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Improvement of squash plants growth growing under cold stress conditions by using calcium and phosphorus forms

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Abstract

Two pots experiments were carried out at Experimental Station of Agricultural Botany Department, Faculty of Agriculture, Moshtohor during 2020 and 2021 seasons on squash plant cv. Eskandarani. Seeds of squash were soaked and foliar spray with calcium at 250 & 500mg/l, nano calcium at 50 & 100mg/l, phosphorus at 250 & 500 mg/l, nano phosphorus at 50&100 mg/l and distilled water (control) to evaluate the effect of treatments on vegetative growth, photosynthetic pigments, anatomical characteristics (out-doors pot experiment) of squash plants **(*Cucurbita pepo* L.)** grown under natural cold stress conditions. Foliar application of squash plants with the previous treatments gave the highest values of vegetative growth of roots (size, length, diameter, fresh and dry weights), stems (length, diameter, fresh and dry weights) and leaves (number, total leaves area, fresh and dry weights), photosynthetic pigments compared with the control especially, nano phosphorus at 50 mg/l followed by nano calcium at 100 mg/l. For the anatomical characteristics, results revealed that different anatomical characteristics of squash roots and stems were improved positively. Also, the most traits of squash anatomical features were increased with different applied treatments. Generally using nanoparticles can be applied to increase squash growth and productivity under cold stress conditions.

Key words: Squash plants , cold stress, calcium, phosphorus, vegetative growth, photosynthetic pigments and anatomical characteristics.

INTRODUCTION

Squash (*Cucurbita pepo* L.) is an important vegetable crop, widely grown and consumed as food and herbal remedies in many countries around the world. It is considered as a natural source of carotenoids, carbohydrates, polysaccharides, flavonoids, alkaloids, phenols, terpenoids, minerals and fatty acids **Ratnam *et al*., (2017) and Salehi *et al*., (2019)**. Fresh fruit had a high content of β-carotene. Although fruits contained high amounts of magnesium, potassium, sodium, phosphorus, manganese, calcium and high content of moisture **Hashash *et al*., (2017)**.The total area harvested of squash in 2020 reached 17477acres, while total productivity was 360210 tons **(FAO, 2020)** in Egypt**.**

Abiotic stresses cause many morphological, physiological, biochemical, and molecular changes in plants that negatively impact growth and productivity, Climate change is among the care problems of recent times, as it is threating global food security **(FAO, 2020)**. Cold stress is perceived by the receptor at the cell membrane. Then a signal is transduced to switch on the cold-responsive genes and transcription factors for mediating stress tolerance. Crop improvement depends on our ability to comprehend the genes involved in the cold stress signaling network and the mechanism of cold stress tolerance **Sudesh, (2010)**. Stresses from the environment cause morphological and physiological changes in plants. These changes help the plants to cope with stress conditions and protect their systems against disturbance. Morphological changes in response to different stresses, clearly appear as a decrease in internodes growth, leaf size, leaf surface area, branching pattern, and growth of shoots and roots **Bano and Singh (2019)**.

Calcium has a very prominent role in the maintenance of cell structure. It recovers the plant from cold injury by activating the enzyme ATPase in the plasma membrane, which pumps back the nutrients lost during cell membrane damage caused by a calcium deficiency. Additionally, calcium functions as calmodulin, which regulates plant metabolic processes and promotes plant growth in conditions of low temperature stress **Waraich *et al*., (2011)**.

Phosphorus is a macronutrient and element that is necessary for many processes, including photosynthesis, respiration, energy transfer and storage and cell division, and expansion. It is also essential for metabolic processes that are necessary for plant growth and maturity. In the early stages of crop maturity, root development, flower formation, seed production, stalk growth, stem strength, and resistance to plant diseases are all supported by an adequate supply of P. Moreover, the availability of P can increase the N-fixing capacity of legumes and support development throughout the plant’s life cycle **Amanullah *et al*., (2012)**.

