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Reproductive bud development and reserves concentrations status of (*Vitis vinifera*, L.) cv. Superior seedless as affected by some nutrient and cytokinin treatments

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Abstract

The study investigated the effect of some foliar nutrients and cytokinins (CKs) treatments on the histological development of latent compound (N+2) in the lab, in addition to relating it with the reserves concentration status of vines as indicators for the fruitfulness of the cv. Superior seedless. The treatments were T1: as a control, T2: (K_2SO_4 at 1g /l as a source of K), T3: mono-ammonium phosphate (MAP at 1 g/l as a traditional foliar phosphate fertilizer;12-61-0), T4: nano-calcium phosphate (Nano-Ca-P at 0.333 g/l as nano fertilizer;18% P_2O_5), T5 and T7: (Kinetin at 10 ppm), T6 and T8: (benzyladenine at 20 ppm) as two types of Cytokinins. Each year treatments (T2 to T6) were sprayed 5 times, while T7 and T8 were sprayed three times only. The histological parameters were determined at the two stages of development of latent compound bud (N+2); early during the initiation phase (in spring) and, at the end of the differentiation phase (before winter pruning). The percentage of initiated buds, potential fertile buds, potential bud fruitfulness, and actual bud fruitfulness, besides the length and width of the latent bud in the two stages, were estimated. Additionally, the total nitrogen and carbohydrate in roots and canes, and starch content of buds were estimated. T6 showed notable superiority of (N+2) buds entered the initiation phase, also, the dimensions of (N+2) of T6 overrated those of the other treatments in the two samples date. The latent buds of T6 and T8 exhibit better differentiation performance along the season expressed by the highest significant actual fruitfulness, accompanied by improved reserves concentration. Foliar application of CKs after harvest was effective in improved fertility because it coincides with the best active period for stimulating anlagen to differentiate to inflorescence primordial promote divisions into sub-branches to ensure cluster, not tendril formation and improving reserves concentration status finally improving fertility.

Keywords: Anlagen, Cytokinins, Differentiation, Initiation, Nutrients, Reserves concentration

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1. Introduction

Grapevine is one of the most managed crops in horticulture with multicultural practices during the season, and any part of the management program was directed at ensuring and controlling the fruitfulness of latent bud (N+2), which is a key component of the reproductive cycle of grape. Several reports discussed the inflorescence formation and reported that the development pathway of anlagen is regulated by environmental factors; temperature, light, water stress, and macronutrient availability during the differentiation of latent bud and trials conducted to quantify these factors as a physiological stimulus for fruitfulness [1,2,3,4]. In addition to endogenous factors involved; carbohydrate reserves, hormonal balance and hormonal control and genetic factor as reviewed by [5,6,7]. According to [8] temperature may exert its effect on flowering by modifying the level of cytokinins (CKs) and confirmed this hypothesis through manipulated exogenous (BA or PBA) on rootless cuttings. The normal differentiation of inflorescence primordial (IP) later confirmed that CKs produced by roots are involved in the regulation of flower development expansion along two growth seasons. Also, [7] revealed that warmer and dryer weather from May to June when floral induction and differentiation occurred had a good effect. This may be due to that higher temperatures may have increased the supply of nutrients to differentiating anlagen, such as the supply of carbohydrates (CHO), minerals even hormones [9, 10]. The process of flowering in grapevine is an output of integration more than factor together because inflorescence formation is regulated at two levels of initiation and that of differentiation of anlagen which requires a specific balance between hormones, nutrition status, and optimum climate conditions in woody plants. Both reserve nitrogen and

carbohydrates are the controllers for the initial growth and development of grapevine in spring, as they provide energy and building blocks for new growth. Vines started its new growth depended on the use of reserves storage in perennial organs during the previous season before any net carbon assimilation and significant root uptake of N takes place [11,12,13]. Adding to this [14] support the view that the carbohydrate reserves of node and internode tissue in mid-winter are positively related to fruitfulness in the following spring of the Sultana cultivar. In the same line, [15] in Sauvignon Blanc grapevine mentioned that CHO content of a cane or its proportion of starch is correlated to its volume or mass suggesting that cane cross-sectional area may be an indicator of the CHO status of the cane/vine. Since the number of IP determines 60% of the fruitfulness of annual variation [6]. Furthermore, grape growers could be using bud dissection results to estimate yield whereas, studies have found that bud dissections may explain from 50% to 90% of the variation in actual yield [16]. Therefore, the goal of researchers and grapevine grower directed to try to maximize number of differentiation inflorescence primordial (IP) by application an effective agriculture practice led to increase the number of cluster per vine and finally maximize yield. Although the use of nutrients and synthetic cytokinins (CKs) are found to be widely acceptance as a common agriculture practice among grape growers vineyard. However, the effect of foliar nutrients and CKs on fruitfulness through its impact on differentiation stages of latent bud (N+2), as well as its effect on reserves concentration in storage wood needs more investigation. Superior seedless is one of the most important early cultivars in the local and export market in Egypt, but, it is known for its decreasing fertility over the years eventually giving low yield. The study aimed to Evaluate the effect of some nutrient and cytokinins (CKs) treatments on the two main stages of bud development; the initiation stage (around inflorescence emergence), and after full differentiation (before winter pruning), by comparing the middle latent compound buds (N+2) of the different foliar treatments through some histological parameters using bud dissection. Determine the effect of the same treatments on the reserves concentration level of the vines and its distribution before winter pruning that highly correlated with the fruitfulness of vines. Provide a recommendation to grape growers of economic cultivars to efficiently increase fruitfulness and yield which is mainly determined in the first season.

2. Materials and Methods

Twenty-four Superior Seedless vines were chosen for this experiment which were arranged in a complete randomized block design they were divided into 7 treatments beside the control, and each treatment included 3 replicates and each one vine plus one vine guard as each end. The treatments were T1: as a control, T2: (K_2SO_4 at 1g /l as a source of potassium K), T3: mono-ammonium phosphate (MAP at 1 g/l as a traditional foliar phosphate fertilizer;12-61-0), T4: nano-calcium phosphate (Nano-Ca-P;18% P_2O_5 at 0.333 g /l) as a nano form of phosphorus (preparation and characterization described and measured according to methods described by [17], T5 and T7: (Kinetin at 10 ppm) and finally, T6 and T8: (benzyladenine at 20 ppm) as two types of cytokinins (CKs). Each season treatments from T2 up to T6 were sprayed 5 times: emergence of cluster, full bloom (FB), 80-90% berry set, two weeks after fruit set and after harvest (end of July), while T7 and T8 were sprayed three times only at 80-90% berry set, two weeks after fruit set and after harvest. In order to determine the effect of different treatments on initiation and differentiation stages and the development of latent bud, three canes were tagged and collected for every treatment in two samples dates: (1) during spring around fruit set, (2) in winter at pruning time, buds of the middle part of the canes killed and fixed at once by F.A.A. as mentioned by method of [18]. The eyes of each considered dates were sectioned longitudinal as possible at 15 -20 µ thick were cut with a rotary microtome, then stained with saffranine and fast green pigments [19], mounted with Canada balsam and dried in an electric oven at 55°C for 48 hours slides were microscopically examined, and images of dissections were taken using the LEICA ICC50 HD Microscope equipped with a camera connected to the computer for image capture.

2.1. Histological parameters of development latent compound bud (N+2)

The following histological parameters were measured by using longitudinal sections of buds in the lab:

2.1.1. Percentage of initiated buds (%)

By distinguishing the change in the apical meristem indicating the first stage of the reproductive stage during the first sample date at the initiation stage.

2.1.2. Length and width of the latent bud (µ)

During the first sample date at the initiation stage.

2.1.3. Potential fertile bud (%)

During the second sample date percentage of differentiated buds with at least one cluster according to the bud dissection before winter pruning [20].

2.1.4. Potential bud fruitfulness

The number of inflorescences primordial per bud measured in the fully differentiated bud before winter pruning [20].

2.1.5. Length and Width of full-differentiated latent bud (µ)

Estimated during the second sample date (before winter pruning).

2.1.6. Actual bud fruitfulness

The average number of clusters per bud in the following spring.

2.2. Reserves concentration status

To assess whether treatments influenced nutrient storage in buds, woody tissues (canes), and roots, a sample of subsamples of pruning wood and fine roots was collected before one week of pruning time during the two seasons of the study to determine the following measurement.

2.2.1. Cane vigor measurements

Nine grape canes were determined from each treatment before winter pruning which can be quantified as cane diameter, and internode length. Whereas cane diameter (mm) and internode length (mm) were measured at the mid-point between two nodes in the middle position of the cane (from bud number 6 up to bud number 9) from one-year-old mature canes.

