Effect of NAA and Chitosan in rooting branches resulting from stem nodes plantation of Kumquat (*Citrus japonica) in vitro*

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Abstract:

The research was conducted in a Plant Tissue Culture Laboratory, Agricultural Research Department, Ministry of Science and Technology, from December 10/1/2019 to December 30/1/2021, to study the effect of adding auxin NAA at concentrations (1, 2, and 3) mg L^{-1}, and interaction with Chitosan at concentrations (10, 15, 20 and 25) mg L^{-1}, to the culture media MS, to determine the optimal concentration in rooting the branches resulting from the multiplication of micro branches of the Kumquat plants (*Citrus japonica*), produced from *in vivo* development of the stem nodes. The results of the study showed that the planting of Kumquat branches in MS media supplemented with 2.0 mg L^{-1} NAA was a high significant response on rooting percentage (92.0%) and roots number (3.20 plant $root^{-1}$), added 15 mg $L^{-}1$ chitosan to the culture media prepared with 2.0 mg L^{-1} NAA was a significantly increased in same parameters (rooting percentage 80% and roots number 5.60).

Key words: Kumquat (*Citrus japonica*), Chitosan, rooting branches.

Introduction

Citrus fruits are among the fruits of high nutritional value, it is grown in tropical and subtropical regions of the world. The genus Citrus includes more than 162 species of citrus, belongs to Rutaceae family, Kumquat (*Citrus japonica*) is one of the most important types of citrus,. Its name came from the Chinese language, which was two words, the first being Kum, which means golden, the second is Quat, which means good fortune [1].

The method of propagation of kumquat by seeds is not favored by Orchids owners, its difficult to grow plants from seeds, because it needs a long time for the purpose of vegetative growth, called Juvenile phase, plants resulting from planting seeds were late in fruiting, when compared with plants produced by vegetative propagation, early in fruiting [2].

Many researchers have resorted to the method of *in vitro* micropropagation, it has many advantages, including obtaining large numbers and matching the mother plant, the resulting plants were early fruiting, Propagation by tissue culture technique, under sterile and controlled conditions, therefore, it produces plants that are free of pathogens, was an advantage in addition to the advantages of *in vitro* propagation [3, 4].

Chitosan is a compound with biological activities, antimicrobial, antioxidant and growth stimulant, it is a natural carbohydrate polymer derived from the deacetylation of chitin consisting of N-acetyl-D-glucosamine and D-glucosamine residues They are bound together by the ß-1,4-glycosides bond [5]. Chitosan is a powerful reducing agent, it has an important role in plant resistance to diseases and defense mechanism an important and influential role in plant growth [6].

Kanchanapoom *et al*. [7] indicated the effect of chitosan at different concentrations (10, 15 and 20 mg L^{-1}), in organogenesis of the resulting callus, from the cultivation of sexual embryos of the oil palm plant Jacq. var. tenera, the concentration is 15 mg L^{-1} added to the culture media, gave the highest response to indirect callus organogenesis (16%). The combination of TDZ at a concentration of 1 mg L^{-1} and 2,4-D at a concentration of 5 mg L^{-1} gave the highest percentage of response to rooting (75%).

Sopalun *et al*. [8] showed the effect of adding different concentrations of chitosan (0, 5, 10, 15, 20, 25, 50 and 100 mg L^{-1}) to MS cultuer media with half the strength of MS salts on altering the vegetative branches of *in vit*, they found that the concentration 5-15 mg L^{-1} to the culture media led to the formation of the highest average number of vegetative branches resulting from the offspring (3.1 vegetative branches).

Dastjerd *et al*. [9] they study combinations of chitosan at concentrations (0, 20, 40, 60 and 120) mg L^{-1}, with BA at concentrations of 0.5 and 1.5 mg L^{-1}, with gibberellic acid at concentrations (0.1, 0.2 and 0.3) mg L^{-1}, which were added to the MS food medium prepared for micropropagation of apple origin M26 (*Malus domestica*) *in vitro*.The combination of 120 mg L^{-1} chitosan with 0.5 mg L^{-1} BA, it gave the highest mean of virulence after 12 weeks of cultivation, as for not adding chitosan to the MS culture media prepared with BA. It resulted in the highest average number of vegetative branches resulting from the succession, the branches were stunted and dense in growth, which resulted in the formation of yellow leaves, while when adding chitosan at a concentration of 120 mg L-1 with GA at concentrations 0.1-0.3 mg L^{-1}, led to the production of long branches with green leaves tilted to yellowish and small. The addition of mixtures consisting of chitosan at a concentration of 120 mg L^{-1} with low concentrations of growth regulators, led to an increase in the succession of vegetative branches of the rootstock of apple M26.