Nanofertilizers play a significant role in physiological and biochemical processes by improving nutrient availability, which can help in the enhancement of metabolic processes and stimulate meristematic activities, resulting in increased apical growth and photosynthetic area. It is crucial for increasing vegetative growth, improving reproductive growth and flowering and so increasing productivity, product quality, and shelf life of fruits Nanofertilizers balance the release of nitrogen, phosphorus and other macronutrient fertilizers absorbed by the plant. It also avoids nutrient losses and unwanted interactions of nutrients with water, air and especially microorganisms **Blois and Lay- Ekuakille (2018)**.

Calcium nanoparticles (CaNPs) like other nanoparticles can act as nano fertilizer to supply nutrients such as Ca and make available some bound minerals via pH, organic matter and cation exchange capacity modification In addition to reducing soil salinity, calcium is a macronutrient that is necessary for plant, photosynthesis, plant cell walls, membranes, hormone systems, enzyme activities, antioxidant activity, chemical reactions, and the delivery of coupling messages that are responsible for extracellular signals and intracellular physiological response **Tombuloglu *et al*., (2019); Aziz *et al*., (2019); Hu *et al*., (2018)and Esposito *et al*., (2019)**.

This present study was designed to investigate the effect of nano calcium, calcium, phosphorus and nano phosphorus on the possibility to improve the ability of squash to tolerate cold stress.

MATERIALS AND METHODS:

Two pots experiments were carried out at Experimental Sation of Agricultural Botany Department, Faculty of Agriculture, Moshtohor, Benha university during 2021and 2022 seasons on squash (*Cucurbita pepo* L. cv. Eskandarani) secured from national company, Cairo, Egypt.

Treatments were as follows:-

- Calcium 250&500 mg/l in form of (chelated calcium 10%).
- Nano calcium 50&100 mg/l particle size range (6.4-9.3nm).
- Phosphorus 250&500 mg/l in form of (phosphoric acid 96.9%).
- Nano phosphorus 50&100 mg/l particle size range (7.9-11.3nm).
- Distilled water (control).

The applied treatments were used as seeds soaking for 4 hours and as foliar spray on squash plants in subjected to one of the following treatments.

Materials sources

1- Calcium and phosphorus was exported from Algomhoria Chemical Company, Egypt.
2- Nano particles was exported from Nano fab company, Giza , Egypt.

Cultivation

Treated seeds were sown and as foliar on 1th of December in pots (30cm) in a randomized complete block design (RCBD) with three replicates for each treatment, with mixture of clay and sandy soil (2:1 by volume). Every agricultural technique for cultivating squash plants was followed, including distributing fertilizer and water equally and controlling pests and diseases. During the growth and development plants were sprayed 3 times with different assigned treatments, the first one was at 30 days after sowing and repeated each 15 days intervals, the spraying solution volume was spraying until the solution run off from the plant.

Measurements and Recorded Data:-

1-Vegetative Growth Parameters:-

Different morphological characteristics of squash plants at 70 days after sowing were measured as following:-

-Size of the root system (cm^3) was determined according to **Hanson and Churchill (1968) /** root diameter (cm) from the crown area of the root by 5cm / root length (cm)/ plant.

-Stem diameter (cm) at the first internode , stem length (cm) ,No. of leaves plant / total leaf area (cm^2) / plant by using disk methods according to **Derieux *et al*., (1973) ,** Fresh and dry weights of roots, stems and leaves.

Then the samples were deried in the oven at 65ºC for 48 hours until the weight stability then dry weights for different parts were recorded

2-Photosynthetic pigments:-

Using the techniques outlined by **Nornal (1982)**, colorimetric measurements of the third apical leaves of the squash plant were used to determine the levels of chlorophyll a, b, and carotenoids. The results were then expressed as mg/g fresh weight.

3- Anatomical characteristics:-

It was intended to carry out a comparative anatomical characteristic on stems and roots of treated plants and those of the control at 70 days after sowing (flowering stage).

Specimens of stems were taken from the 3 apical internodes of the main stem. These vegetative specimens were killed and fixed in F.A.A. (5 ml formalin, 5 ml glacial acetic acid and 90 ml ethyl alcohol 70%), washed in 70% ethyl alcohol, dehydrated in a series of ethyl alcohols 80, 90, 95 and 100%, infiltrated in xylene embedded in paraffin wax with a melting point 60-63°C, sectioned 15 microns in thickness for stem and 20 microns for the leaf **(Sass, 1951),** stained with the double stain method (Erythrosin and crystal violet), cleared in xylene and mounted in Canada balsam (**Johanson, 1940**). Four sections treatment were microscopically inspected to detect histological manifestations of noticeable responses resulted from treatments. Counts and measurements (µ) were taken using a micrometer eye piece. Averages of readings from 3 slides/ treatment were calculated.