2.2.2. Total nitrogen (mg/ g D.W)

Samples were cut into small pieces, cleaned with tap water, dried at 70 C to constant weight, and finally ground as a powder, wet ashed in H_2SO_4 – H_2O mixture according to [21], digests were brought to 50ml. nitrogen was estimated by the macro-Kjeldahl method as described by [22].

2.2.3. Total carbohydrates in pruning canes and in roots (g/100g D.W.)

Were measured according to methods described by) [23] and [24], in this method, the concentrated sulpheric acid breaks down any polysaccharides, oligosaccharides and disaccharides to monosaccharides. This forms a green-colored product with phenol and has an absorption maximum of 490 nm.

2.2.4. Starch content in buds (mg/ 100g D.W.)

Storage content in buds was quantified as the method described by [25] from 300 mg powdered grape buds samples, and extracted three times with 9 mL of 80% ethanol incubated in a water bath (60 °C) for 1 h and then centrifuged (4000 × g) for 10 min at 16 °C. The dry residue was used for starch analysis. Starch was determined in the sugar-free residual tissue prepared as described by [26]. The tissue was oven-dried at 70 °C for 18 h, and then the dried tissues were homogenized in 60% (v/v) perchloric acid for starch hydrolysis. The liberated glucose was quantified utilizing the anthrone colorimetric method [27 and 28], Read the absorbance of samples and anthrone reference using UV visible spectrophotometry (Jenway-Japan) at 750 nm wavelength.

2.3. Statistical analysis

A statistical analysis of data as a complete randomized block design with three replications for each treatment was carried out using the software SPSS version 15.0, when analysis of variance showed a statistical effect of treatment ($P \leq 0.05$), means were separated by the Dunken test.

3. Results and discussion

3.1. Histological parameters of development latent compound bud (N+2) of Superior Seedless grapevine under foliar treatments of nutrients and cytokinins

Due to complicated nature of grapevine compound bud and the several names mentioned in literature which are used for all bud generations of compound buds. [29] conduct a unique naming and coding consist of N letter plus number to avoid miss communication as follow; N is the shoot itself. While shoot initials which have been formed in every node in the axil of each leaf coded N+1 indicated to (summer lateral, lateral shoot, prompt and axillary shoot); N+1 carries the bud N+2 it represents the main part of compound bud which responsible for initiation and differentiation of inflorescence primordial (IP), it has different names (latent bud, dormant bud, primary bud or flower bud). In the axil of N+2 there were two type of buds $N+3_1$ which indicated to the secondary bud that may or may not form inflorescence primordial (IP), and $N+3_2$ indicated to tertiary bud always vegetative. N+2, $N+3_1$ and $N+3_2$ together indicated to (winter bud, dormant bud, compound bud or eye). To avoid confusing in the study we used the coding names of [29]. The timing and stage involved in anlagen initiation and differentiation of Superior grapevine have been defined in detail previously (data not shown). The comparison effect of different foliar treatments on the main stages of compound latent buds (N+2) in the point of anatomy will be discussed in this paper by comparing middle buds, using longitudinal bud dissection of the cane for different foliar treatments for two-time points, the initiation stage (around berry setting) and after full differentiation (before winter pruning). Then (N+2) buds were assessed for some histological parameters. Results of bud dissections showed significant differences between most of the parameters. Data in Table (2) showed that T6 gave the highest percentage of compound buds (90%) entered the initiation phase as a change from vegetative to fruitful bud by distinguishing the change of apex, this change was indicated by the primordial becoming more circular in cross-section rather than being elliptical as they appear in the dormant bud, followed by T5 (75%) and T8 (69%), whereas T7 showed the least initiation buds (28 %) reflecting the vegetative status of most buds that they still in it with no change in the apex and it may be increase later. Generally, the observation showed that CKs treatments promoted early and higher percentage of initiated buds in the first sample date rather than nutrients treatments as revealed in Fig. (1, 2, and 3), where T8 showed the most unequal division of the growing apex pointed to the more progressive stage of initiation Fig. (3). The difference between treatments in initiation (%) may be related to the effect of nutrients and cytokinins treatments on vine vigor. Concerning the anatomical change of (N+2) from vegetative to fruitful bud which is distinguished by the division of apex to form anlagen declaring the beginning of the initiation phase, was described by previous work of [30] and [31] on Thompson seedless. According to [32] when worked on Sultana and two other cultivars indicated that differences in the timing of primordial development related to vine vigor. Similarly, [5] mentioned that the critical factors for inflorescence initiation are closely associated with vine vigor, which includes temperature, light, and water stress, in addition to nutrient availability and hormone balance, which represent the studied treatments. The data in Table (2 and 3) represented the average dimensions of the compound bud at

the first sample date (initiation stage) and second sample date (before winter pruning) which the samples for chemical analysis were taken. Although T5, T6, and T8 gave the significantly largest bud length and width in the two sample dates, T6 gave the highest increase in bud length measured in the second sample date about 19%. In general, CKs treatments were more significant than nutrients except T5 which was lower than control. It is obvious that between the two sample dates there was a clear increase in length and width of (N+2) for all treatments. This increase was probably due to the activity of primary bud to complete its differentiation along the season until winter pruning. There was an increase in cell length and number coincided with the active period of primordial cluster differentiation underwent considerable cell division, and enlargement resulting in numerous sub-branches increase in the latent bud(N+2) size as was revealed by the histology parameters. Finally, leaf primordial (LP), (IP) and tendril primordial (TP) will exist in (N+2) by the winter pruning. Regarding Potential fertile buds (%) and potential bud fruitfulness, data showed that T6 recorded the highest significant value of potential fertile buds (35.47%) and potential bud fruitfulness (1.8). Generally, they were enhanced by cytokinins treatments rather than nutrient treatments. We suggested that the high potential fruitfulness of T6 and T8 may be related to the effect of BA in accelerated vines to the entrance initiation stage and creates a high opportunity for primary bud to differentiate more IP during the growing season compared to other treatments. This agreed with [33] suggested that the BA doses may cause better effects in two or three applications, especially the crucial period of changing from vegetative buds to flowering buds to avoid (Filagen) occurring, which means the reversion of partially differentiated (IP) to tendrils. The mode of action of cytokinins on improving fruitfulness of grapevine interpreted previously by [30] mentioned that the control of inflorescence formation in grapes hinges upon the control of branching of anlagen or tendril. This hypothesis was confirmed by isolated tendrils treated with BA, PBA, or zeatin riboside [34]. Regarding phosphorus treatments (T3 and T4) it gave a little increase than control (T1), this could be due to the effect of phosphorus on promoting fruitfulness through the synthesis of higher rates of ribonucleic acid (RNA) in the buds [35]. There was a difference in potential and actual fruitfulness between treatments. Actual fruitfulness was lower than potential fruitfulness in all treatments involved in the control, but T6 and T8 relatively kept their actual fruitfulness in the high rank compared with other treatments which were 1.45 and 1.25 for T6 and T8 respectively, as revealed in Figure. (4, 5 and 6) indicating to the IP developed in the transverse section. [7] identified bud fruitfulness as a manifestation of the productive capacity of the grapevine; the higher the fruitfulness, the higher the productive potential in the following season, and it is expressed by the number of inflorescence primordia per winter bud. The better performance of T6 and T8 showed a higher potential and actual fruitfulness would be realized in the following spring, this may be due to the direct hormonal effect of Kinetin and BA treatments on the success of IP differentiation process in addition to a low portion of blind eyes in following spring. Whereas, Srinivasan and [36] suggested that flowering in grapes is controlled by the gibberellin: cytokinin balance, as the formation of the inflorescence axis (the anlagen), is gibberellin controlled, but subsequent differentiation into flowers is regulated by cytokinin. Many researchers agreed that Superior grapevine in general recorded a low fertility both potential and actual, in this concern [37] agreed that the mean of potential fertility was higher than the real fertility of Superior Seedless. In the same line, [20] found in evaluating different fertility parameters of 17 grapevine varieties that Superior seedless (Sugraone) presented very low values of potential bud fruitfulness (0.51) and potential fertile buds (46.0%), while the actual bud fruitfulness (0.40-0.80) and actual fertile buds was (43.4%) in almost all nodes. Also, they indicated that seedless varieties presented low values of bud fruitfulness below 1.00. Our results highlighted that CKs treatments (T6 &T8) showed higher potential and actual fertility than other treatments. Our findings are opposite t [33] when observed the real fertility of Thompson seedless grapes grafted on Ramsey rootstock when treated one time with foliar BA at (60, 120, and 240 mg/l) 42 days after pruning, neither affected the actual fertility nor the potential fertility and had similar results to the control, the same trend was obtained in case of the percentage of sprouted buds. We suggested that the difference between the potential and actual fruitfulness of Superior Seedless among all studied can be explained by vine age and the warm weather. This confirms the results of [7] that showed the positive effect of the warmer and dryer weather from May to June on the increase of fruitfulness due to the increased supply of nutrients to differentiating anlagen, such as the supply of carbohydrates, hormones or minerals [10]. From our histology results in (Table 2), bigger buds of T6 and T8 have higher potential and actual fruitfulness. [5] concluded a positive relationship between bud size and carbohydrate level. He suggested that the difference in bud size is attributed to the difference in (CHO) supply transported to the developing compound buds via the vascular connection between the leaf and the compound buds. Therefore, a sequence of measurements of bud size as bud length from the top of the bud at the point of attachment of the cane to the base of the associated leaf scar, this length would then include all of the vascular tissues associated with the compound bud and subtended leaf. In other words, consequently, any reduction of the vascular connection would reduce the supply of CHO to developing buds which could reduce fruitfulness. [38] support this conclusion when tracking and timing the inflorescence development in latent buds and found a relationship between latent bud length, cane diameter beneath the bud, and bud fruitfulness. They added that 1 or 2 IP were reached when bud length was more than 2.2 mm or cane diameter was more than 4.2 mm that certain the relation between the fruitfulness of compound buds and quantity of reserves in the cane loaded the buds. Similarly, [13] suggested that the area of IP was more correlated to bud carbohydrate level. All the above will direct us to the next part of the investigation, the effect of different treatments on the amount of reserves concentration stored during winter as a determinable and detector factor of fertility in the following season.

