Effects of Auxin and Cytokinin on Morphological and Physiological Factors in Soybean *(Glycin max L)*

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Abstract: Auxins and Cytokinins are of plant growth regulators. These two hormones are interfered in various physiological growth processes such as (activation and meiosis, tropism and morphology and …). Soybean is the most important sources providing world protein and plant oil and considering to food consumption for human and animals and medicine functions has high economic importance. For this reason, determining affects of plant hormones, Auxins (NAA) and Cytokinin (BA) was done on growth factors and then affect on the level of chlorophyll carotenoid and sugar and protein. Three different concentration of Auxins (NAA), Cytokinin (BA) hormones and three different concentration of equal combination from these two hormones were used. The studies showed that in the treated plants due to using different levels of (NAA) and (BA) concentrations as independently and treatment (NAA + BA) it was seen to increase in wet weight because of enhancing the concentration of hormone up to 1.5 mg/l and then to decrease wet weight. The dry weight verses wet weight increased by using hormones with enhancing hormones concentration, but through using combination of these two hormones along with enhancing concentration, it has been decreased. So, the most wet and dry weight of stem to treatment (NAA + BA) was 0.5 mg/l and the least wet and dry weight of stem to treatment (NAA) was 0.5 mg/l. In associated with dry weight of root, it was seen increasing hormone concentration NAA caused decreasing dry weight of root. But in treatments (BA and NAA + BA), it was seen increasing up to 1.5 mg/l and then decreasing dry weight. In associated with increasing treatment concentration (NAA), it was seen decreasing wet weight of root, and by increasing concentration, it was seen decreasing and then increasing and at last by increasing concentration (NAA + BA), it has been seen increasing wet weight. Also, the most wet weight root related to NAA treatment was 0.mg/l and the least was 1.5 mg/l for (NAA) treatment and the most dry weight of root was 1.mg/l for (NAA + BA) treatment and the least amount was 0.5 mg/l for (NAA + BA) treatment. In general, by increasing treatment concentration it was seen to shorten stem length and also in associated with root length, by increasing treatment concentration, it was seen increasing trend in root length, therefore the most stem length and root was dedicated to (NAA) treatment, 0.5 and (BA) 2.5 mg/l and the least stem and root length to (NAA + BA) treatments, 2.5 and (NAA) 0.5 mg/l, respectively. By increasing hormone concentration NAA and combination hormone, first increasing sugar concentration to treatment 1.5 mg/l and then decreasing sugar concentration amount were seen and in treatment with BA, by increasing hormone concentration, first decreasing and then increasing sugar concentration were seen, the most amount of carbohydrate in NAA treatment, 1.5 mg/l and the least amount in (NAA + BA) treatment, 0.5 mg/l were seen and in related with protein, by increasing hormones concentration of (NAA + BA), increasing protein concentration was seen. But by increasing combination hormones concentration, decreasing concentration amount has been seen, so the most amounts was seen in (BA) treatment, 2.5 and the least amount in control treatment. In associated with protein there was a statistically significant difference among control and all used treatments except (NAA + BA), 2.5 mg/l. The studies has been shown that in related to photosynthetic pigments, by increasing NAA hormone concentration, the amount of chlorophyll a has increased and in BA treatment up to 1.5 mg/l it was seen increasing and then decreasing and in combination treatment of two hormones, by increasing hormones, the amount of chlorophyll a has been decreased and about chlorophyllb and total chlorophyll and carotenoids, by increasing hormones concentration used, increasing was seen, respectively.
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Introduction

Soybean is from class Faboidiea, Fablese row, Fabaceae or Leguninoea family and Papilionoidea and sub-family Glycinmax species. This plant has branched branches, with to purple flowers and one to five seeds in each sheath, this plant is native of tropical areas but is planting in moderate areas also and naturally it is very sensitive to low temperatures and easily damaged against cold weather. Its chromosomes number is 40 (2n = 40). The flower arrangement is clustery. Russian researcher are among first individuals who found the beneficent of soybean, these researchers realized that soybean has more protein necessary amine acids for

body, calcium and fiber, iron, phosphorus, magnesium, zinc, vitamin group B and the called the following beneficent for soybean: heart health and decreasing cardiac diseases, bones health, preventing from cancer, decreasing menopause complications, skin and hair health, preventing from anemia, fiber caused in decreasing weight, decreasing cholesterol and triglyceride and increasing function of colon and preventing cancer in colon. Scientists succeeded to make a kind of cheap anti biotic from soybean that called Moro mix act for killing harm bacteria of intestine. The plant is found with concentration range 1 to 100 mg auxin per 1 kg plant fresh weight, the young tissues and plantlets that fast grow and elongate contain high concentration of auxin than adult tissues and it is believed that the younger tissues are more sensitive than this growth regulator, physiological effects of auxin are complex and different tissues differently response to them and their reasons are unknown. In addition, separating primary effects from secondary effects are often difficult. The classic effect of auxin is to increase growth or stimulate length growth of cells formed a certain tissue. Additionally, when the effect of its different concentrations is tested, the straight line of this effect becomes complex very soon. The diagrams of different tissues response to concentrate show that addition to their role in elongation growth of cells, the influence on cell mitoses, so it stimulates cambium activation and causes to distinguish vena in vertex region of new branches, they are factor of establishing in the lateral roots and stimulate forming abnormal roots in stem and leaf cuttings. However, cytokinines were identified as cell division factors, but these hormones can have stimulus and inhibitor effects on different physiological, metabolic, biochemical and growth processes. And it is clear that endogenous cytokinines have significant role in regulating these processesin healthy plants. Cytokinines are defined as compounds that have bioactivity similar to Transzatin that interfere in some activities such as inducing cell division in the cell of callous in presence of auxin, stimulating formation of root and bud in planting callous when moll ratio to auxin is proper, delaying to become old leaves an stimulating to enlarge cotyledon in dicotyledonous plants, inducing formation bud in moss and genetic glands, more chemicals that have cytokinineial activity have been tested the analysis of these compounds was provided a good view about necessary structure for activating cytokinine. Almost all active materials as cytokinine are amino purines that their azoth number 6 has been substituted such as Banzil Adenin (BA).

Materials and methods

In order to implementing this research, the seed of soybean plant (Glycin max) canola species has been provided from the agriculture office of Babolsar city. Expected survey were done in plant sciences laboratory of Islamic Azad University in 1391 and has been independently examined the effect of two hormones, auxin (NNA) and cytokinine (BA) and a compound of both of them with concentrations (0.5- 105- 2.5 mg/L) on photosynthesis pigments and amount of carbohydrate and protein of soybean plant.