5-Statistical analysis:-

According to **Snedecor and Cochran (1980)**, the morphological and chlorophyll content data were statistically analyzed, and the means were compared using the least significant difference test (L.S.D.) at 5%.

RESULTS AND DISCUSSION

1-Vegetative Growth Parameters:-

Data in **Table(1)** demonstrate that different treatments were applied with calcium at 250 & 500 mg/l, nano calcium at 50 & 100 mg/l, phosphorus at 250 & 500 mg/l, nano phosphorus at 50 & 100 mg/l as well as foliar spray treatments significantly increased the growth parameters of squash (*Cucurbita pepo* L.) cv. Eskandarani roots (size, length, diameter, fresh and dry weights), stems (length, diameter, fresh and dry weights) and leaves (No. of leaves, total leaves area, fresh and dry weights)estimated at 70 days after sowing, when compared to the untreated plants in both seasons. The highest values of these characteristics were found by nano phosphorus at 50 mg/l followed by nano calcium at 100mg/l.

In this respect, these results are in agreements with the findings of **Upadhyaya *et al*., (2017)** showed that the nanoparticles increased the growth rate and affect the physiology of the plant. Calcium phosphate nanoparticles may help in the formation of new nano growth promoter and nano-fertilizers for agricultural use. Therefore, it could potentially help in reduction of the quantity of fertilizer applied to crops and contributing to precision farming as it reduces fertilizer wastage and in turn environmental pollution due to agricultural malpractices. Nano fertilizer helps improve seed germination, it has an impact on plant growth. which has a favorable impact on physical characteristics **Abdel Wahab *et al*., (2019)**. Calcium is crucial for plant growth and development. It also promotes cell metabolism, preserves cell function, and aids in plant growth and development **Pathak *et al*., (2020)**.

The application of nanotechnology in agriculture has reduced the potential risks associated with biotic and abiotic stresses while simultaneously improving plant growth. Thus, a critical analysis of the role that nanoparticles play in contributing to agriculture has been presented in order to identify the available nanoparticles used in the field of agriculture and the alleviation of abiotic stresses through the advantageous properties of nanoparticles **Janasekaran *et al*., (2023).**

2-Photosynthetic pigments:-

As shown in **Table (2)** all applied treatments significantly enhanced all Photosynthetic pigments content of plants (i.e., chlorophyll a & b, Chl.(a +b) and Carotenoids) during both seasons. The highest values of total leaves content of photosynthetic pigments were obtained with nano phosphorus at 50 mg/l followed by nano calcium at 100 mg/l While, the lowest significant increase was existed with calcium at 500 mg/l during both seasons. These results are in agreement with the finding of **Abdel Wahab *et al*., (2019)** showed that the incensement of chlorophyll may be due to the role of nano particle in improvement of leaves photosynthesis and decreasing the respiration rate. Calcium maintains photosynthesis by controlling the expression of genes linked to chlorophyll synthesis in leaves **Zhang *et al*., (2020).**

Table(1):-Effect of calcium nanoparticles, calcium, phosphorus nanoparticles, and phosphorus on growth characteristics of squash plants grown under cold condition at 70 days after sowing during 2020 and2021 winter season.

