T. (2024). Softwood Grafting of Clementine Mandarin as Affected by Thidiazuron Treatment and Rootstock Type. Indian Journal of Agricultural Research. 58(4): 664-669. doi: 10.18805/IJARe.AF-853.

Submitted: 27-01-2024 **Accepted:** 03-04-2024 **Online:** 29-04-2024

Thus, study aim to demonstrate the effect of treatment with Thidiazuron and rootstock type in the success per cent of softwood grafting in Clementine mandarin and some vegetative growth attributes of resulting saplings.

MATERIALS AND METHODS

Site and experimental characterization

The present investigation was carried out in the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar, Ramadi, Iraq, during 2022/23 under lath house conditions. A factorial experiment (3 × 4) was employed within a randomized complete block design (RCBD) with three replicates per treatment and ten saplings per experimental unit (Al-Mohammedi and Al-Mohammedi, 2012). The first factor involved dipping the scion sticks basal in the thidiazuron (TDZ) solution, active ingredient 95%, manufactured by Xi'an Wison Biological Technology Co., Ltd., China, at three different concentrations (0, 10 and 20 mg L^{-1}), denoted as TDZ_0, TDZ_{10} and TDZ_{20}, sequentially. The second factor consisted of four types of citrus rootstocks: Sour orange (*C. aurantium* L.), Rough lemon (*C. jambhiri* Lush.), Rangpur lime (*C. limonia* Osbeck) and Swingle citrumelo [*C. paradisi* (Duncan) Macf. X *P. trifoliata* (L.) Raf.], represented as R_1, R_2, R_3 and R_4, sequentially. These rootstocks were obtained on October1, 2022, from Government nursery at the age of six months and were planted in Polybags of 4 kg capacity.

Selection of scions

For the purpose of preparing scions from the mother plants of Clementine mandarin, mature and robust branches free from diseases and insect infestations were chosen. The selected branches, aged 4-5 months, were defoliated 9-12 days prior to the grafting process (Kumar *et al.*, 2012; Jinalben *et al.*, 2021).

Scion stickswith a diameter of 2-3 mm and a length of 8-10 cm were equipped on the morning of September 15, 2022 after separation them from mother plant (Patel *et al.*, 2010). They were wrapped in a wet cloth and transported to experiment location. The scion sticks basal was dipped for five minutes in a solution of growth regulator (TDZ) and grafting process was carried out directly.

Softwood grafting technique

The apex of rootstocks were head back at a height of 8-12 cm above the surface of polyethylene bags. Leaves were removed and leaving 2-3 leaf under graft union. A cut of 1.5-2 cm in length, was made at the center of the rootstock apex. Additionally, by slicing off the bark and small pieces of wood from two equal and opposing sides, the basal of scion stick was repaired to a 1.5-2 cm wedge.

Then scion stick was inserted into the incision and graft union was tied with a polyethylene strip (Hartmann *et al.*, 2011). After finishing the grafting process, grafts were covered with polyethylene tubes to protect the graft union from irrigation water, also to increase the success per cent and early sprouting (Bhilare *et al.*, 2018).

Measurements

For the initiation and completion of sprouting, observations were recorded daily.

The following formula was used to calculate the graft success:

$$\text{Graft success per cent} = \frac{\text{Number of sprouted grafts}}{\text{Total plants grafted}} \times 100$$

After eight months of grafting, all observations were recorded, including:

$$\text{Plant survival per cent} = \frac{\text{Survived plants}}{\text{Graft success plants}} \times 100$$

Height of plant (cm), scion and rootstock diameter (cm), leaves number (leaf $plant^{-1}$) and branches number (branch $plant^{-1}$).

RESULTS AND DISCUSSION

The dipping in Thidiazuron had a significant effect on the initiation and completion of sprouting, percentage of graft success and percentage of survival (Table 1), particularly, the treatment with a concentration of 20 mg L^{-1} (TDZ_{20}), which achieved the best outcomes through early initiation of sprouting (15.50 days), least number of days for complete sprouting (24.50 days), highest percentage of graft success (87.50) and highest percentage of survival (91.10), with a significant superiority over the remaining treatments. In contrast, the treatment with a concentration of 0 mg L^{-1} (TDZ_0) recorded the Maximum days for first sprout and to complete sprouting and the lowest percentage of graft success and survival, reaching 20.75 days, 34.42 days, 70.00 and 80.00 respectively.

The results also showed significant differences in the number of days for sprouting initiation and completion, as well as grafting success and survival per cent due to the influence of rootstock type. Rough lemon rootstock (R_2) achieved superior results, with respective values of 12.33 days, 21.78 days, 90.00% and 93.20% sequentially with significant superiority over the rest of the rootstocks. Conversely, Swingle citrumelo rootstock (R_4) exhibited delayed sprouting initiation (24.65 days) and completion (38.33 days), coupled with a lower graft success percentage (73.30) and a decreased survival per cent (78.00).