Preparation and culturing seeds

First the healthy seeds with same size were chosen and sterilized with 5% Hypochlorite solution (bleach) for 3 minutes and well washed by distilled water. Then sterilized seeds were cultured on two layers filter of Vatman number 1 in medium Petri dish (number 8) and added about 20 ml stilled water to each Petri dish in order to occur its primary growth and transferred to germinator with temperature 25℃ to bud seeds under dark conditions and fixed temperature. After spending that time and implementing bud process, they are transferred to a light environment to occur natural growth, when the roots of seed reach 1.5 cm and the color of cotyledon became green, they were transferred to pots with washed sand. After this step, they watered 5 to 7 days and then the sand environment has no food ingredients for faster growth and strengthening seedlings, it was added Hugland to food environment.

Surveying growth factors

After, the soybean has 13 to 15 leaflets or 24 to 5 leaves, the growth parameter include root length, stem length, wet weight and dried weight and seedlings' root were measured by three replicate of each treatment. Stemle and rootlet length were measured by millimeter ruler and wet and dry weight of stem and root by digital scale. For measuring dry weight, samples were put for 24 hours in temperature 70℃ in oven and then the dry weight of samples was measured by digital scale.

Determining the amounts of photosynthesis pigments

For measuring chlorophyll, it was used the Lichtenthler's method (1987), for this purpose 0.1 gr from the green leaf of plant was taken and scaled and well-grinned in porcelain mortar with acetone 80% in order to extracting its pigments. The grinded materials filtered by vatman's filter paper N. one and the filtered sample was transferred to test tubes. Then attraction in wave length of 663 nanometers for chlorophyll and wave length 646 nanometers for b chlorophyll and 470 nanometers for carotenoid were read by spectrophotometer. The control (blank) is acetone in this measurement. After reading waves length, the resulted numbers were put in the formulas and related calculations were done: (concentration in

microgram on milliliter from plant extraction was determined).

Measuring carbohydrate

For measuring carbohydrate, it was used Nelson's method (1943). 0.05 gr from dried leaves grinded with 10 ml distilled water and the grinded samples poured in test tube then filtered by vatman's filter paper N. one. After adding solutions of copper sulphate and acid molybdic, the tubes were shacked to appear the blue color in them, after this step, their absorption was read in wave length 600 nanometers in spectro photo meter apparatuand concentration of carbohydrate was calculated by standard curve of glucose in mg on gr.

Measuring protein

For measuring protein, it was used Lowery's method (1951). Based on this method, it was used 0.05 gr from fresh leaves of soybean and grinded by 7 ml Tris buffer in a porcelain mortar and transferred in centrifuge tube, the higher solution transferred to test tube for extracting protein. After adding D and E reagents it was read in wave length 625 nanometers by spectro photo meter apparatus, for providing standard curve and calculating samples protein, the serum of cow albumin was prepared in different concentrations and all process were repeated on them and their absorption was read.

Statistical Analysis

The tests were implemented with three replications in a full-accidental plan form. The amounts of three replicate were obtained and calculated variance and the standard deviation of means and the difference among means by variance analysis. Surveying the result of tests and drawing curves were implemented according to comparing means and standard deviation (Mean ± SE). Grouping treatments in the level of 5% ($p \leq 0.05$) was done by Tukey test. Statistical analysis of data was done by SPSS software and for drawing diagrams, it was used EXCEL software.

Results

The wet weight of stem: by using different levels of Auxin (NAA) and cytokinine (BA) concentrations as independent and compound treatments (NAA + BA), it was seen increasing in wet weight by increasing hormone concentration amount to 1.5 mg in liter and decreasing weight in 2.5 mg in liter as in (NAA + BA) 0.5 mg was the most wet weight and the least amount in (NAA) treatment was seen 0.5 mg in liter and in the it has been seen a significant difference in statistical level. The difference of wet weight among control and all treatments of (NAA) have been meaningful. (Table 1, Diagram 1).

Table 1: Analyzing variance of hormones effect (NAA), (BA) and (NAA + BA) on soybean's morphological parameters

Source of variations (SOV)	Degree of Free (df)	Mean square					
		Wet weight of stem	Wet weight of root	Dried weight of stem	Dried weight of root	Stem length	Root length
total	29	13.6**	6.9**	75.62**	31.42**	184.91**	16.957**
treatment	9						
repeat	2						
Test error	20	1.17	0.39	0.728	0.0163	39.9	4.9
Coefficient of variations		16.12	46.29	37.03	13.12	29.14	22.07

The dried weight of stem: the dried weight of seedlings opposite of wet weight is increased by using auxin and cytokinine hormones and increasing in hormones concentrations, but by simultaneous action or compound of two hormone as (NAA + BA) has been decreased by increasing concentration so in concentration of (NAA) 0.5 mg in liter was the least amount and in (NAA + BA) treatment, 0.5 mg in liter was the most amount, they had a significant difference in statistical level of 0.001. The difference of dried weight among control and all treatments has been meaningful. (Table 1, Diagram 2).

The wet weight of root: The wet weight of seedlings root in (NAA) treatment has been reached the least amount 2.5 mg/l and the most amount 0.5 mg/l. in general, by increasing concentration of (NAA) treatments, it has been seen to decrease in wet weight andby increasing in (BA) concentration first decreasing and then increasing and at last by increasing (NAA + BA) has been seen to increase wet weight. There has been a significant difference in the statistical level of 0.01. The difference of wet weight of root among control and all treatments except (NAA + BA) 1.5 mg/l has been meaningless (Table 1, Diagram 3).

The dried weight of root: the least dried weight of root was seen 0.5 mg/l in (NAA + BA) treatment, so the most amounts were 1.5 mg/l in (NAA + BA). Totally, by increasing hormone concentration (NAA) occurs decreasing the dried weight of root, but in (NAA + BA & BA) treatments till concentration of 1.5 mg/l and in concentration of 2.5 mg/l was seen increasing and decreasing, respectively. They had a significant difference in the statistical level of 0.01. The difference of the dried weight of root among the control and all treatment except (NAA + BA) treatment in 1.5 mg/l and (BA) treatment in 0.5 mg/l has been meaningful. (Table 1, Diagram 4).