Characteristics / Treatment	Root					Stem				Leaves			
	Size (cm^3)	Length (cm)	Diameter (cm)	fresh weight (g)/plant	Dry weight (g)/plant	Length (cm)	Diameter (cm)	Fresh weight (g)/plant	Dry weight (g)/plant	No of leaves /plant	Total leaves area(cm^2)/plant	Fresh weight (g)/plant	Dry weight (g)/plant
2020 season													
Control 0.0	7.90	10.00	0.667	1.78	0.176	20.50	0.66	13.27	1.66	14.00	1157.66	57.38	8.77
Nano calcium at 50 mg/l	9.16	12.80	0.733	2.53	0.177	26.50	1.13	30.63	3.04	16.33	1174.66	73.93	9.33
Nano calcium at 100 mg/l	10.30	13.50	0.967	3.28	0.283	32.00	1.43	35.17	3.06	19.00	1494.66	80.38	8.88
Calcium at 250 mg/l	8.50	10.16	0.733	2.18	0.180	25.75	0.73	27.46	1.93	14.66	1272.66	63.42	8.88
Calcium at 500 mg/l	9.83	11.66	0.733	3.18	0.260	28.00	0.95	31.45	2.82	15.00	1395.33	79.57	9.37
Nano phosphorus at 50 mg/l	12.90	14.90	1.667	4.49	0.293	34.00	2.00	39.75	3.63	22.00	1821.00	96.64	11.88
Nano phosphorus at 100 mg/l	10.00	13.50	0.900	2.26	0.260	30.63	1.00	31.96	2.31	17.66	1303.66	74.41	9.33
Phosphorus at 250 mg/l	9.50	12.33	0.967	3.31	0.240	29.50	0.86	27.60	2.51	18.66	1297.33	79.79	9.49
Phosphorus at 500 mg/l	8.00	13.16	0.767	2.31	0.277	27.50	0.96	29.60	2.69	17.66	1238.00	78.97	9.03
L.S.D at 0.05	2.1	1.2	0.1	0.5	0.03	1.9	0.2	3.5	0.6	2.5	140	12	1.9
2021 season													
Control 0.0	8.33	16.83	0.490	1.88	0.117	18.50	0.43	11.89	0.577	11.66	925.33	84.65	5.00
Nano calcium at 50 mg/l	10.16	20.50	0.600	2.92	0.240	28.33	0.63	17.95	0.993	12.00	1183.66	139.14	7.75
Nano calcium at 100 mg/l	11.00	23.50	0.730	3.06	0.240	30.50	0.73	22.24	1.313	13.00	1171.66	140.52	8.15
Calcium at 250 mg/l	8.83	16.83	0.533	2.04	0.157	19.00	0.60	12.87	0.787	11.33	959.66	88.19	5.44
Calcium at 500 mg/l	9.00	20.33	0.600	3.06	0.197	18.83	0.73	15.26	1.200	11.66	1095.00	118.05	7.28
Nano phosphorus at 50 mg/l	13.83	25.50	0.933	3.95	0.323	33.00	0.90	31.56	1.610	14.66	1300.33	157.15	10.00
Nano phosphorus at 100 mg/l	10.93	22.83	0.600	2.84	0.183	29.60	0.63	14.62	1.040	12.00	1261.33	143.17	6.24
Phosphorus at 250 mg/l	9.93	19.33	0.533	2.95	0.240	26.63	0.63	21.06	0.797	12.33	1151.33	99.31	5.84
Phosphorus at 500 mg/l	8.50	19.33	0.617	1.97	0.200	24.33	0.68	18.47	0.940	12.66	1013.00	137.64	7.80
L.S.D at 0.05	2.3	1.5	0.2	0.8	0.03	2.9	0.15	2.8	0.29	1.2	127	21.9	1.2

Table (2): Effect of calcium nanoparticles, calcium, phosphorus nanoparticles, and phosphorus on photosynthetic pigments of squash plants grown in cold conditions, at 70 days after sowing during 2020–2021winter seasons.

Characteristics / Treatments	2020 season				2021 season			
	Chlorophyll a mg/g f .w.	Chlorophyll b mg/g f .w.	Chlorophyll (a + b) mg/g f .w.	Carotenoids mg/g f .w.	Chlorophyll a mg/g f .w.	Chlorophyll b mg/g f .w.	Chlorophyll (a + b) mg/g f .w.	Carotenoids mg/g f .w.
Control 0.0	0.497	0.403	0.90	0.483	0.520	0.447	0.967	0.510
Nano calcium at 50 mg/l	0.600	0.497	1.09	0.534	0.560	0.490	1.05	0.537
Nano calcium at 100 mg/l	0.700	0.570	1.27	0.657	0.810	0.623	1.43	0.720
Calcium at 250 mg/l	0.580	0.460	1.04	0.577	0.620	0.493	1.11	0.630
Calcium at 500 mg/l	0.533	0.420	0.95	0.497	0.537	0.447	0.984	0.520
Nano phosphorus at 50mg/l	0.857	0.600	1.41	0.790	0.933	0.743	1.67	0.870
Nano phosphorus at 100mg/l	0.620	0.453	1.07	0.610	0.710	0.510	1.22	0.677
Phosphorus at 250 mg/l	0.577	0.467	1.04	0.544	0.637	0.480	1.12	0.530
Phosphorus at 500 mg/l	0.600	0.493	1.09	0.583	0.687	0.563	1.25	0.680
L.S.D at 0.05	0.13	0.08	0.56	0.13	0.10	0.12	0.24	0.14