The results indicated a significant interaction between study factors. The treatment $TDZ_{20}R_2$ achieved the best results, with values of 10.33 days, 17.67 days, 96.70% and 96.30%,sequentially. Conversely, treatment TDZ_0R_4 recorded values of 28.00 days, 44.33 days, 70.00% and 71.40% for sprouting initiation, completion, graft success and plant survival, respectively.

The findings demonstrate a significant increase in plant height, scion and rootstock diameter, branches number and

Table 1: Effect of Thidiazuron treatment and rootstock on number of days to sprouting initiation and completion, success of graft and plant survival of softwood grafting in the clementine mandarin.

	Number of days till initial sprouting	Number of days till complete sprouting	Graft success (%)	Plant survival (%)
		Thidiazuron (TDZ) mg L^{-1}		
TDZ_0	20.75	34.42	75.00	80.00
TDZ_{10}	18.08	30.17	80.00	84.20
TDZ_{20}	15.50	24.50	87.50	91.10
L.S.D.	3.61	3.68	4.66	6.25
		Rootstock type (R)		
R_1	18.56	30.00	76.70	85.70
R_2	12.33	21.78	90.00	93.20
R_3	17.00	28.67	83.30	83.40
R_4	24.56	38.33	73.30	78.00
L.S.D.	4.17	4.25	5.54	7.29
		Thidiazuron × Rootstock		
TDZ_0R_1	21.33	34.67	70.00	79.40
TDZ_0R_2	14.67	25.00	83.30	91.70
TDZ_0R_3	19.00	33.67	76.70	77.40
TDZ_0R_4	28.00	44.33	70.00	71.40
$TDZ_{10}R_1$	17.67	30.33	73.30	85.70
$TDZ_{10}R_2$	12.00	22.67	90.00	91.70
$TDZ_{10}R_3$	17.33	28.00	83.30	83.30
$TDZ_{10}R_4$	25.33	39.67	73.30	76.20
$TDZ_{20}R_1$	16.67	25.00	86.70	92.10
$TDZ_{20}R_2$	10.33	17.67	96.70	96.30
$TDZ_{20}R_3$	14.67	24.33	90.00	89.60
$TDZ_{20}R_4$	20.33	31.00	76.70	86.30
L.S.D.	7.23	7.37	9.32	12.50

leaves number with increasing levels of dipping in growth regulator solution (Table 2). TDZ_{20} concentration achieved the maximum values, reaching 46.86 cm, 0.46 cm, 0.58 cm, 5.42 (branch $plant^{-1}$) and 46.41 (leaf $plant^{-1}$), respectively. In contrast, the lowest values for these traits were observed in the TDZ_0 treatment, with 35.26 cm, 0.35 cm, 0.46 cm, 2.12 (branch $plant^{-1}$) and 21.74 (leaf $plant^{-1}$), respectively.

The type of rootstock demonstrated a significant impact on the vegetative growth characteristics, particularly with rootstock (R_2) exhibiting the highest values at 50.44 cm, 0.46 cm, 0.58 cm, 4.67 (branch $plant^{-1}$) and 40.92 (leaf $plant^{-1}$). Conversely, rootstock (R_4) recorded the lowest plant height, scion and root diameter, number of branches and leaves, measuring 32.75 cm, 0.34 cm, 0.46 cm, 2.72 (branch $plant^{-1}$) and 26.70 (leaf $plant^{-1}$), respectively. While in these characteristics, the rootstocks R3 and R1 did not differ significantly from one another.

The results indicated a significant interaction between study factors on growth traits (Table 2). Treatment $TDZ_{20}R_2$ attained the highest values, at 57.02 cm, 0.52 cm, 0.65 cm, 6.67 (branch $plant^{-1}$) and 57.33 (leaf $plant^{-1}$). Conversely, the lowest values were observed in treatment TDZ_0R_4, with 27.16 cm, 0.29 cm, 0.40 cm, 1.33 (branch $plant^{-1}$) and 14.55 (leaf $plant^{-1}$), respectively.

The early initiation and completion of sprouting, as well as the increase in the percentage of graft success and survival, resulting from dippingin TDZ, may be due to its role in stimulating the proliferation of callus, cambium activity and vascular reconnection in the graft union, as well as stimulating the transport of nutrients towards the treated tissue (Gou *et al.*, 2011; Immanen *et al.*, 2016; Nanda and Melnyk, 2018; Sharma and Zheng, 2019; Ibrahim *et al.*, 2023).