The stems length: it was seen the least amount of stem length related to (NAA + BA) 2.5 mg/l and the most amount related to (NAA) 0.5 mg/l. in general, by increasing treatment concentration, it has been seen to increase in stem length, so compound treatment of (NAA + BA) in all concentrations related to this treatment (0.5 – 1.5 – 2.5 mg/l) than the other treatments had shorter size. They had significant difference in the statistical level of 0.01 and the difference of stem length among control and all treatments except (NAA + BA) treatment has been meaningful in 0.5 mg/l. (Table 1, Diagram 5)

The root length: the least amount of the root length related to (NAA) treatment was 0.5 mg/l and the most amounts related to (BA) was 2.5 mg/l. in general, by increasing treatment concentration, it has been seen increasing trend by increasing the concentration of treatments. They had significant difference in the statistical level of 0.01 and the difference of root stem among control and all treatments except (BA) treatment with 1.5 mg/l and in (NAA + BA) treatment with 0.5 mg/l were meaningful. (Table 1 and Diagram 6)

The amount of chlorophyll (A): by increasing different concentration of hormone of NAA, the amount of chlorophyll A was increased in NAA treatment and in BA treatment increased to 1.5 mg/l concentration and then decreased in concentration of 2.5 mg/l and in the compound treatment of both hormones by increasing hormones concentration, the chlorophyll amount A decreased and it was shown to increase in chlorophyll amount A in the concentration of 2.5 mg in liter. So, they has been dedicated the least amount of chlorophyll in control sample and the most amount in NAA treatment of 2.5 mg/l. they had a significant difference in the statistical level and there has been a significant difference in the difference of Chlorophyll level A among control and all treatment levels. (Table 1 and Diagram 3)

The amount of chlorophyll (B): by increasing the concentration of used hormones, the concentration of existing chlorophyll increased in leaves, so the BA treatment with 2.5 mg/l has dedicated the least amount of chlorophyll B and (NAA + BA) treatment with 2.5 mg/l has had the most amount of chlorophyll. They had significant difference in statistical level of 0.01and difference was meaningful in the chlorophyll levels B among control with all treatments level except (0.5 BA mg/l) and 0.5 mg/l) and (NAA 0.5 and 1.0 mg/l). (Table 1 and Diagram 4)

The total amount of amount: the least amount of total chlorophyll was related to control treatment and the most total of chlorophyll was related to NAA 2.5 mg/l. by increasing the concentration of hormones, the total amount of chlorophyll has been also increased. They had significant difference n statistical level of 0.01 and the difference of chlorophyll level of all control samples with all treatments has been meaningful. (Table 1, Diagram 2)

The amount of Carotenoid: by increasing the concentration of hormones, the amount of carotenoid was also increased, so the most amount was related to (BA) treatment with 2.5 mg/l and the least amount related to (NAA) treatment with mg/l and they had significant difference in statistical level of 0.01and the difference of carotenoid level in control sample with all treatments except NAA and BA and (NAA + BA) with mg/l has been meaningful. (Diagram 5)

The amount of carbohydrate: by increasing the concentration of NAA hormone and compound hormone (NAA + BA), first increasing glucose concentration to treatment 1.5 mg/l and then decreasing glucose concentration was seen and in treatment with BA hormone, by increasing concentration of hormone, first it was seen decreasing and then increasing glucose concentration. The most carbohydrate amount was related to

(NAA) 1.5 mg/l and the least amount of carbohydrate was related to (NAA + BA) 0.5 mg/l, and the difference in the amount of carbohydrate has been meaningful among control and all treatments. (Table 1 and Diagram 1)

The amount of protein: by increasing in the concentration of BA and NAA hormones, it was seen to increase in concentration of protein, but by increasing the concentration of compound hormones, it was seen to increase the concentration of protein. The most amount of protein was related to (BA) treatment with 2.5 mg/l and the least amount of protein was related to the control treatment. And there was no any meaningful difference in statistical level of 0.01. There has statistically been a significant

difference among control and all level of treatments used except (NAA + BA) with 2.5 mg/l (Table 1 and Diagram 2).

Table 2: Analyzing variance of hormones effect (NAA), (BA) and (NAA + BA) on soybean's Physiological parameters (n.s: it is meaningless, there is no difference. *: It is meaningful in the level of 1%. **: It is meaningful in the level of 5 %.)

Source of variations (SOV)	Degree of Free (df)	Mean square					
		Chlorophyll (A)	Chlorophyll (B)	Chlorophyll (T)	carotenoid	protein	carbohydrat
total	29	13.10**	205.2**	32.41**	11.24**	1.66n.s	279.73**
treatment	9						
repeat	2						
Test error	20	19.76	7.15	9.022	6.13	0.231	2.41
Coefficient of variations		15.54	15.81	13.30	32.23	10.12	89.94

Discussion

By increasing the amount of NAA hormone concentration, the amount of wet weight of stem was increased and also by increasing the amount of BA hormone concentration, the amount of wet weight of stem was increased, so the amount of this increasing was more than the case of treating with NAA. By applying compound of both hormones, in case of increasing it was seen to decrease and then increase in the amount of wet weight of stem. The amount of wet weight of root has been decreased by increasing NAA hormone concentration and by increasing BA hormone to 1.5 mg/l has had increasing and then decreasing and in compound treatments of both hormones it has been seen to increase in wet weight of root by adding concentration. By using NAA and BA hormones and by increasing hormones concentration, the dried weight of stem also has increased and in the case of using compound hormones of both of them, it has been seen the dried weight of stem. By increasing NAA hormone concentration it was seen to decrease in the dried weight of root and about treatment with BA hormone first it was seen to increase in dried weight and then decrease and by simultaneously using both hormones, first it was seen to increase and then decrease. By applying treatments NAA and BA and NAA+BA, it has been seen to decrease the stem length by increasing concentration. Increasing the amount of NAA and BA and NAA+BA, it was seen to increase in the length of root. It was implemented several research about this parameter on different plants that as an example it can be pointed to a research in rapidly sinking in 30 mm concentration of nitrogen along with 500 mg/l NAA that has the highest amount of creating root (salehi Najaf Abadi 1378, planting miniature Rose into glass, Iran agricultural research, N. 15, 15-67), so in the present research, NAA with 0.5 mg/l has dedicated the highest wet weight of root and with 1.93 mg/l had the lowest amount of wet weight of stem and with 0.482 g had the least amount of dried weight of stem that indicate the opposite auxin effect in aerial and ground organs, so has been consistent with this research. (Davies – 1995) reported that Auxin is able to stimulate cell division similar to cytokinine and or in partnership with it and the high concentration of Auxin in seeds can produce more cytokinine in seeds growing and or in the other research the highest directly creating new branch lets were seen in NAA medium with 0.1 mg/l and 0.5 mg/l BAP and also creating root from separate parts was seen in NAA medium with 0.5 mg/l. Cytokinine treatment accelerates to extend additional cell without causes to increase in dried weight of treated cotyledon. By increasing the amount of BA from 0.5 to 1 mg/l, the percentage of branching will increase and then adding it to 2 mg/l will not alter branching studying the effect of micro-samples and the concentration levels of Auxin and cytokinine in de-morphology of Soybeen, SHarifi – Bageri – Vesal, 1382). Increasing the amount of cytokinine from 0.5 to 2 mg/l will increase the number of branches, but decrease the length of branchlets and wet and dried weight of aerial organs, so in this research it has been dedicated the highest amount of wet and dried weight that has been consistent with this research.