The current study aims to stimulate root formation on propagated kumquat vitro using different concentrations of NAA auxin and Chitosan

Material and methods

The research was conducted in the Plant Tissue Culture Laboratory, Agricultural Research Department, Ministry of Science and Technology, the shoots resulting from the planting stage were used, one of the best treatment for BA, planted after cutting into 2 cm, in test tubes containing MS culture media for vegetative multiplication, it consists of the components of the culture media with a difference in the concentration

of BA and chitosan, one branch was planted in each tube, 10 replicates were used for each treatment. The cultures were incubated in the growing room under the light conditions of 1000 lux and temperature21C°, observations were taken after six weeks of planting in terms of the number and length of branches. The replication experiments included the following:

Good plants growth resulting from the previous multiplication stage were selected with a length of 3 cm, for cultivation in rooting media, it was taken into account that the growth of the selected plants is as homogeneous as possible in terms of length, number of leaves and thickness of stems. Rooting experiments included studying the effect of auxin NAA at concentrations (1, 2 and 3) mg $L^{-1,}$ and Chitosan at concentrations (10, 15, 20 and 25) mg L^{-1} each and in individual experiments, conducted using Complete Randomized Design (CRD). The results were analyzed after angular transformation, range test at a probability level of 0.05 [10]. One branch was planted in each tube, each tube was a replicate, (10 replicates for each concentration). The cultures were incubated in the special incubation room under the same previous conditions, data were taken on rooting percentage, number of roots, lengths, fresh and dry weight, six weeks after planting.

Results and Discussion

Rooting percentage (%)

Table (1) shows that adding chitosan to culture media had a significant effect on the percentage of rooting of kumquat plants multiplied *in vitro*, the concentration treatments (15 and 20) exceeded mg L^{-1} of chitosan, gave the highest values for that trait (60.00 and 62.00%), without any significant difference between them. The concentration treatment of 25 mg L^{-1} of chitosan gave the lowest value (48.00%), did not differ significantly from the 10 mg L^{-1} concentration treatment, no significant difference between the concentration treatments (10 and 15) mg L^{-1} of chitosan. The superiority in this trait is the result of treatment with chitosan, it may be due to promoting the growth and emergence processes of cultures germinated outside the living body, by entering the biosynthesis pathway of endogenous hormones (Auxins), it enhances the production of tryptophan, the bio-initiator of auxin, contributes to the growth and division of plant cells [11], or as mentioned by Kanchanapoom *et al*. [7], who indicated that chitosan is one of the compounds with biological activity, which has been approved as a growth regulator that aids in the development and growth of plants. As indicated by Hamel and Beaudoin [12] that chitosan has the ability to stimulate the formation of root nodes.

There was a significant effect of adding NAA to the culture media, the treatment of the concentration of 2 mg.L^{-1} NAA was superior to it by giving it the highest rate (70.00%), compared to the 1 mg L^{-1} NAA treatment that gave the lowest rate (44.00%). It may explain the significant superiority induced by the growth regulator NAA, to its positive effect in inducing and stimulating the roots of plants through its local effect on plant tissues, contributes to achieving good rooting of the vegetative branches, in addition to its high ability to stimulate the division of specialized cells,

leads to root formation. The superiority may be explained by the role of the auxin NAA in stimulating the activity of the enzymes Ornithine decarboxylase and Arginine decarboxylase, this results in high levels of polyamines in plant tissues, its effect was positively reflected in stimulating the formation of roots, in addition to the slow movement of auxins within plant parts, low mobility and chemical stability, the NAA auxin is more stable in plants than other auxin, causes a continuation and prolongation of the effect of auxin, which gives a higher probability of influence, the lack of movement of this auxin, means that the compound will remain or be retained near the area of use or application [13]. Or, the reason may be due to the efficiency of the concentration of 2 mg L^{-1} in stimulating the formation of roots on the branches compared to the other concentrations used in the research experiment.

The interaction between chitosan and NAA had no significant effect on the percentage of rooting.

Table (1): Effect of chitosan and NAA and interaction on rooting percentage (%) of kumquat plant grown *in vitro*.

Chitosan (mg L^{-1})	NAA (mg L^{-1})			Chitosan means
	1	2	3	
10	44.00	60.00	52.00	52.00
15	44.00	80.00	56.00	60.00
20	52.00	76.00	60.00	62.70
25	36.00	64.00	44.00	48.00
NAA means	44.00	70.00	53.00	
L.S.D$_{0.05}$	**Chitosan**	**NAA**	**Chitosan× NAA**	
	10.38	8.99	N.S	

Number of roots (root^{-1}):

Table (2) shows that adding chitosan powder in several concentrations, it did not significantly affect the number of roots formed on kumquat branches multipliedin vitro, there were no significant differences between the concentrations used of that substance in the number of roots.

