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# EFFECT OF SPRAYING WITH TRYPTOPHAN AND GLYCINE ON PROTEOTYPE ESTIMATION OF KUMQUAT AND LOCAL LEMON SEEDLINGS

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## ABSTRACT

This study was conducted in the cloth canopy of the College of Agriculture, University of Basrah, during the growing season 2021-2022 on seedlings of kumquat and local lemon, totaling 54, 27 seedlings for each plant type, in three replications, as the seedlings were sprayed six times with three concentrations (0, 50, 100) mg l-1 of the amino acids tryptophan and glycine. The most important results of the study were the appearance of between (3-8) protein bundles depending on the type and conditions of treatment. The number of protein bundles differed between the comparison treatments for the two types of kumquat and lemon seedlings, as the comparison treatment of lemon seedlings showed 7 protein bundles, while 8 protein bundles appeared in the treatment; in comparison to kumquat seedlings, there were 6 protein bundles in the G50 treatment for both types. The treatment with tryptophan at a concentration of 100 mg l-1 caused some protein bundles to disappear, and only three protein bundles remained in the kumquat leaves for treatment T100 and G50.

**Keywords**: protein pattern; protein bundles; kumquat; lemon.

## INTRODUCTION

Citrus is one of the perennial fruit trees belonging to the Rutaceae family and includes a number of species, the most important of which is Citrus, and the species belonging to it are widely spread worldwide due to their adaptation to a wide range of environmental conditions. It includes many species with wide varieties and strains (Abbas et al. 2016; Ismail and Zhang 2004).

Citrus Limon L. belongs to the genus Citrus, which belongs to the Rutaceae family. It is its original home in northeastern India and southwestern China (Al-Khafaji et al., 1990). Lemon fruits are used for fresh consumption, making juices and flavorings for many foods, and their practical effect in treating many diseases ( Forte et al., 2011). The local variety is one of the most desirable varieties in Iraq because its fruits are juicy, small in size, and have thin skin, and the acidity percentage is lower than the rest of the international varieties (Al-Khafaji et al., 1990).

Kumquat, whose scientific name is Citrus japonica L. is considered one of the citrus fruits and belongs to the Rutaceae family. It is one of the species of the genus Fortunella, which includes many species and regions of India and East Asia, the home of its

cultivation, and then its cultivation spread to all parts of the world. It is commonly used because of the vitamins and fibers it contains (Hasan et al., 2016).

Amino acids are a source of important organic nitrogenous compounds for building proteins in ribosomes (Davies, 1982), and among the important amino acids is tryptophan, which is one of the structural units and important building blocks of enzymes and proteins and the main factor for building auxins (IAA) in some plant tissues. The amino tryptophan instead of the direct use of auxin to improve the growth and productivity of crops (Ahmad et al., 1999) and glycin, which activates the formation of chlorophyll (Hendry and Stobart 1977) and prevents photorespiration (Taiz and Zeiger, 2002) and has an essential role in protecting the plant from environmental conditions. Different stresses (salinity, heat and drought) (Ashraf and Harris 2004).

The electrophoresis technique (SDS-PAGE) is used to estimate the protein pattern, an important and effective method for studying gene expression during the different stages of plant growth. An increase or decrease in protein bundles may occur due to exposure to the surrounding environmental conditions.

The current study aims to study the effect of foliar spraying with the amino acid tryptophan and the amino acid glycine on proteotype estimation of leaves of kumquat and local lemon seedlings.

## MATERIALS AND METHODS

This study was conducted on seedlings of kumquat and local lemon during the growing season 2021-2022, which amounted to (54) seedlings for each plant type (27) seedlings, two years old, with three replications, planted in small plastic bags, and then transferred to plastic pots with a capacity of (5) kg after mixing them with sandy soil, the mixture before starting the experiment. The seedlings were placed in the cloth canopy. After mixing them well, samples were taken from the soil where the seedlings grew. The soil was analysed to find out the physical and chemical characteristics. The seedlings were sprayed six times with three concentrations (0, 50, 100) mg l-1 of the amino acid tryptophan and its formula. The molecular formula is C11H12N2O2, and its molecular weight is 23.204 g mol-1 (Al-Dawoody, 1990; Taiz and Zeiger 2002), and three concentrations (0, 50, 100) mg l-1 of the amino acid glycine, its molecular weight is 75.05 g mol-1, its molecular formula is C2H5NO2 (Ashraf and Harris 2004; Plimmer 1912). The experimental treatments were as follows:

Table (1) the meanings of symbols

| | |
|---|---|
| T0 G0 | Control |
| T0 G50 | Tryptophan 0 + Glycine 50 mg $l^{-1}$ |
| T0 G100 | Tryptophan 0 + Glycine 100 mg $l^{-1}$ |
| T50 G0 | Tryptophan 50 mg $l^{-1}$ + Glycine |
| T50 G50 | Tryptophan 50 mg $l^{-1}$ + Glycine 50 mg $l^{-1}$ |
| T50 G100 | Tryptophan 50 mg $l^{-1}$ + Glycine 100 mg $l^{-1}$ |
| T100 G0 | Tryptophan 100 mg $l^{-1}$ + Glycine |
| T100 G50 | Tryptophan 100 mg $l^{-1}$ + Glycine 50 mg $l^{-1}$ |
| T100 G100 | Tryptophan 100 mg $l^{-1}$ + Glycine 100 mg $l^{-1}$ |



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Table (2) some physical and chemical properties of the seedling's soil

| Analysis name | rate | unit |
|---|---|---|
| Ec | 5.54 | ds/m |
| PH | 8.13 | PPm |
| Na | 200 | PPm |
| K | 7.25 | PPm |
| N | 233.4 | PPm |
| P | 1.62 | PPm |
| soil texture | | |
| sand | %78 | g kg |
| clay | %10 | g kg |
| silt | %12 | g kg |
| texture | Sandy loam | |

Proteotype estimation method:

Samples of leaves of kumquat and local lemon seedlings were prepared to estimate the protein pattern after spraying with the amino acids tryptophan and glycine. The size, length, and area of the protein bundles were measured in the following method:

1. Freeze samples

The leaf samples were dried using the Freeze Dryer (Lyophilization Technique). The samples to be lyophilized were placed in plastic containers and then placed in the Lyophilization Device (Edwards) model (Prianiso) at a temperature of (-26 C) for a certain period so that most of them were removed. Approximately moisture, then these lyophilized samples were used for the electrophoresis of proteins on a polyacrylamide gel in the presence of SDSSodium Dodecyle Sulfate by SDS-PAGE method as described in Mistrelloet al. (2008).

2. Protein extraction

The protein was extracted from the dried leaves by taking 1 gm of it and placed in a ceramic mortar with 3 ml of (Tris-HCl-buffer (0.1M, pH7.5) solution containing (PMSF) Phenyl methane sulfonyl fluoride at a temperature of (4°C), then the centrifugation process was carried out. Centrifuge at a temperature of (4°C) and a speed of (18000) revolutions for half an hour, then take (40 microliters) of the filtrate into a relay on a Polyacrylamide gel.

3. Electrophoresis preparations

Protein migration was carried out on a polyacrylamide gel using the Slab-Electrophoresis method in the presence of SDS denaturants according to the method of Leammli (1970) and described by Bavei et al. 2011.

4. The solutions used:

4.1. Resolving gel buffer (pH=8.8)

It was prepared at a concentration of 1.5 M Hcl-Tris by dissolving 18.2 g of Tris (hydroxymethyl) methylamine in 80 ml of distilled water and adjusting the pH to 8.8 using 1 M of concentrated hydrochloric acid and completing the volume to 100 ml with distilled water.



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4.2. Stacking gel buffer (pH=6.8)

It was prepared at a concentration of 0.5 M Tris by dissolving 6 g of Tris (hydroxymethyl) methylamine in 40 ml of distilled water and adjusting the pH to 6.8 using 1 M of concentrated hydrochloric acid and completing the volume to 100 ml with distilled water.

4.2. Stacking gel buffer (pH=6.8)

It was prepared at a concentration of 0.5 M Tris by dissolving 6 g of Tris (hydroxymethyl) methylamine in 40 ml of distilled water and adjusting the pH to 6.8 using 1 M of concentrated hydrochloric acid and completing the volume to 100 ml with distilled water.

4.3. SDS solution

It was prepared by dissolving 10 g of Sodium dodecyl sulphate in a quantity of distilled water, then completing the volume to 100 ml with distilled water.

4.4. Electrode buffer (pH=8.3)

Prepared by dissolving 1.5 g of Tris (hydroxymethyl) methylamine and 7.2 g of glycine acid in a quantity of distilled water and bringing the volume to 500 ml with distilled water, adding 5 ml of 10% SDS solution.

4.5. Acryl amide stock solution

It was prepared by adding 29.2 g of acrylamide with 0.8 g of Bis-acryl amide in 60 ml of distilled water, completing the volume to 100 ml of distilled water, then filtering the solution through filter paper No. 1, then adding 4 ml of SDS10% solution to it.

4.6. Ammonium persulfate (Aps)

Prepare immediately at a concentration of 1.5% by dissolving 0.15 g of (Aps) in 10 ml of distilled water.

4.7. (TEMEDN, N, N, N-tetra methylene diamine)

It is ready to use.