Cytokinines cause to increase food movement in leaves and their accumulation and increasing in dried weight. Since that the main weight of cells is related to cellulose existing in their cells and water, therefore it is natural that the wet weight and also the amount of dried material in 0.5 mg/l BA that its cells made sediment on its walls become

more. In concentration of 1.5 and 2.5 mg/l bA, also division cell causes to increase the number of cells and total weight of micro-samples through water absorption rather than increasing the dried material (Lesani- Mojtahedi- 1367- plant physiology of Tehran University). Accumulation of statolyts in root affected Auxin and geo tropism phenomenon are following by increasing dried weight of root (Slevers.Volkman 1976), so decreasing the wet weight of root was seen by increasing Auxin concentration to 2.5 mg/l.

Rafeekher et al (2001) showed that GA_3 and NAA increased the length of internodes and caused elongation of stem in cucumber. The highest mean number and the length of branch in treatment with 0.5 mg/l concentration BA and 0.05 mg/l NAA were obtained, in a test on miniature Rose that was implemented by Salehi Najaf Abadi, the concentration of 2.25 and 0.1 mg/l NAA and BA has been dedicated itself the highest mean number and the lengtj of branchlet (Salehi Najaf Abadi, 1378, planting miniature Rose into glass, Iran agricultural research, N. 15 – 15 -67). Surveying the effect of different concentrations of cytokinine hormone in micro proliferation of zinnia flower in 1388 showed that the most number of leaves was in BA hormone with concentration of 3 mm and the highest concentration in increasing the leaf length was BA hormone with concentration of 2 mm, and the most increasing stem length was related to BA hormone with concentration of 1 mm (Surveying the effect of concentrations and different kinds of cytokinine hormone on micro proliferation of zinnia flower, Mahmoodzade – Abbasi, 1388). Cytokinines can expand cell in some tissues and organs. This effect is clearly seen in bi- cotyledons with wide leaves such as mustard, cucumber and sunflower. Cotyledons of these species grow due to expanding cell during growth of seedling (Ryle et al, 1982). Kinetin more strongly acts in growing stem and root length and BA has had stronger effect on branching (Babaei, A – R – 1381, surveying proliferating two kinds of Antrium flower by using tissue culture system, Gilan University). Cytokinines like as Auxin does not increase formability by acidifying cell wall (Rulan and Ryle, 1982). BAP as a cytokinine more actively acts in branching (Goerge – 1993). Using 2 mm BAP in MS medium causes to create branch and increase its elongation in Zelegans (Lakshni- Sita G 1993). Auxin is usually known as main responsible of increasing cell length and consequently elongating aerial parts of plant, but in real hormone balance obtained is effective in cell responding, so when the ratio of Auxine is more than cytokinine, regeneration process is following by creating branchlet and in the opposite case causes to increase in organs length and to produce root (Banki – T. J & A.A Boe 1975).

In the high concentration of BA, the length of branch let is less than the low concentration (Bertrand.A.m 1999). Simultaneously using Auxin and cytokinine cause to create lateral buds and branches and remove the apical dominance and cause to shorten the plant. Auxines increase the growth of stems and stems sheath, so Auxin will increase expandability of cell walls by withdrawing Protom and acidifying walls. (Jacobs & Ray, 1976) Auxin suppresses the growth of lateral bud in the bean plant and is following by increasing stem length (Wilkins).

Auxin is necessary to begin cell proliferation in environmental circle in order to creating root and growing root (Celenza.et.at. 1995)

Protein: in the soybean, in the case of applying hormone treatment, the level of leaf protein has been increased by increasing NAA concentration. Auxin cause severe cell elongation that are placed under the apical (materials and regulators of plant growth, translated by Ghodratolah Fathi – Behrooz Ismaeil poor). Cytokinines along with Auxin cause cell division in erial organ and root and regulating cell cycle, so the low concentration of cytokinine and the high concentration of Auxin cause to create root that in this research (NAA+BA) 2.5 mg/l has been dedicated the least amount of stem length that is 30 cm among treatments, so are completely consistent with this case.

Growing root will occur in the low concentration of cytokinine that in this research, BA with 2.5 mg/l has been dedicated itself the highest stem length that is, 11.8 cm, so it is seen unconformity in this case. In general, elongation of cell will occurs only by presenting Auxin. Auxin has inhibitory effect on this step of growing, for example some amount concentration of Auxin that causes elongation stem, to decelerate elongation of root. Therefore in this research concentration of 0.5 mg/l Auxin has been dedicated the highest amount of stem length that is, 72.33 and in his concentration, the root length was 6.133 that was completely consistent.

Also, by increasing BA concentration, the leaf protein level will increase, too, so this increasing was more than increasing with NAA treatment, but by simultaneously applying both hormones, the level of protein will decrease. Studies shows that the proper concentration of elements especially elements that have important role in making protein cause to alter stored protein (Randall-P.J.A. Thomps-n.j.A 1979). Auxin is effective in biosynthesis some enzymes such as cellulose - peroxisase. Auxin interferes in the step of copying and in this step it activates RNA polymerase enzyme to make DNA from RNA, in real Auxin does not interfere in