Table 1. The main physical and chemical properties of soil used for the field experiment.

Properties	Particles size distribution			Soli texture	pH 1:2.5	EC dsm^{-1}	Soluble cations (meq^{-1})				Soluble anions (meq^{-1})		
	Sand (%)	Silt (%)	Clay (%)				Na^{+}	K^{+}	Ca^{++}	Mg^{++}	HCO_3^{-}	Cl^{-}	SO_4^{-2}
	62.15	22.95	14.90	Sandy loam	7.70	1.34	8.98	0.46	2.33	1.63	2.61	9.11	1.7

Table 2. Histological effect of spraying some nutrients and cytokinin on the latent bud (N+2) of Superior seedless grapevine at initiation stage

Date of sampling	Histological parameters	T1	T2	T3	T4	T5	T6	T7	T8
First date sample (around fruit set) at initiation phase	Initiated buds (%)	40 e	55 d	53 d	56 d	75 b	90 a	28 f	69 c
	Primary bud length (µ)	4550 f	4979 d	5668 b	5525 c	6071 a	5595 b	4329 g	6123 a
	Primary bud width (µ)	2000 e	2830 b	2360 d	2630 c	2890 b	3220 a	1450 f	3210 a

Table 3. Histological effect of spraying some nutrients and cytokinin on the latent bud (N+2) of Superior seedless grapevine after differentiation stage

Date of sampling	Histological parameters	T1	T2	T3	T4	T5	T6	T7	T8
Second date sample (before winter pruning) after differentiation phase	Potential fertile buds (%)	21 de	21.13 de	19.17 ef	22 d	30.87 b	35.47 a	17.51 f	28.72 c
	Potential bud fruitfulness	1.1 e	1.2 d	1.3 cd	1.3 cd	1.4 c	1.8 a	1.3 cd	1.7 b
	Primary bud length (µ)	6080 e	6530 c	6240 d	6430 c	6690 b	6990 a	6080 e	6890 a
	Primary bud width (µ)	3846 c	4116 b	4356 b	4296 b	4092 b	5424 a	2832 d	5400 a
	Actual bud fruitfulness	0.9 d	1.1 c	1.1 c	1.2 bc	1.2 bc	1.45 a	1.2 bc	1.25 b

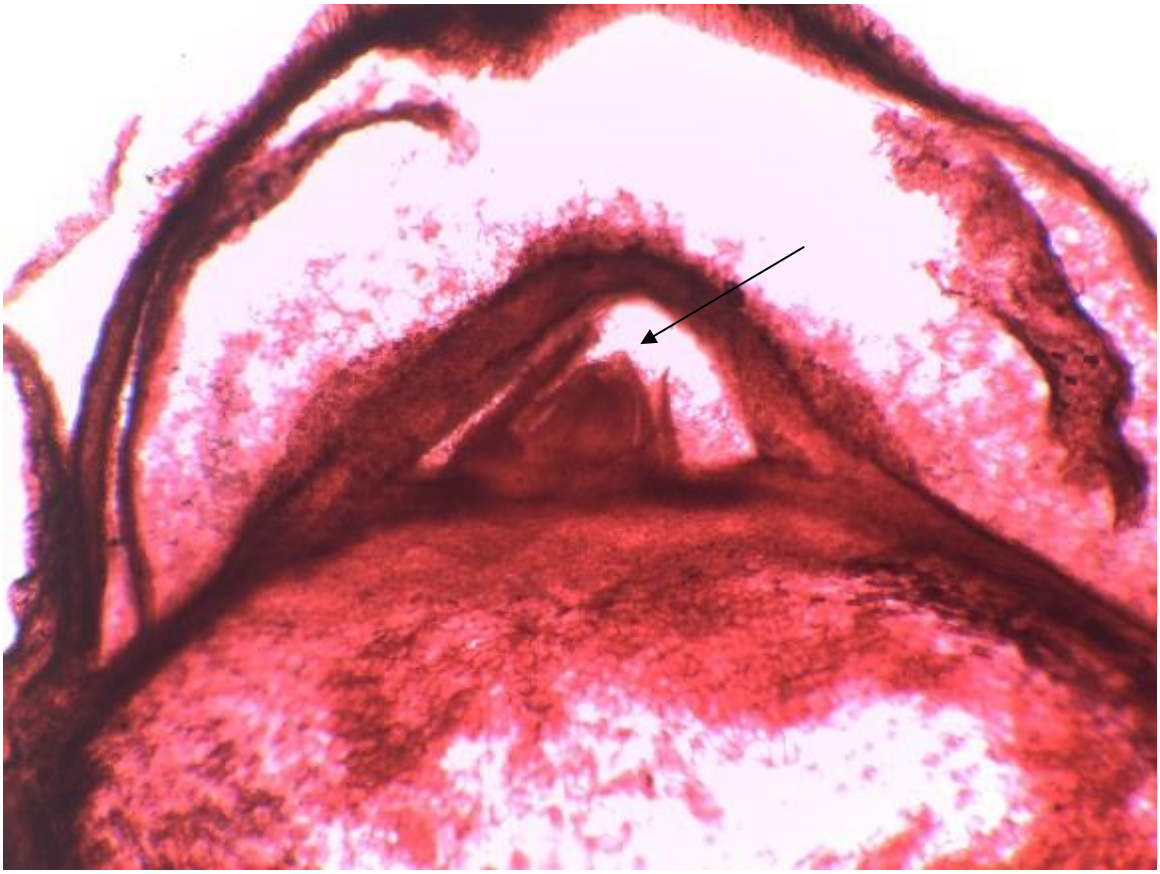

Figure 1. The cross-section in the latent bud (N+2) of T1(control) of Superior Seedless on the first sample date (initiation phase), note the first change in the apex becoming more circular in cross-section rather than being elliptical and division of the apex to form anlagen later as the arrow indicated. (4x magnified)