Table (3): The impact of calcium nanoparticles, calcium, phosphorus nanoparticles, and phosphorus on the histological characteristics of the main root of squash plants after 70 days of sowing in the 2021 season under cold conditions.

Treatments / Characteristics (micron)	Nano calcium at 50mg/l	Nano calcium at 100mg/l	Calcium at 250mg/l	Calcium at 500mg/l	Nano phosphorus at 50mg/l	Nano phosphorus at 100mg/l	Phosphorus at 250 mg/l	Phosphorus at 500mg/l	Control 0.0
Root diameter.	4830.4	3508.2	3756.6	3646.8	1891.8	1367.4	2289.6	2847.6	2847.6
Epidermal cell thickness.	25.20	30.60	24.30	32.40	27.90	16.20	19.80	28.80	28.80
No. of cortex layers.	14	6	7	6	7	5	5	4	3
Cortex layers thickness.	950	360	234	189	198	135	180	135	135
Thickness of vascular cylinder.	2880	2727	3240	3204	1440	1665	1890	2520	2520
No. of vascular bundle in vascular cylinder.	4	4	4	4	4	4	4	4	4
Thickness of xylem in vascular bundle.	540	927	450	1134	810	640	648	990	720
No. of vessels in xylem bundle.	21	26	42	12	18	12	18	21	14
Thickness of widest xylem vessel.	180	162	180	232	135	135	108	126	126
Thickness of cambium layers.	108	90	90	89	89	72	90	90	54
Thickness of phloem.	135	117	120	180	117	90	108	180	90
Parenchymatous pith thickness.	180	207	-	180	90	-	-	-	288
Xylem of pith thickness.	-	-	90.0	-	-	90.0	90.0	90.0	-

Table (4): The impact of calcium nanoparticles, calcium, phosphorus nanoparticles, and phosphorus on the histological characteristics of the main stem of squash plants after 70 days of sowing in the 2021 season under cold conditions.

Treatments / Characteristics (micron)	Nano calcium at 50mg/l	Nano calcium at 100 mg/l	Calcium at 250 mg/l	Calcium at 500mg/l	Nano phosphorus at 50 mg/l	Nano phosphorus at 100mg/l	Phosphorus at 250mg/l	Phosphorus at 500 mg/l	Control 0.0
Stem diameter.	5805.0	5684.4	8119.8	5385.6	6186.6	6611.4	6589.8	7344.0	5274.0
Hollow pith diameter.	540.0	540.0	540.0	900.0	360.0	720.0	900.0	1350.0	1440.0
Epidermal cuticle layer thickness.	9.00	14.4	16.2	15.3	8.1	7.2	14.4	16.2	13.5
Epidermal cell layer thickness.	18.0	28.8	20.7	22.5	16.2	13.5	22.5	28.8	18.0
No. of collenchyma layers.	5.0	4.0	4.0	5.0	4.0	5.0	7.0	6.0	7.0
Thickness of collenchyma layers.	135.0	90.0	108.0	117.0	90.0	99.0	180.0	135.0	180.0
Thickness of cortical parenchyma layers.	810.0	900.0	1170.0	720.0	450.0	900.0	900.0	990.0	540.0
Thickness of fibers layers in cortex.	45.0	135.0	90.0	117.0	117.0	108.0	108.0	108.0	81.0
Length of large vascular bundle.	630.0	864.0	1035.0	621.0	972.0	918.0	900.0	729.0	720.0
Thickness of upper phloem in v. bundle	108.0	90.0	135.0	90.0	180.0	189.0	180.0	180.0	135.0
Thickness of cambium layers.	72.0	108.0	90.0	81.0	117.0	90.0	90.0	90.0	90.0
Thickness of xylem in vascular bundle.	270.0	576.0	720.0	360.0	540.0	459.0	450.0	279.0	360.0
No. of xylem rows in vascular bundle.	7.0	6.0	13.0	9.5	12.0	6.0	7.0	9.0	7.0
No. of vessels in the xylem row.	5.5	7.0	9.0	3.5	7.0	5.5	5.5	4.0	5.0
Thickness of lower phloem in v. bundle	180.0	90.0	90.0	90.0	135.0	180.0	180.0	180.0	135.0
Thickness of widest xylem vessel in vascular bundle	90.0	189.0	90.0	72.0	189.0	166.5	112.5	54.0	90.0
Parenchymatous pith thickness.	720.0	540.0	1350.0	630.0	1260.0	900.0	720.0	990.0	630.0