biosynthesis DNA, but affects on the step of copying. In general, Auxin causes to copy RNA from DNA and consequently to increase to synthesis protein. Cytokinines are effective on tn the general metabolism of plant especially enzymes – coenzymes and in appearing intra-organs of cell and transferring for synthesizing RNA and DNA and protein and generally for whole parts of plant. Cytokinines can regulate synthesis of protein: there are good evidences that show cytokinines play important role in synthesizing protein, when cultured soybean cells treated with cytokinine (Zeatin), during 15 minutes, increasing in the content of cell polyribosome. This increasing occurs due to movement of single free ribosome (monosomes)of polyribosome. (Tapfer and Fosket, 1978). Since polyribosomes contain such ribosomes that actively make protein, the results show that cytokinines cause to synthesis total protein in these cells (Tapfer and Fosket, 1978). Cytokinines not only are able to increase the speed of synthesizing protein, but also alter the spectra of made protein by plant tissues, this case proves by marking proteins of culture soybean cell with mytonin { S^{35} } and separating them by electrophorus of polyAcryl Amid gel. Gel auto-radiograms showed that treatment with cytokinines will increase to synthesis some proteins, so inhibits to synthesis the other protein (Fosketo and Tapfer, 1978). The tissues of treated to baccowith cytokinine indicates to alter synthesizing protein paradigm that shows hormones are necessary for synthesizing some proteins and inhibiting to synthesis the other protein (Aik Holes et al, 1983). Cytokinines of tRNA can play important role in synthesizing protein, tRNA that contain these compounds, cytokinines occupy sensitive location near to Anti Codones that may influence in tRNA to mRNM (Hal, 1970). In general, cytokinines has sensible effect on the speed of protein making and any kind of made protein by plant cells, especially cytokinines stimulate to synthesis certain chloroplast protein that code by nuclear genes and make by cytoplasm ribosome. Based on existing evidences and results obtained from different research, cytokinines have a significant role in synthesizing proteins that will increase by adding concentration of this hormone. In this research the highest protein was seen in BA with 2.5 mg/l and the lowest amount in the Control sample that are completely consistent with the results of other researches.

Carbohydrate: by increasing the amount of NAA concentration to 1.5 mg/l, the amount of leaf carbohydrate increased and in the case of applying hormones with BA, increasing the amount of hormone concentration has decreased the amount of carbohydrate, by using simultaneously both hormones also was seen to increase up to 1.5 mg/l concentration and then decrease. Sugars cause to regulate osmosis pressure and also stability in membranes and existing proteins in cell, this action can occur by forming hydrogen bonds between carboxylic groups of sugar and polar chains of proteins and at last stabilizing proteins (Koster.K.L-Leopold A.C 1988). Whatever the process of photosynthesis is done with more speed in plants, primarily metabolites including proteins and carbohydrates increase in the same proportion. Cytokinines existing in the wooden vena often are nucleotides, as soon as nucleotides reach leaves, may alter to free base or glycosides. Glycosides of cytokinine accumulate in large amount in leaves and there are even existing considerable amounts of them in old leaves. Cultured Cotyledons of radish in medium containing cytokinine with Banzyl Adenin base of cultured cotyledon easily destroy hormone and alter it to different glycosides, BAP, Ribonocloizides BAP, and Ribonocloides BAP. When cotyledons reentered to medium lack of cytokinine, the speed of growth, BAP concentration, ribonocloizid and Ribonocloitid BAP decrease in tissues and in this case the amount of glycoside BAP remains constant. Generally, cytokinines accelerate to get chloroplast, so the result of this action is to increase photosynthesis process and then more providing the photosynthesis production including sugars that in this research the highest amount of protein was seen in BA treatment with 2.5 mg/l that was consistent with this principle.

Photosynthesis pigments: By increasing the amount of NAA hormone concentration, the amount of chlorophyll has been increased and by treating with BA hormone and increasing the concentration of hormone to border of 1.5 mg in liter, it was shown first to increase in the amount of chlorophyll and then decrease in the amount of chlorophyll. It was shown to decrease in the amount of chlorophyll with increasing the hormone concentration to the border of 1.5 mg/l and with concentration of 2.5 mg/l to increase by applying compound of both hormones. In related to carotenoid, it has been seen to increase in the amount of carotenoid in leaves by increasing concentration of BA and the by increasing VAA hormone concentration first it was seen to decrease and then to increase and by applying compound of two hormones. It was seen to increase in the amount of hormones concentrations. Cytokinines causes elongation of cells in leaves and cotyledons that this case itself causes to increase in wet and dried weight and to increaseinthe amount of photosynthesis micro pigment including chlorophyll (Koochaki–GholamhossainSarmadnia). Grow thre gulators, especially cytokinines are responsible of keeping

pigments of petal and leaf, Jibrolines also interfere in biosynthesis carotenoids and Antosyanines and delaying chlorophyll decomposition. (Mutui et al, 2001) researchon chrysanthemum morifolium showed that Banzyl Adenin caused to keep the color of petals. (Petridoe et. al 2003) In the old leaves the ratio of chlorophyll a up to b will increase due to decomposition of chlorophyll b and its alteration to chlorophyll a that indicates the activity of pre-Enzymes of the path of breaking chlorophyll. (Harpaz – saod.al 2007) cytokinines affected on many growth aspects of regulating with light includes differentiation of chloroplast and simultaneously with it making chlorophyll- growth and self culture metabolism- enlarging cotyledon of leaf. (Tyze Zyger) Tobin et al studied on the role of cytokinines in chloroplast maturity of humpty blue lenticels. In this research, the most amount of carotenoid pigments was seen in BA treatment with 2.5 mg/l equals with 9.258 mg/l that were consistent with this research, but about chlorophyll pigments, the most amount is seen in NAA with 2.5 mg/l equals with 29.828 and the least amount in BA with 2.5 mg/l.

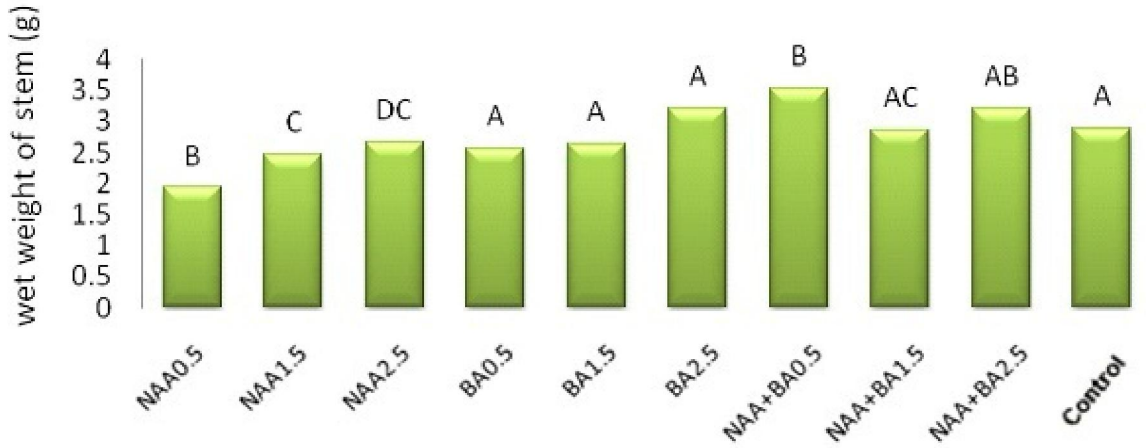

Diagram 1: The effect of difference treatments (NAA; BA; NAA+BA) on the wet weight of stem (g)