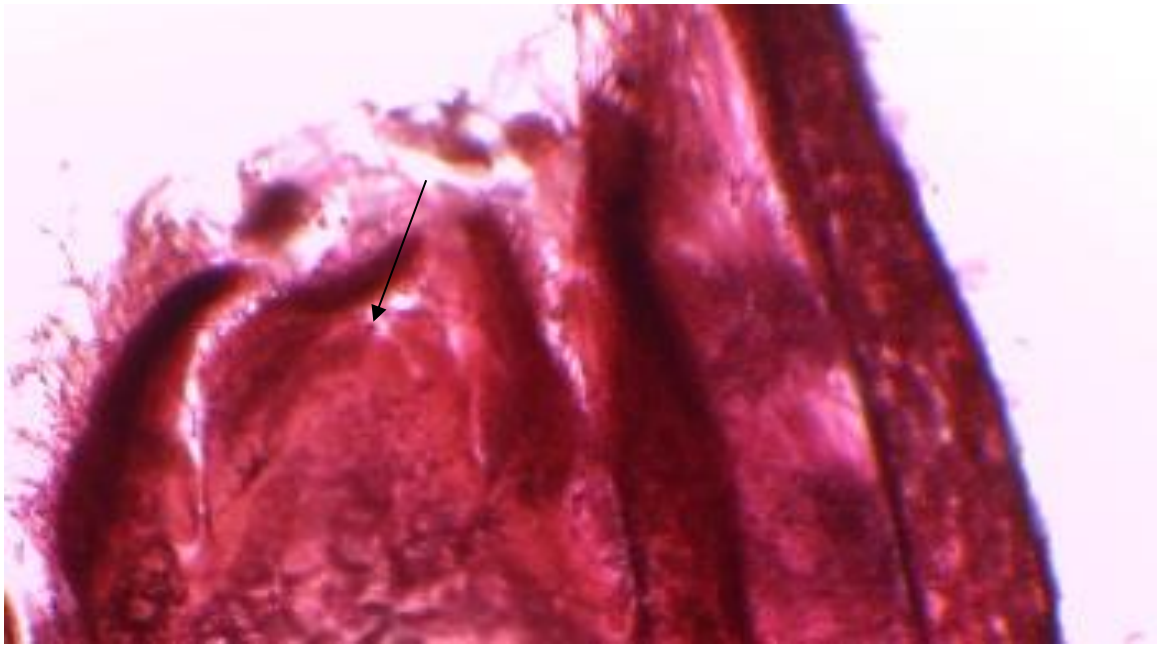

Figure 2. A cross-section in the latent bud (N+2) of T5 of Superior Seedless on the first sample (initiation phase). Indicate to the division of apex. (4x magnified)

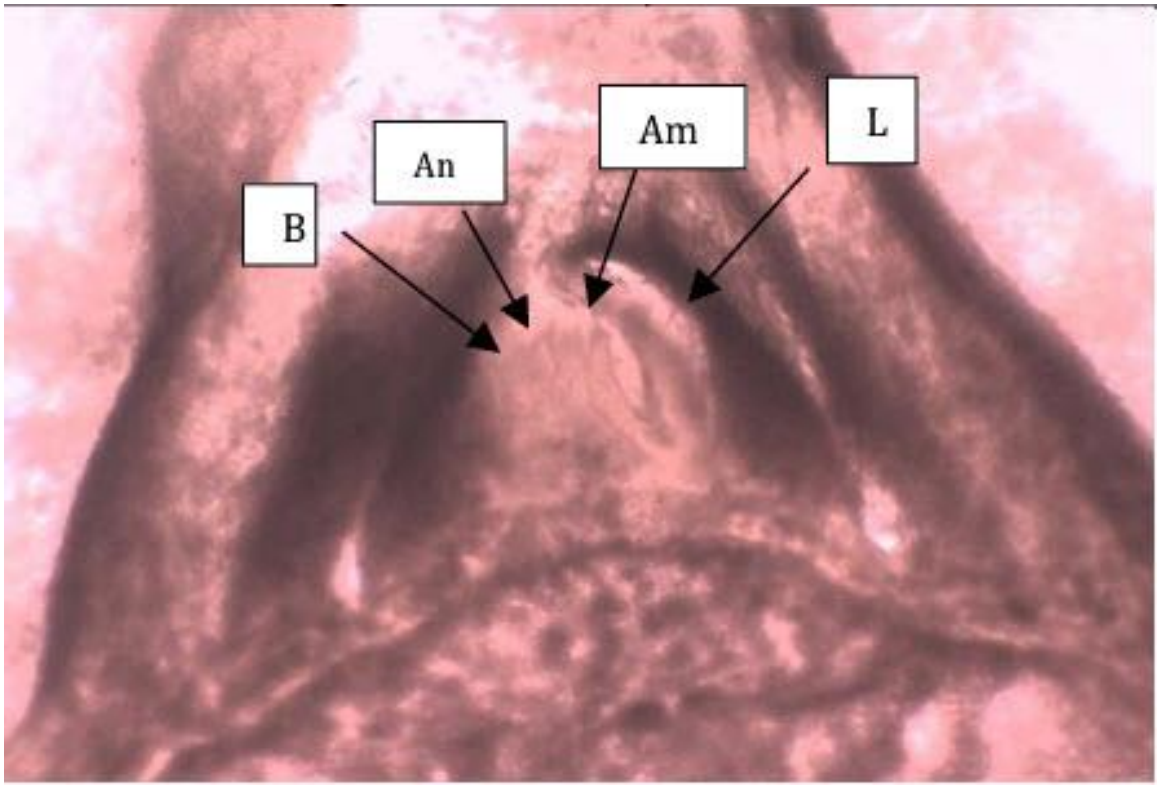

Figure 3. A cross-section in (N+2) of T8 on the first week of May (initiation phase) of Superior Seedless, arrows indicating to the change in apex. Observe the division of apical to three parts (bract (B), anlagen (An) and the apical meristem (Am), while leaf primordial (LP) in the right, indicating a clear advancement in development compared to the others (4 x magnified).

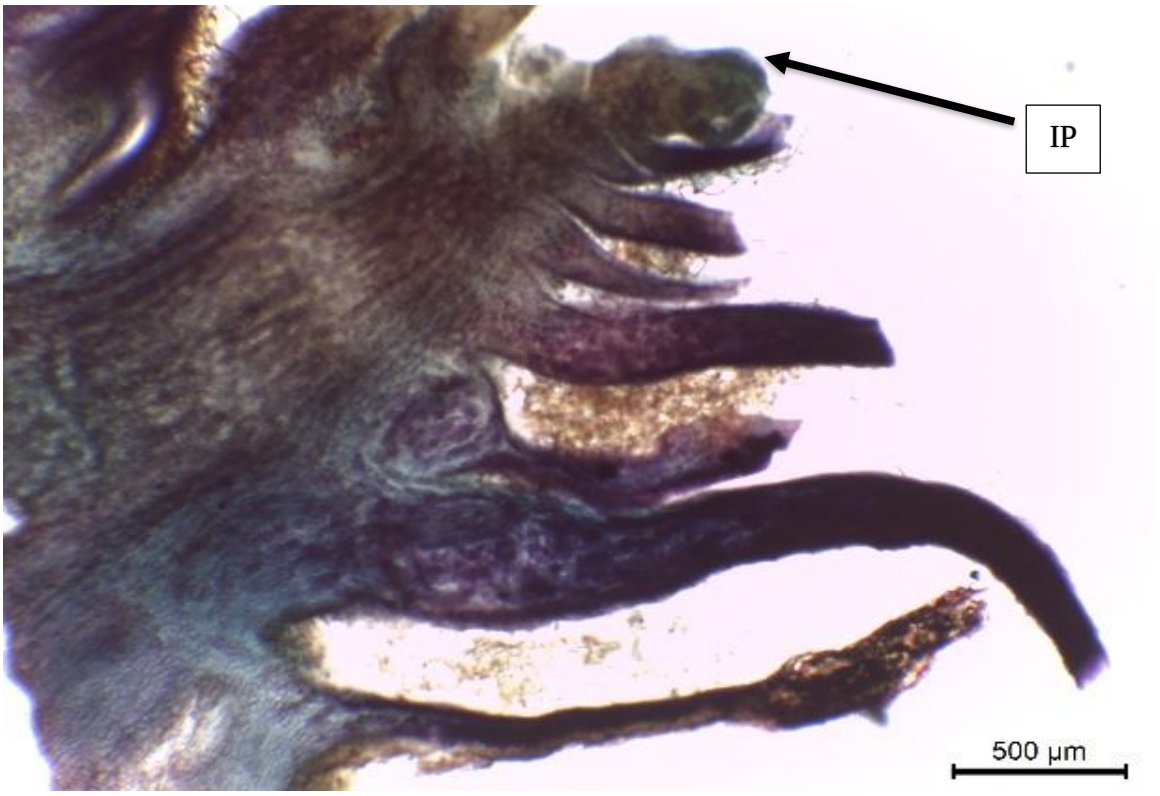

Figure 4. A transverse section in (N+2) of T1 of Superior Seedless after differentiation before winter pruning, arrow indicate to, inflorescence primordial (IP). (4x magnified).