The reason for increase in vegetative characteristics as a result of treatment with a growth regulator (TDZ) may be attributed to its effective role in aspects of growth and development of plant, which include promoting cell division, apical meristem activity, bio-synthesis of proteins, nucleic acids and polyamines in lateral buds and their growth (Wang *et al.*, 1986; Nisler, 2018), translocation of photosynthesis products and mineral elements towards growth points in plant, also initiation and development of primordial leaves (Beck, 1996; Gou *et al.*, 2011; Ahmed and Faisal, 2018). These results are consistent with results of Sunitha *et al.* (2016) on Thompson Seedless grape, Farsi *et al.* (2018) on walnut cv. Chandler and Jamal, Al-Janabi and Aubied (2021) on Nagami Kumquat, Kumawat *et al.* (2021) on Kesar Mango cultivar, Fayek *et al.* (2022) on grapes cv. Flame Seedless and Early sweet and Al-Karboli and Al-Janabi (2024) on local lemon.

Table 2: Effect of Thidiazuron treatment and rootstock on vegetative growth characteristics of softwood grafting in the clementine mandarin.

	Plant height (cm)	Scion diameter (cm)	Rootstock diameter (cm)	Number of branches (branch. plant^{-1})	Number of leaves (leaf. plant^{-1})
Thidiazuron (TDZ) mg L^{-1}					
TDZ_0	35.26	0.35	0.46	2.12	21.74
TDZ_{10}	40.36	0.40	0.51	3.46	32.46
TDZ_{20}	46.86	0.46	0.58	5.42	46.41
L.S.D.	3.45	0.03	0.04	0.86	3.39
Rootstock type (R)					
R_1	38.21	0.41	0.51	3.50	31.21
R_2	50.44	0.46	0.58	4.67	40.92
R_3	41.91	0.41	0.53	3.78	35.33
R_4	32.75	0.34	0.46	2.72	26.70
L.S.D.	3.98	0.04	0.05	1.00	4.53
Thidiazuron × Rootstock					
TDZ_0R_1	32.91	0.35	0.44	2.00	20.88
TDZ_0R_2	44.23	0.41	0.54	3.00	27.55
TDZ_0R_3	36.75	0.36	0.47	2.17	24.00
TDZ_0R_4	27.16	0.29	0.40	1.33	14.55
$TDZ_{10}R_1$	37.83	0.41	0.51	3.33	30.11
$TDZ_{10}R_2$	50.06	0.44	0.56	4.33	37.88
$TDZ_{10}R_3$	40.84	0.40	0.52	3.67	34.77
$TDZ_{10}R_4$	32.72	0.34	0.47	2.50	27.11
$TDZ_{20}R_1$	43.90	0.47	0.58	5.17	42.66
$TDZ_{20}R_2$	57.02	0.52	0.65	6.67	57.33
$TDZ_{20}R_3$	48.15	0.46	0.59	5.50	47.22
$TDZ_{20}R_4$	38.37	0.40	0.52	4.33	38.44
L.S.D.	6.90	0.06	0.08	1.73	6.78

As for the reason for the difference in success per cent of grafting, per cent of survival, additionally the date of initiation and completion of sprouting among types of rootstocks, it may be due to the variations of genotype, physiological status such as content of promoters, inhibitors and nutritional reserves, which leads to the difference in quantity and speed of callus formation and vascular tissues differentiation necessary for union between rootstock and scion (Hartmann *et al.*, 2011).

The supremacy of Rough lemon compared to the rest of rootstocks in all growth traits may be attributed to the genotype difference and physiological status, which comprises mineral elements uptake, nutrients transport, stimulants production and use it for growth (Hartmann *et al.*, 2011; Yulianti *et al.*, 2021), as Rough lemon is considered a vigorous rootstock that possess A large and profound root system, which reflects favorably on grafts growth (Lacey *et al.*, 2012; Bowman and Joubert, 2020). These results are consistent with the results from studies on Khasi mandarin cultivar by Dubey *et al.* (2004), Alphonso mango cultivar by Patil *et al.*, (2008), on mandarin cv. Khasi by Patel *et al.* (2010) and local sweet orange by Al-Janabi (2020).

CONCLUSION

From the results obtained, the best performance was for all traits, such as sprouting initiation and completion, per cent of graft success, per cent of survival, plant height, diameter of scion and rootstock, branches number and leaves number, when soaking clementine mandarin bud sticks in Thidiazuron solution at a concentration of 20 mg L^{-1} and grafted them on Rough lemon rootstock.

ACKNOWLEDGEMENT

The authors would like to acknowledge the contribution of the College of Agriculture, University of Anbar (www.uoanbar. edu.iq) *via* their prestigious academic staff for providing the necessary infrastructure and academic support to carry out the research.

Conflict of interest

Authors declare none competing/conflicting interests.

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