Diagram 2: The effect of difference treatments (NAA; BA; NAA+BA) on the wet weight of root (g)

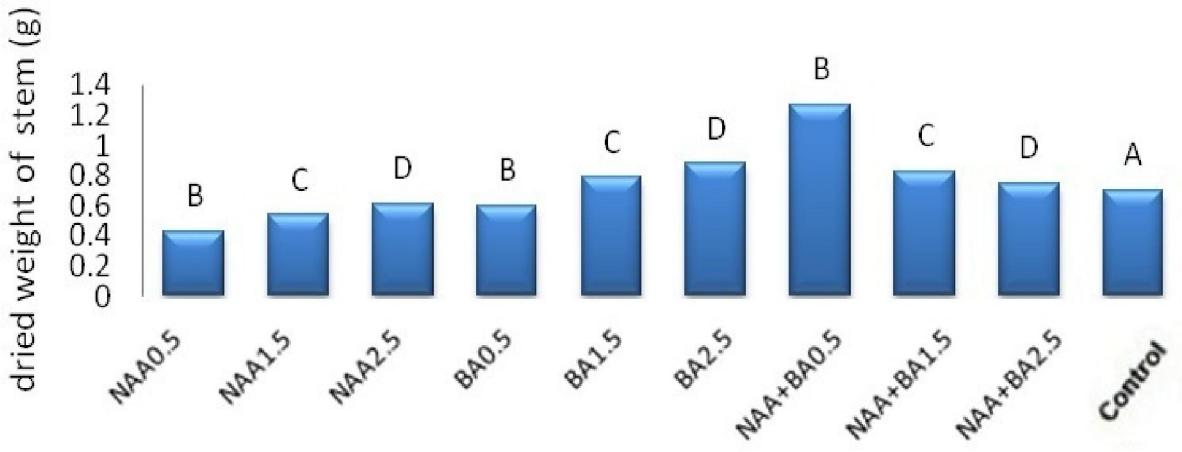

Diagram 3: The effect of difference treatments (NAA; BA; NAA+BA) on the dried weight of stem (g)

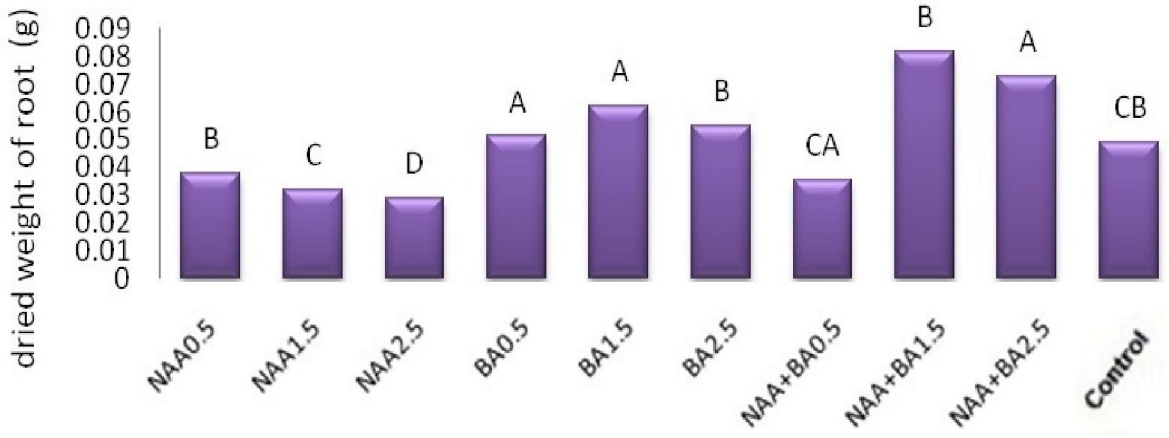

Diagram 4: The effect of difference treatments (NAA; BA; NAA+BA) on the dried weight of root (g)

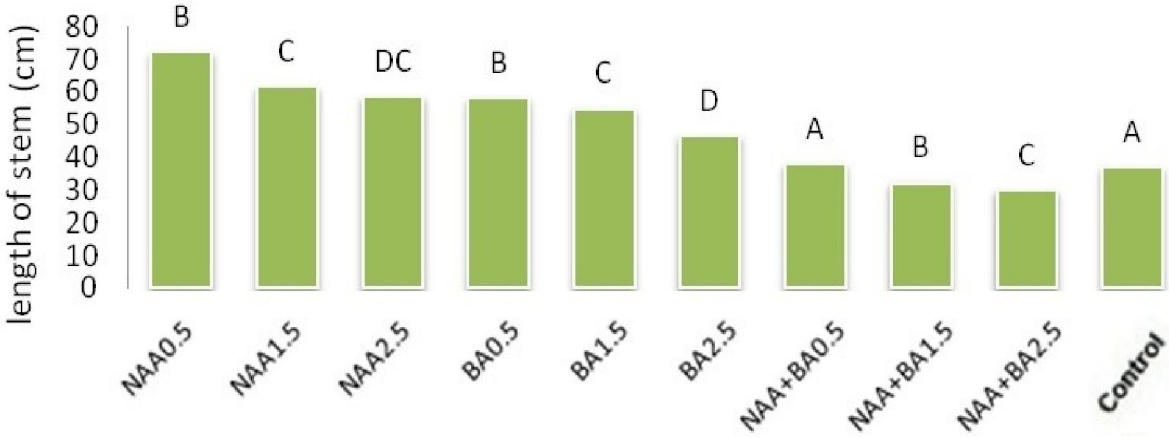

Diagram 5: The effect of difference treatments (NAA; BA; NAA+BA) on the length of stem (cm)