The results of the same table showed the significant effect of adding NAA to rooting medium on the number of roots, the treatment of concentration of 2 mg L^{-1} of NAA was significantly superior by giving it the highest rate of number of roots (5.10 root.branch^{-1}), compared to a concentration treatment of 1 mg L^{-1} of NAA, which gave the lowest average (1.90 root.branch^{-1}), which did not differ significantly with the treatment of concentration 3 mg L^{-1} NAA (2.45 root.branch^{-1}). This may be attributed to the fact that the NAA auxin increases the number of meristem sites at the base of the treated vegetative branch, the process of dedifferentiation of specialized tissues, convert them to meristematic cells, contributes to an increase in the number of roots formed [14]. The high efficacy of the NAA growth regulator is that it has the highest number of double bonds, in addition to the shortness of the acid side chain

that is connected to it [15], or it may be due to the appropriateness of the concentration of 2 mg L^{-1} of NAA in stimulating root formation compared to other concentrations.

The interaction between the two experimental factors had no significant effect on the characteristic of the number of roots.

Table (2): Effect of chitosan and NAA and interaction on the number of roots formed of kumquat plant grown *in vitro*.

Chitosan (mg L^{-1})	NAA (mg L^{-1})			Chitosan means
	1	2	3	
10	1.60	4.40	2.40	2.80
15	2.00	5.60	2.80	3.47
20	2.20	5.60	2.40	3.40
25	1.80	4.80	2.20	2.93
NAA means	1.90	5.10	2.45	
L.S.D$_{0.05}$	**Chitosan**	**NAA**	**Chitosan× NAA**	
	N.S	0.589	N.S	

Figure (1): Roots formed on branches grown in full strength MS media of salt supplemented with 2 mg.L^{-1} NAA.

Roots length (cm):

Table (3) shows that there were significant differences caused by the addition of Chitosan compound on the root length of kumquat plants multiplied *in vitro*, the concentration treatment of 20 mg L^{-1} of chitosan was superior to giving it the highest values (2.60 cm), without a statistical difference from the treatment of concentration 15 mg L^{-1} of chitosan (2.58 cm), while the concentration treatment of 10 mg L^{-1} of chitosan recorded the lowest values (2.42 cm), which did not differ significantly from the

treatment of concentration 25 mg L^{-1} of chitosan, which gave an average of 2.44 cm. The reason for the superiority in the used chitosan concentrations may be attributed to what was mentioned by El-Hadrami *et al*. [16], that chitosan improves the indicators of the root system by increasing the number and length of roots, then improve the strength of plants, by stimulating it to build the chitin compound inside the plant when treated with it, it can also be explained to its role in providing a source of organic nitrogen in the food environment. The chitosan compound contains a high percentage of nitrogen, up to about 6.89%, which helps cells to grow and divide because it contains an amino group (NH_2), which is the backbone of this compound. The amine group is more readily absorbable than inorganic nitrogen [17]. Confirmed by Zhao *et al*. [18] that the amine group in the compound chitosan, it gives many special advantages to this compound, that make it easily applicable in agricultural fields.. Although the effects of chitosan were not completely clear, however, it has a clear role, because they are positively charged ions and strongly bond with nucleic acids, DNA and RNA, it also binds to proteins that carry negatively charged groups. [13, 19].

The results of the same table showed the significant effect of adding NAA to rooting media on root length characteristic, the treatment of the concentration of 2 mg L^{-1} NAA was superior to it by giving it the highest rate of root length (4.00 cm), this was followed by treatment with a concentration of 3 mg L^{-1} at a rate of 2.04 cm, compared to the concentration treatment of 1 mg L^{-1} NAA, which gave the lowest average root length (1.50 cm). The reason for the superiority can be attributed to the role of auxin in increasing the elasticity of cell walls, to cause it to increase in elongation and increase in size, by stimulating and increasing the activity of many enzymes, which was responsible for increasing the softness of the cell walls and increasing their permeability, reflected in the increase in the lengths of the roots, or for its role in stimulating the division, elongation and expansion of cells, by increasing the elasticity and ductility of the cell walls, contributes to lowering the wall's tensile resistance, response of the walls to the osmotic pressure, as a result of the presence of dissolved substances, or in other words, an increase in the osmotic nutrients dissolved in the cell juice [20].