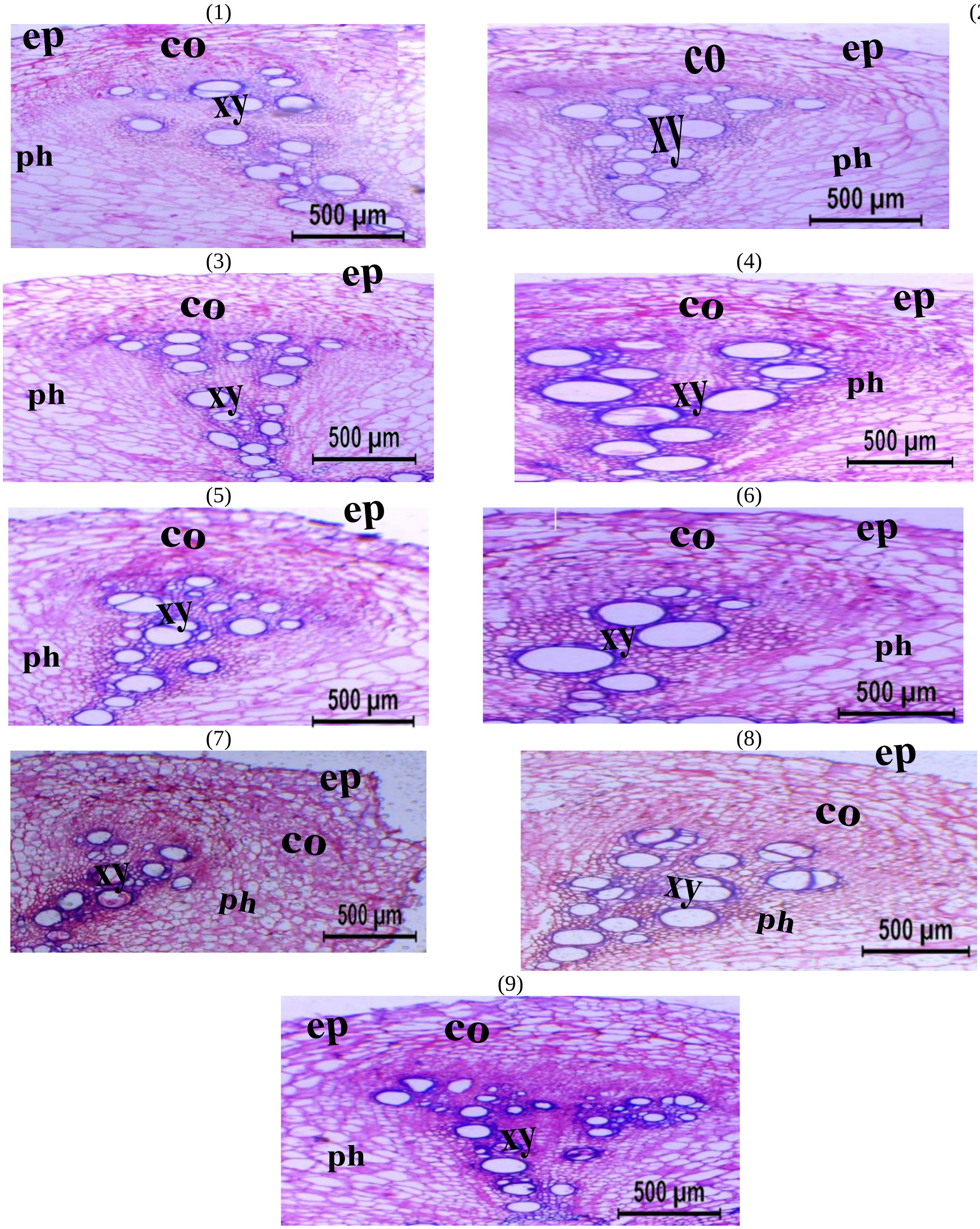

Fig. (1): Transverse sections (X = 40) about 5 cm after the crown area of squash roots plants at 70 days after sowing as affected by different applied treatments.

Where: (1): Nano calcium at 50 mg/l (2): Nano calcium at 100 mg/l. (3): Calcium at 250 mg/l
(4): Calcium at 500 mg/l. (5): Nano phosphorus at 50 mg/l, (6): Nano phosphorus at 100 mg/l.
(7): phosphorus at 250 mg/l, (8): phosphorus at 500 mg/l (9):control.
ep= Epidermis co= Cortex xy= Xylem tissue ph= Phloem tissue