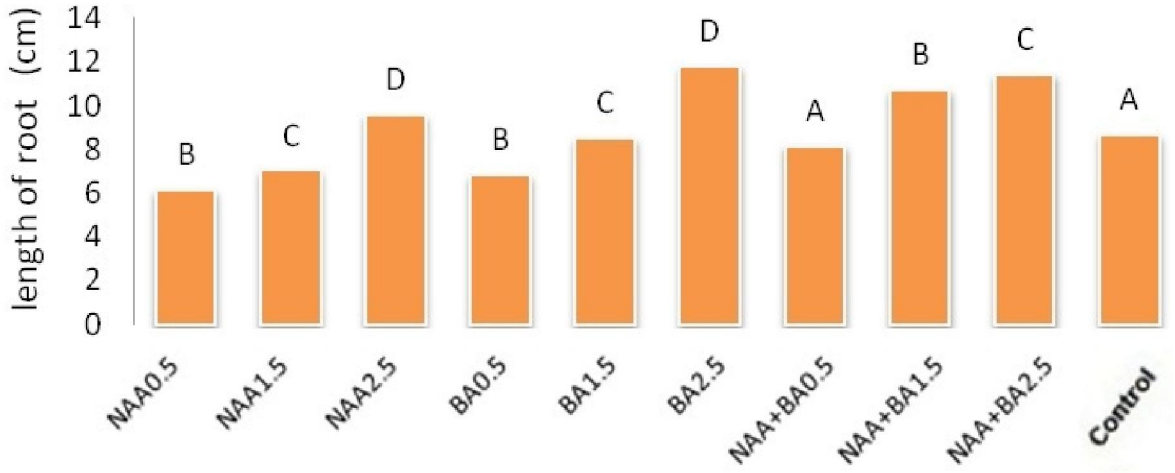

Diagram 6: The effect of difference treatments (NAA; BA; NAA+BA) on the length of root (cm)

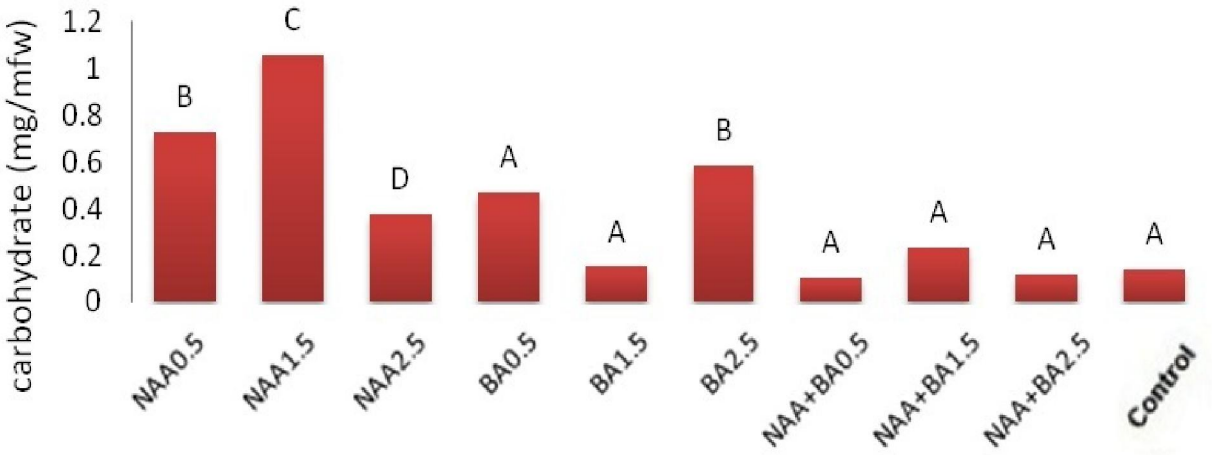

Diagram 7: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of carbohydrate (mg/mfw)

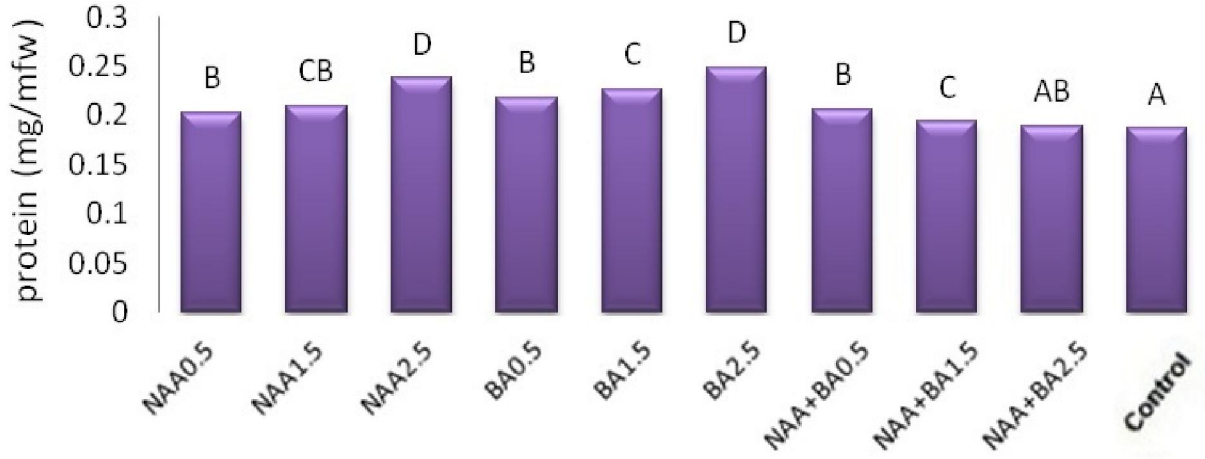

Diagram 8: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of protein (mg/mfw)

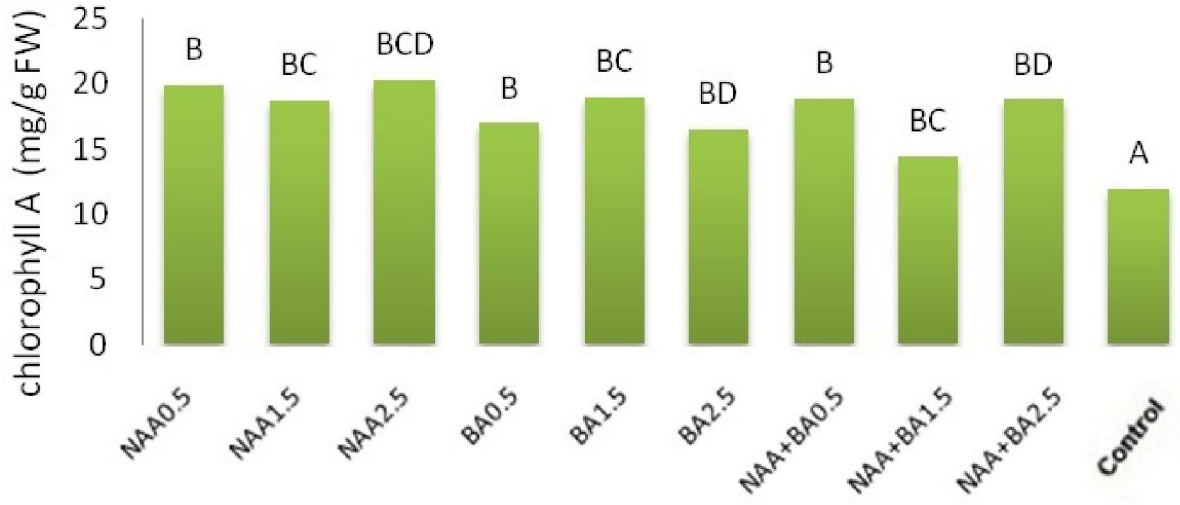

Diagram 9: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of chlorophyll A (mg/g FW)