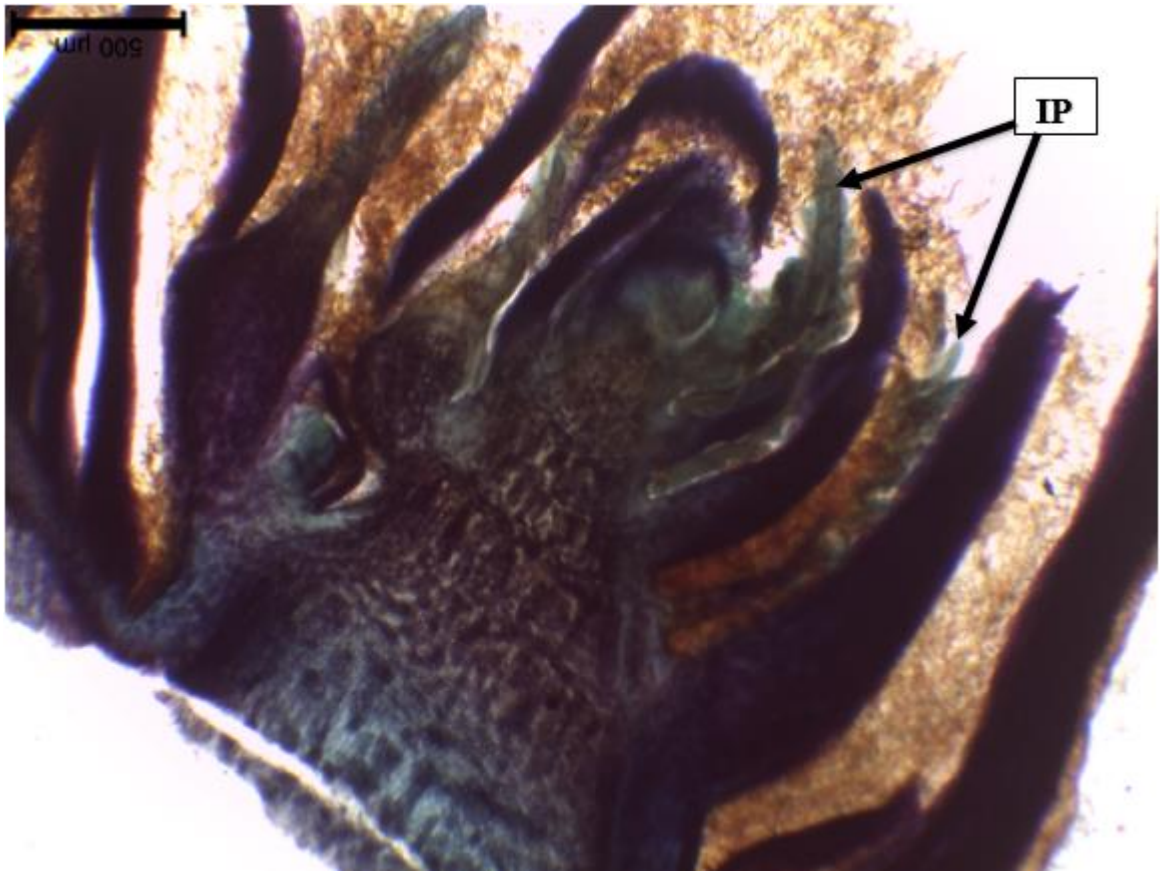

Figure 5. A transverse section in (N+2) of T6 of Superior Seedless indicated two differentiated inflorescences primordial (IP) before winter pruning as arrows indicated (4x magnified).

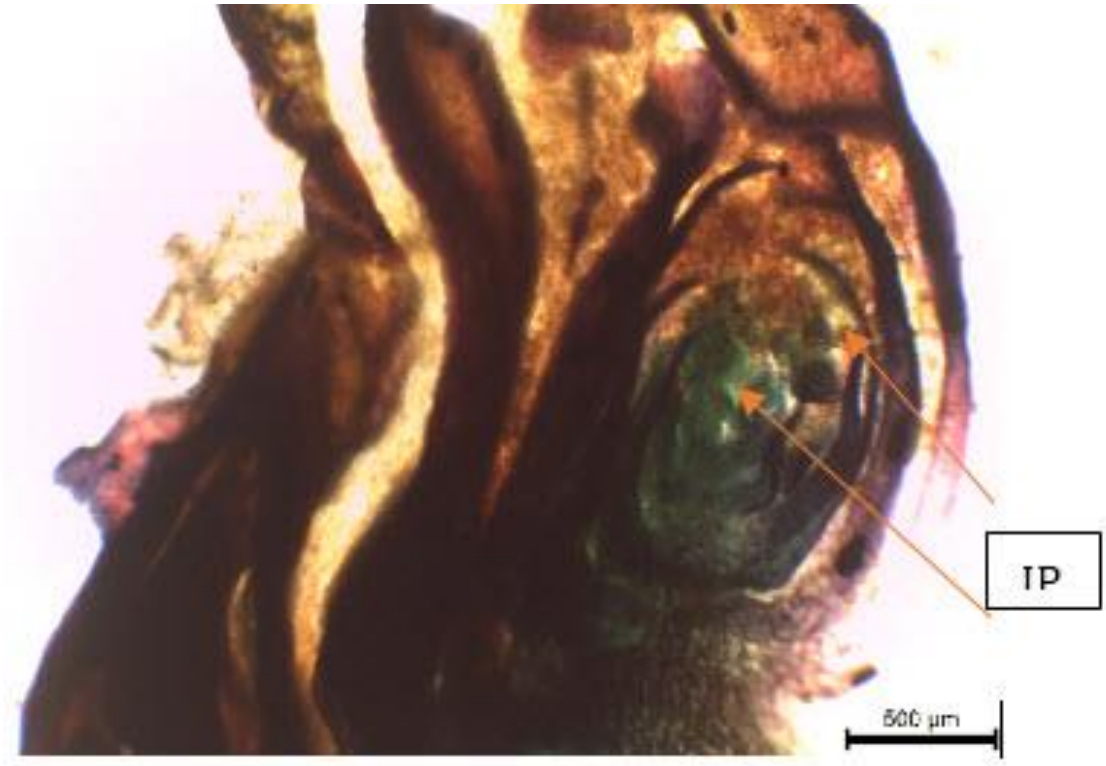

Figure 6. A transverse section in (N+2) of T6 of Superior Seedless after differentiation before winter pruning, arrows indicated to two inflorescence primordial (IP) (4x magnified).

Table 4. Effect of some nutrients and cytokinins on cane vigor of Superior grapevine

Treat.	Internode length (cm)		Cane diameter (cm)	
	1st season	2nd season	1st season	2nd season
T1	10.67 a	10.50 a	0.46 d	0.68 c
T2	9.17 b	9.67 ab	0.64 c	0.74 b
T3	9.83 ab	8.50 b	0.81 a	0.70 c
T4	9.6 ab	9.50 ab	0.65 c	0.75 b
T5	9.2 ab	9.83 ab	0.74 b	0.75 b
T6	9.77 ab	9.83 ab	0.74 b	0.75 b
T7	9.67 ab	9.07 ab	0.71 b	0.83 a
T8	9.50 ab	9.50 ab	0.82 a	0.81 a

T1: control (water only); T2: K_2SO_4 (1g/l), spraying 5 times; T3: MAP (1g/l), spraying 5 times; T4: Nano-Ca-P (0.333g/l), spraying 5 times; T5: Kin-Kinetin-(10ppm), spraying 5 times; T6: BA-benzyl adenine- (20ppm), spraying 5 times; T7: Kin- Kinetin-(10ppm), spraying 3 times; T8: BA-benzyl adenine- (20ppm), spraying 3 times.