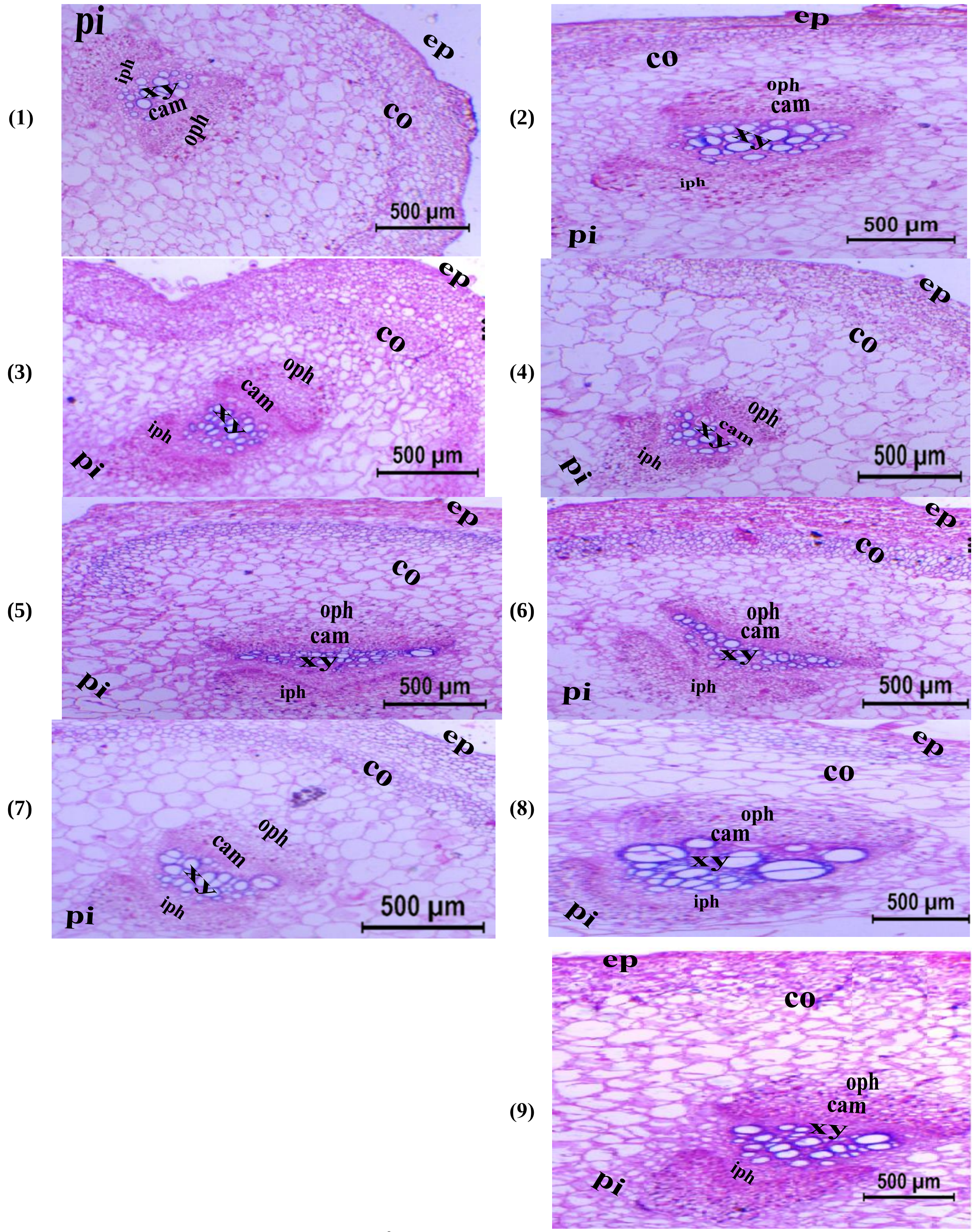

Fig. (2): Transverse sections (X = 40) through 3 th internode of the main stem of squash plants at 70 days after transplanting as affected by different applied treatments

Where: (1): Nano calcium at 50 mg/l (2): Nano calcium at 100 mg/l, (3): Calcium at 250 mg/l , (4): Calcium at 500 mg/l, (5): Nano phosphorus at 50 mg/l, (6): Nano phosphorus at 100 mg/l, (7): phosphorus at 250 mg/l, (8): phosphorus at 500 mg/l and (9):control.

ep= Epidermis	**co= Cortex**
pi= pith	**oph= outer phloem tissue**
iph=inner phloem tissue	**xy= Xylem tissue cam= Cambium**

Anatomical characteristics:

Tables (3,4 and fig1,2) indicate the effect of treatments upon different estimated anatomical features in cross sections in the root and stem.

1-Anatomical changes in squash roots in response to various applied treatments:

Data in **Table (3)** and **fig(1)** indicates that different applied treatments increased the diameter of whole section. This increase reached maximum with CaNps at 50 mg/l followed by Ca at 250 mg/l (4830.4 and 3756.6 μ), respectively. Meanwhile, phosphorus nano at 50 mg/l gave the lowest increase in this respect (1891.8μ).

Additionally, it was observed that the increases in diameter throughout each section were reversed upon different layers. Since, thickness of each of epidermis, cortex, phloem zone and xylem zone as well. In addition, the number of xylem rows in the vascular cylinder also increased with different applied treatments as well as the number of vessels in each row. The same positive effect of applied treatment upon wall thickness of widest xylem vessel and pith diameter was also existed.

2-Anatomical changes in squash stems in response to various applied treatments:

Data in **Table (4)** and **fig (2)** presents that different applied treatments increased the diameter of whole section. This increase reached maximum with calcium at 250 mg/l followed by phosphorus at 500 mg/l. That reached (8119.8 and7344µ) respectively. Meanwhile, control gave the lowest increase in this respect (5274.0μ).

Furthermore, it was noted that increases in the overall diameter were reversed on various layers that make up each section. Since, thickness of each of epidermis, cortex, phloem zone and xylem zone as well. In addition, the number of xylem rows in the vascular cylinder also increased with different applied treatments as well as the number of vessels in each row. The same positive effect of applied treatment upon wall thickness of widest xylem vessel and pith diameter was also existed. These results are in line with the findings of **Agamy (2004) and Mohammed (2005)** the increase in size of vascular bundles due to the used treatments may be as a result of the enhancement of the activity of cambium to form and differentiate new vascular bundles. The effects of applied treatments on cambium activity may be responsible for the stimulatory effects observed in the anatomic features of treated plants. Auxins and cytokinins in particular, which are endogenous hormones, may be the primary cause of increases in cambium activity **Ismaeil and Abd El-Aal, (2011).**

Conclusion

Finally, the results of this study indicate that it could be recommended to spray squash plants with nano phosphorus at 50mg/l and nano calcium at 100 mg/l three times to enhance the growth ,photosynthesis pigments and anatomical features in roots and stems.

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