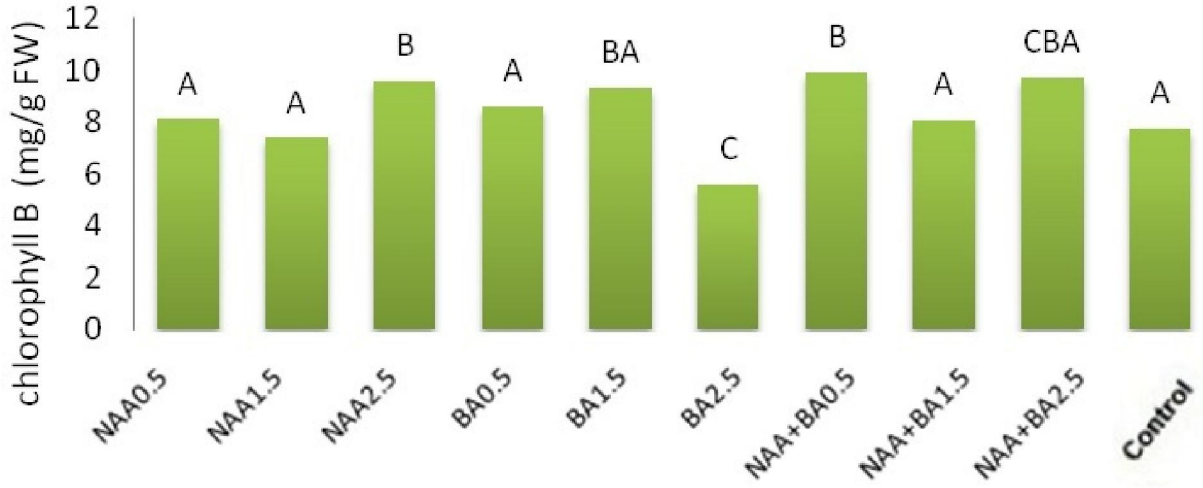

Diagram 10: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of chlorophyll B (mg/g FW)

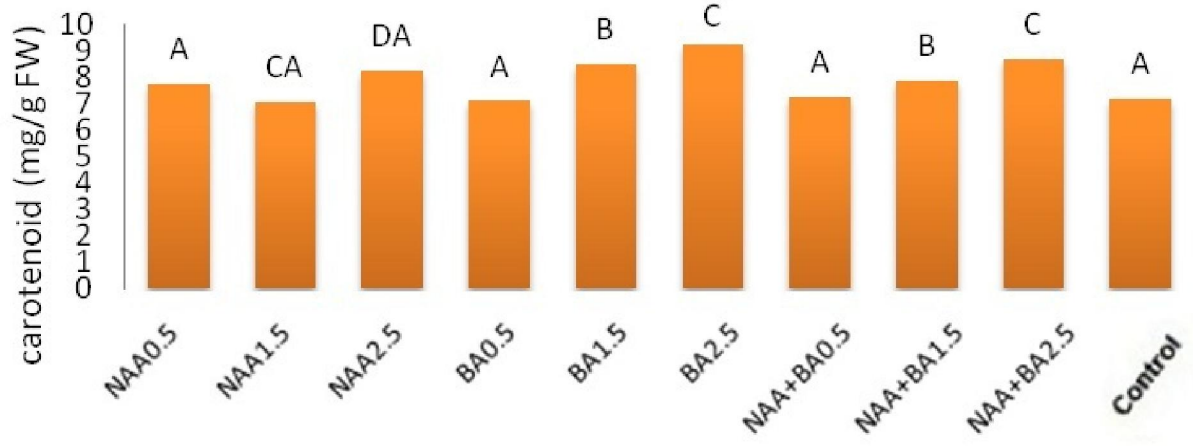

Diagram 11: The effect of difference treatments (NAA; BA; NAA+BA) on the amount of carotenoid (mg/g FW)

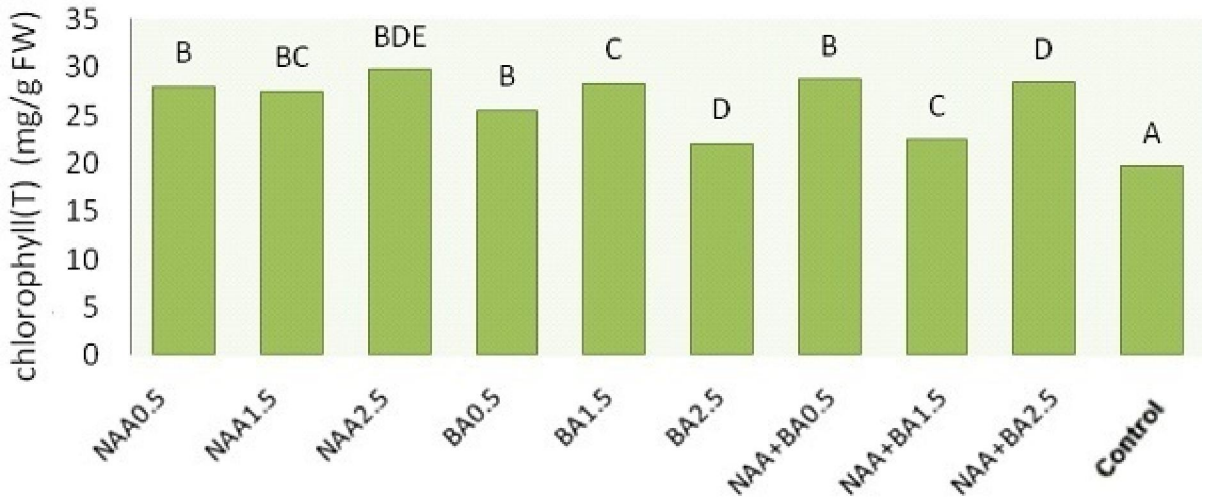

Diagram 12: The effect of difference treatments (NAA; BA; NAA+BA) on the total amount of chlorophyll (mg/g FW)

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