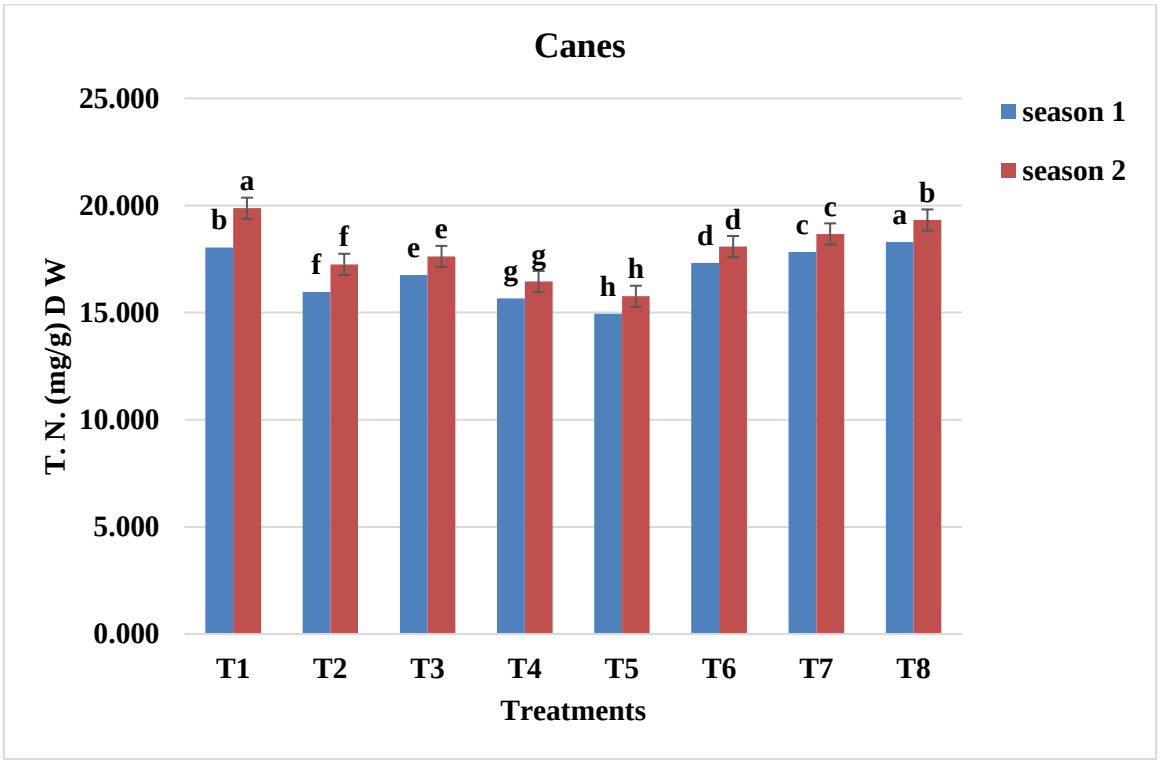

Figure 7. Levels of total nitrogen in canes of Superior grapevine in 2019 and 2020 seasons on some nutrients and cytokinins spraying T1: control (water only); T2: K_2SO_4 (1g/l), spraying 5 times; T3: MAP (1g/l), spraying 5 times; T4: Nano-Ca-P (0.333g/l), spraying 5 times; T5: Kin-Kinetin-(10ppm), spraying 5 times; T6: BA-benzyl adenine- (20ppm), spraying 5 times; T7: Kin- Kinetin-(10ppm), spraying 3 times; T8: BA-benzyl adenine- (20ppm), spraying 3 times.

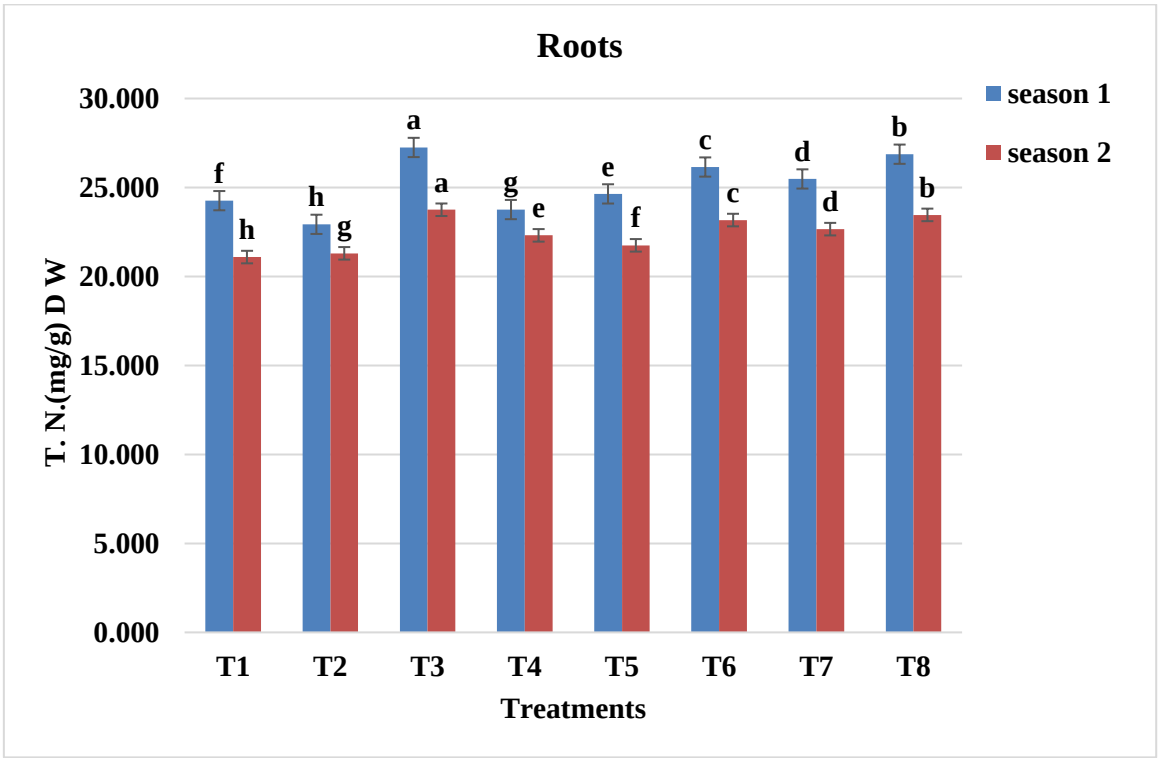

Figure 8. Levels of total nitrogen in roots of Superior grapevine in 2019 and 2020 seasons on some nutrients and cytokinins spraying T1: control (water only); T2: K_2SO_4 (1g/l), spraying 5 times; T3: MAP (1g/l), spraying 5 times; T4: Nano-Ca-P (0.333g/l), spraying 5 times; T5: Kin-Kinetin-(10ppm), spraying 5 times; T6: BA-benzyl adenine- (20ppm), spraying 5 times; T7: Kin- Kinetin-(10ppm), spraying 3 times; T8: BA-benzyl adenine- (20ppm), spraying 3 times.

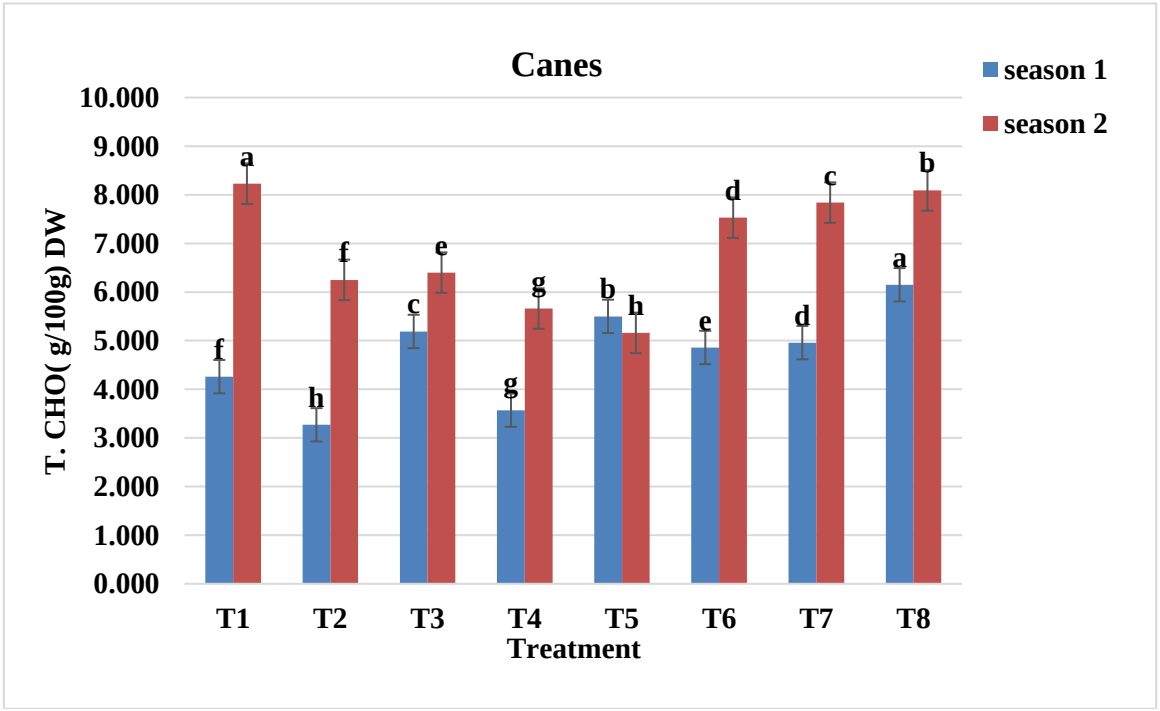

Figure 9. Levels of total carbohydrates in canes (reserves concentration status) of Superior grapevine in 2019 and 2020 seasons of some nutrients and cytokinins spraying T1: control (water only); T2: K_2SO_4 (1g/l), spraying 5 times; T3: MAP (1g/l), spraying 5

times; T4: Nano-Ca-P (0.333g/l), spraying 5 times; T5: Kin-Kinetin-(10ppm), spraying 5 times; T6: BA-benzyl adenine- (20ppm), spraying 5 times; T7: Kin- Kinetin-(10ppm), spraying 3 times; T8: BA-benzyl adenine- (20ppm), spraying 3 times.