The interaction between the experimental factors (chitosan and auxin NAA) had a significant effect on this trait, the interaction treatment (20 mg L^{-1} of chitosan with 2 mg L^{-1} NAA) gave the highest average root length (4.24 cm), without a statistical difference with the interaction treatment (15 mg L^{-1} of chitosan with 2 mg L^{-1} NAA), which gave an average of 4.20 cm, whereas, the dual interaction treatment (10 mg L^{-1} of chitosan with 1 mg L^{-1} NAA) gave the lowest rate of root length at 1.42 cm, they did not differ significantly with the interaction coefficients (15, 20, and 25 mg L^{-1} of chitosan with 1 mg L^{-1} NAA), which recorded averages (1.54, 1.56, and 1.48 cm), respectively. The concentration of 20 mg L^{-1} of chitosan overlapped with the concentration of 2 mg L^{-1} of the growth regulator NAA. The optimum concentrations may be in recording the highest rate of root length formed on the vegetative branches of kumquats, or, the chitosan compound may have an additional effect with the auxin used in the research experiment in stimulating the formation of radicals produced in vitro. Or,

the reason could be due to the small number of roots formed (Table 2), thus, it can benefit from the nutrients present in the culture media, which contributes to an increase in their average lengths [21].

Table (3): The effect of chitosan and NAA and interaction on the length of the formed roots (cm) of the kumquat plant *in vitro*.

Chitosan (mg L^{-1})	NAA (mg L^{-1})			Chitosan means
	1	2	3	
10	1.42	3.68	2.18	2.42
15	1.54	4.20	2.02	2.58
20	1.56	4.24	2.00	2.60
25	1.48	3.88	1.96	2.44
NAA means	1.50	4.00	2.04	
L.S.D$_{0.05}$	**Chitosan**	**NAA**	**Chitosan× NAA**	
	0.13	0.12	0.24	

Fresh weight of the root (gm):

Table (4) shows that adding chitosan to rooting media with different concentrations did not have a significant effect on the fresh weight of kumquat branches multiplied in vitro.

The results of the same table showed a significant response in this trait with the effect of adding the growth regulator NAA to the culture media. The concentration treatment of 2 mg L^{-1} NAA was significantly superior by giving it the highest average fresh weight (0.219gm), compared to the treatment of 3 mg L^{-1} NAA which gave the lowest fresh weight average (0.097gm), which did not differ significantly with the treatment of concentration 1 mg L^{-1} NAA, which recorded a mean of 0.168gm.

The interaction between the two studied factors chitosan and NAA had no significant effect on the fresh weight of kumquat branches multiplied in vitro.

Table (4): Effect of chitosan and NAA and interaction on the fresh weight of roots (g) of kumquat plant *in vitro*.

Chitosan (mg L^{-1})	NAA (mg L^{-1})			Chitosan means
	1	2	3	
10	0.100	0.206	0.100	0.136
15	0.116	0.212	0.134	0.154
20	0.343	0.255	0.087	0.228
25	0.113	0.205	0.066	0.128
NAA means	0.168	0.219	0.097	
L.S.D$_{0.05}$	**Chitosan**	**NAA**	**Chitosan× NAA**	
	N.S	0.085	N.S	

Dry weight of the root (gm)

Table (5) shows that adding chitosan to the food media did not have a significant effect on the dry weight of kumquat branches multiplied *in vitro*, there were no significant differences between the used concentrations of that substance in the dry weight of the plant branches.

The same table show that there was a significant effect of adding the growth regulator NAA to the culture media, the treatment of concentration of 2 mg L^{-1} NAA was significantly superior to the rest of the treatments by giving it the highest average dry weight (0.076 gm), followed by the treatment of 1 mg L^{-1} at a rate of 0.043gm, compared to the treatment of 3 mg L^{-1} NAA, which gave the lowest dry weight for root growth at an average of 0.017 gm. The reason for the superiority at the concentration of 2 mg L^{-1} NAA may be due to the increase in the indices of radical traits, positively reflected in the increase in the fresh and dry weight of the roots formed on the kumquat plant.

The interaction between the two experimental factors had a significant effect, the interaction treatment (20 mg L^{-1} of chitosan with 2 mg L^{-1} NAA) gave the highest dry weight of the branches which was 0.130gm, while the interaction treatment (20 mg L^{-1} of chitosan with 3 mg L^{-1} NAA) gave the lowest dry weight (0.014 gm).

Table (5) Effect of chitosan and NAA and interaction on root dry weight (gm) of kumquat *in vitro*.

Chitosan (mg L^{-1})	NAA (mg L^{-1})			Chitosan means
	1	2	3	
10	0.026	0.065	0.019	0.037
15	0.025	0.053	0.022	0.033
20	0.035	0.130	0.014	0.060
25	0.085	0.054	0.015	0.051
NAA means	0.043	0.076	0.017	
L.S.D$_{0.05}$	**Chitosan**	**NAA**	**Chitosan× NAA**	
	N.S	0.024	0.048	

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