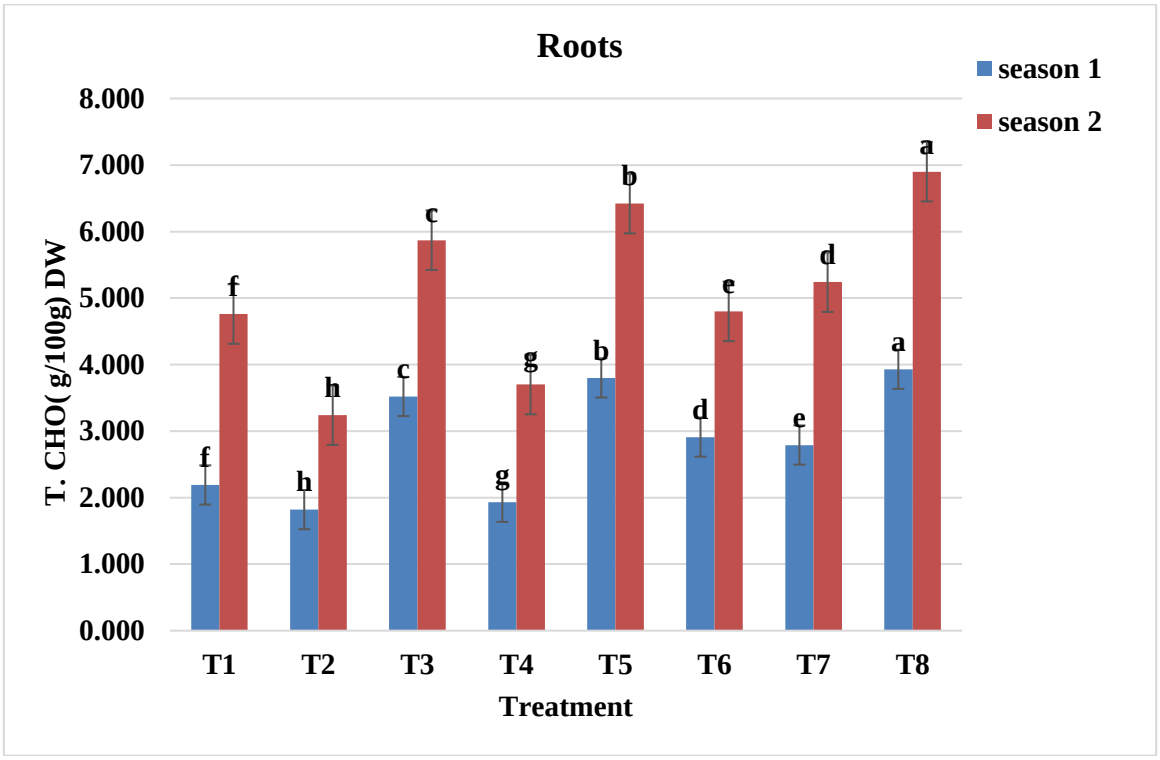

Figure 10. Levels of total carbohydrates in roots (reserves concentration status) of Superior grapevine in 2019 and 2020 seasons of some nutrients and cytokinins spraying T1: control (water only); T2: K_2SO_4 (1g/l), spraying 5 times; T3: MAP (1g/l), spraying 5 times; T4: Nano-Ca-P (0.333g/l), spraying 5 times; T5: Kin-Kinetin-(10ppm), spraying 5 times; T6: BA-benzyl adenine- (20ppm), spraying 5 times; T7: Kin- Kinetin-(10ppm), spraying 3 times; T8: BA-benzyl adenine- (20ppm), spraying 3 times.

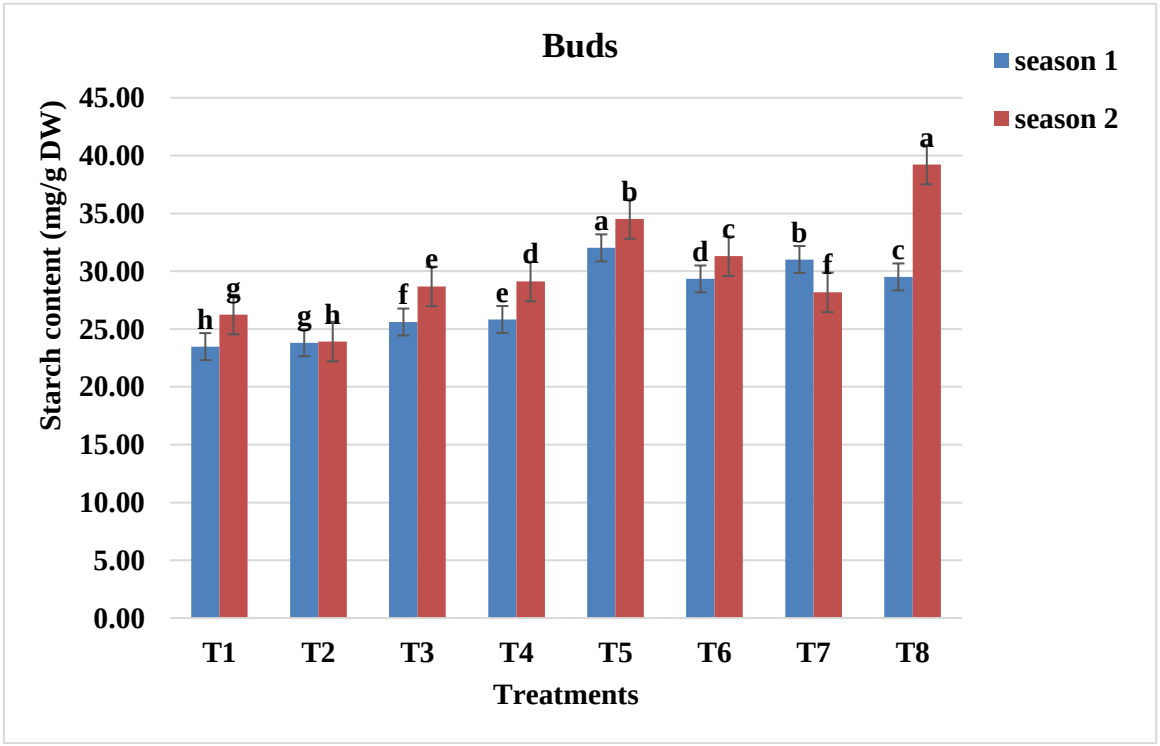

Figure 11. Starch content in buds of Superior grapevine in 2019 and 2020 seasons of some nutrients and cytokinins spraying T1: control (water only); T2: K_2SO_4 (1g/l), spraying 5 times; T3: MAP (1g/l), spraying 5 times; T4: Nano-Ca-P (0.333g/l), spraying 5

times; T5: Kin-Kinetin-(10ppm), spraying 5 times; T6: BA-benzyl adenine- (20ppm), spraying 5 times; T7: Kin- Kinetin-(10ppm), spraying 3 times; T8: BA-benzyl adenine- (20ppm), spraying 3 times.

3.2. cane vigor and reserves concentration status of Superior Seedless grapevine under some foliar treatments of nutrients and cytokinins

3.2.1. Cane vigor

The data in Table (3) showed the cane vigor before winter pruning. Where control gave the tallest internode length but, didn't differ significantly from other treatments. There was no clear trend between treatments. (T8) offered an increase in cane diameter along the two seasons (0.82 & 0.81 cm), respectively. Although our results of internode diameter didn't show a clear difference between treatments, we couldn't separate the effect of different treatments on cane vigor without neglecting the involvement role of reserves concentration, especially CHO content on increasing cane diameter, and its relation with fruitfulness, because they are sequence for each other. Our results showed a little improvement in the internode thickness for most of the treatments compared with the control (Table 3). [34] mentioned that fertilizer, especially K-treated plants have a direct relationship with total CHO, K-treated plants affecting metabolic intensity consequently contain a greater total mass of reserves eventually reflected on the diameter of the internode. [39] reported a relation between starch content and increased wood/pith ratio as long as CHO is flowing rapidly to the buds and not to the new growth in Pinot noir and seven other varieties. This opinion was supported by [10] who observed that less vigorous thinner shoots initiated fewer inflorescences per node than larger latent buds. The same suggestion obtained by [40] who found a positive relation between the increase in cane thickness of Superior grapevine and bud fertility due to the higher content of total carbohydrates in canes at dormant seasons.

3.2.2. Total nitrogen

Data in Fig. (7) showed the effect of studied treatments on total nitrogen content in canes before pruning. The results demonstrated that control and CKs treatments exerted higher significant in total nitrogen, T8 reached (18.29, 18.04 mg/g DW) in the two seasons respectively, then T7 and T6, while T5 was always the least. Regarding total nitrogen content in roots before pruning, the observed results of (Figure. 8) displayed that (T3) recorded the highest nitrogen content in roots (27.25 & 23.75 mg/g DW) in the two seasons respectively, followed by T8 and T6 noting that T5 was always the least. Regarding the effect of different treatments on total N in canes and roots, data showed that total N was higher in roots than canes for all treatments (Fig. 7, 8). The same finding was reported by [41] that N concentration in the trunks and canes represented approximately half of that measured in the roots of grapevine Chasselas. T3 showed an outstanding effect of T.N in roots compared with other treatments, this may be due to the known effect of phosphorus in stimulating the formation and growth of roots [42]. Regarding CKs treatments, T8 followed by T7 and T6 gave a high significant T.N. in roots in canes and roots. [43], attributed the positive results of BA and yeast treatments to the number of metabolites that enhance sink activity and endogenous growth hormones levels, which in turn increase the net amount of photosynthetic assimilate production and transport from the site of synthesis in leaf tissue source to site of accumulation in storage organs, which represent (canes, roots or cluster) in case of our study. In general, treatments of CKs with high T.N. level have a high potential for fertile buds (%). This agreed with [44] when reported that N is an important element for carbohydrate metabolism, and low levels of this element reduce the number of fertile buds in grapevines. According to [12], both vegetative growth and fruiting of young Concord vines are largely determined by reserve nitrogen, not by reserve carbohydrates, and this contrasts with the conventional view that vine growth in the spring is largely determined by reserve carbohydrates.

3.2.3. Total carbohydrate

Data in Fig. (9) showed the effect of studied treatments on reserves concentration which is mainly determined by total carbohydrates (CHO) in canes and roots before pruning. The results showed that CKs treatments gave more satisfactory CHO reserves than nutrients treatments, whereas, K_2SO_4 (T2) usually gave the least reserves values, also Nano-Ca-P (T4) was more effective than MAP (T3) and was the best among nutrients treatments (5.19 &6.40 g/100g DW) in canes and (3.52 & 5.87 g/100g DW) in roots. Sprayed BA 3 times (T8) was the best in providing vines with constant and adequate (CHO) along the two seasons reached (6.15 & 8.09 g/100g DW) in canes and (3.93 & 6.90 g/100g DW) in roots while control treatment (T1) was the least. Many researchers such as [45,5,13] agreed that switching from flowering to fruiting vines needs a considerable amount of energy, which in woody plants obtained from main ways; accumulated reserves or by photosynthesis in initiated leaves or inflorescences, and any reduction in the transport of photo-assimilation into developing buds contributes to a reduction in the fruitfulness. Furthermore, CHO act as a floral stimulus it also represents an energy source, and Researchers investigated the importance of floral initiation and intensity of flowering and linked them with measuring levels of stored carbohydrates, or imposing treatments such as girdling that modified the level of stored carbohydrates [46], or by altering canopy management and linked it with bud CHO level and size of inflorescence primordial [13]. In addition, the inflorescences number that appear in spring is dependent upon the extent of reserve replenishment during the previous year [47] and [7]. This somehow agreed with CKs treatments in our study (T8, T5, and T6), especially (T8) was an example of this relationship that recorded higher CHO reserves associated with higher fertility parameters which has been indicated in (Table 2). Also, spraying with Kin (T5) showed high significant CHO content in canes and roots and higher fertility, this agreed with what [48] reported in their trial on young olive trees that spraying with kinetin gave a significant superiority of total carbohydrates in branches content by increase dose. Although T6 wasn't the best of CHO or starch content (Fig. 3 & 5) as discussed previously, but was the best in the value of histological fertility parameters (table 2). We suggested that the amount of CHO reserves stored in the T6 were enough to support bud initiation, and thus fruitfulness

was not limited. The positive effect of CKs treatments may be related to its indirect effect on fruitfulness by enhancing CHO content as a flowering stimulus or may have a direct effect through molecular mechanisms regulating bud fruitfulness. Many researchers supported our results about the positive effect of exogenous cytokinins on CHO reserves, it is known to be a strong mobilizer of photosynthates in grapes [30]. Similarly, [49] recorded an increase in photosynthate influx to BA treated organs (the sink effect) especially with treatments at the earlier growth stages being more effective in promoting photosynthate efflux from grapevine leaves which adjacent to the application sites. Additionally, [43] discussed the positive results of BA and yeast treatment on increasing and enhancing vegetative growth and amount of metabolites which include total CHO and crude protein in leaves, finally enhancing sink activity in sugar beet. It is worth noting that P treatment (T3) was more forceful than nutrient treatments in CHO reserves in canes and roots along the two seasons. As known that P is the best nutrient for stimulating the formation and growth of roots, this may be due to P being involved in many biochemical processes such as cell division, development of meristematic tissue, carbon fixation, intermediary metabolism, breakdown of the CHO, utilization of sugars and starch and transfer the energy within the plant and its role in nucleic acids and activity in biological energy change via adenosine triphosphate (ATP). [42, 50].

3.2.4. Starch content in buds

Results of Figure. (11) showed the effect of different treatments on the starch content of buds, CKs treatments was more powerful than nutrients treatments, (T2) had the least starch content in buds partly like the trend of total CHO content in canes, except (T5) record high starch content in buds opposite to low CHO in canes, and control was inverse, high CHO in canes with low starch content in buds. All known the importance of starch and soluble sugars is crucial for the development of new primary shoots and inflorescences as an indicator for fruitfulness as proved by the works of [51] when they take samples of Thompson seedless apical bud and latent bud in spring and fall. They observed an increase in cell diameter, nucleus, and nucleolus diameter on latent buds and observed an intensive existence of starch granules in the apex of latent bud during initiation. The same suggestion obtained by [14] stated that starch concentration decreased at higher node position in a cane correlated with a decrease in IP per shoot at the top of the canes, thus confirming the hypothesis of high starch content in the bud is a signal to the high fertility of the bud. Research of [52] noted when collected latent buds of *Vitis vinifera* L. cv. Merlot during its dormancy phase, the microscopical observations showed a gradient of starch content in different regions of bud in which the leaves primordia and scales were richer in starch. Our results showed a partly resemble between the trend of CHO in canes and starch content in buds, indicating that the accumulation of reserves in the perennial parts of the vine varied over the years, i.e. (T1, T5, and T7) showed variation trend between the two years of the study. This agreed with the results of [31] and [7] found a difference between the starch trend among node and internode in two seasons of the study, higher starch content was found in the node in the first season, while in the second season, it was higher in the internode. (T2) had the least starch content in buds, this agreed with [53] observed that foliar spraying of Potassium sulfate had no significant effect on the amount of starch in grape buds and only the sampling stages had a significant effect on starch. On the opposite, (T8) showed the highest positive effect on starch content in the bud, which may be due to BA playing a permissive role in the regulation of various growth processes in the plants [54]. Based on our result concerning the reserves concentration and histological fertility parameters, our study suggested that starch content in buds was more reliable in fertility prediction than total CHO in canes.

4. Conclusions

Results of our study revealed that spraying (T6) benzyladenine (BA) at 20ppm five times showed remarkable superiority of latent compound buds (N+2) entered the initiation phase. The dimensions of buds of treatment (T6) overrated those of the other treatments in the second sample date. The latent bud sprayed with T6 (BA at 20ppm five times) and T8 (BA at 20ppm three times) exhibited better performance in completing its differentiation along the season compared with other treatments and gave the highest significant actual fruitfulness. Cytokinins treatments especially (T8) increased the level of reserves concentration representing by highest total carbohydrate in canes and roots, and starch content in buds. We suggested that foliar application of nutrients or cytokinins after harvest was very important because it coincided with the best active period for stimulating anlagen to differentiate to (IP) and promote more divisions into sub-branches to ensure finally cluster not tendril formation, eventually, leading to improve fertility. While total nitrogen and CHO in canes and starch content in buds may be an indicator for good reserves status for reproductive growth of Superior Seedless in the next year, starch content in buds was more reliable in fertility predicting than total carbohydrate in canes. In addition, any cultural practice promoting reserve concentration would be a useful tool for improving the fruitfulness of low-fertility cultivars.

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