4.8. Dyeing solution ((0.1%)Staining solution

Prepared by dissolving 0.25 g of Coomassie Brilliant Blue R-250 in 250 ml of a mixture composed of acetic acid: methyl alcohol: distilled water in a ratio of 4:1:5, respectively.

4.9. Destaining solution

It consists of a mixture of acetic acid: methyl alcohol: and distilled water in a ratio of 5:4:1, respectively.

4.10. Bromophenol blue dye solution. Bromophenol Blue

It was prepared by dissolving 0.25 g of bromophenol blue in a 50% glycerol solution.

4.11. Sample buffer

It consists of Tris solution with a concentration of 0.5 M, SDS with a concentration of 10%, bromophenol blue with a concentration of 0.5%, mercaptoethanol with a concentration of 0.5%, and sucrose with a concentration of 20%, which was prepared by mixing 0.5 ml of distilled water with 0.2 ml of a 10% SDS solution and 0.1 ml of a gel solution lead, 0.5 ml of 0.5% bromophenol blue dye solution, 0.2 ml sucrose, and 0.05 ml mercaptoethanol.



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5. The method of work

5.1. Sample preparation

The samples were prepared by dissolving the precipitated protein after the precipitation treatment in the sample's buffer solution, placed in a water bath for 5 minutes at a boiling point, and left to cool at the laboratory temperature to leave the sample later.

5.2. Prepare the separation gel.

Separation gel 7.5% acrylamide was prepared by mixing 14.55 ml of distilled water, 7.5 ml of alkyl amide-biz acrylamide solution, 7.5 ml of gel separation buffer and 0.3 ml of SDS solution, and 150 µl of ammonium persulfate solution and 15 µl of sodium chloride were added. Stretch, and leave to harden for an hour and a half.

5.3. Prepare the stacking gel.

The stacking gel was prepared by mixing 12.2 ml of distilled water with 5 ml of gel stacking buffer solution, 2.6 ml of acrylamide solution, 0.2 ml of SDS solution, 50 µL of ammonium persulfate solution, and 10 µL of tmid and left the gel to solidify for an hour and a half to form Ready for electric migration.

5.4. Turn on the device

The components of the separating gel were mixed and then carefully squeezed with a medical syringe between two glass plates in the Chamber repository (Cleaver Scientific) electrophoresis device of English origin, and the gel was left for a period to complete solidification, then the stacking gel was added and the comb designated for the purpose of forming pits in the gel was placed To bluish the sample and leave the gel to complete solidification, then lift the comb carefully to prevent deformation in the pits formed, then the samples are blotted with a micro syringe measuring 50 microliters, and after completing the process of bluing the samples, the reservoir is placed in the electrophoresis device and an electrode buffer solution is added to it, then the device is sealed and connected With the power supply, by means of wires attached to the device, the power supply was set to 2.5 milliamps (70 volts) in the model stacking stage and 5 milliamps (100 volts) in the separation stage, and the relay process lasted 3-4 hours.

5.5. Peel off the gel

Carefully from between the two glass plates by adding a little water with a syringe to avoid rupture of the gel, then add the dyeing solution and leave for 24 hours, then remove from the dyeing basin and add the dye removal solution to wash the gel until the bands appear. They were photographed with a British-made Gel documentation device.

Experiment design

The experiment was designed according to a randomized complete block design (R.C.B.D) as a factorial experiment with three factors (2 * 3 * 3) and three replicates for each treatment. The first factor represents the plant type (kumquat and lemon) and the second factor represents the amino acid tryptophan with three concentrations ( 0, 50, 100) mg l-1 and the third factor, the amino acid glycine, with three concentrations (0, 50, 100) mg l-1. Thus, the number of experimental units for each plant species is 27, and the total number of units for the study is 54. The statistical analysis was conducted for the experiment data, and the statistical program GenStat was used to analyze the



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variance. The Least Significant Difference (L.S.D) test was used to compare the means at a significant level of 0.05, according to what was stated in (Al-Rawi, and Khalaf Allah, 2000).

Results and Discussion

From observing the results of the protein pattern of the leaves of kumquat and lemon seedlings under study (Fig. 1- a, b, c, d, e and f) and Fig. (2), we find that there are differences between the seedlings of the two types for all study conditions, as the specifications of the protein bundles differed in terms of size, area, and height, by type and treatment.

As noticed from Figure (2) that there are differences between these seedlings in the number, locations, and specifications of the protein bundles on the polyacrylamide gel; the number of protein bundles ranged between (3-8) protein bundles depending on the type and treatment conditions, it was seven protein bundles in lemon seedlings (comparison), and eight protein bundles in kumquat seedlings (comparison) and this difference in the number of protein bundles is due to the genetic difference between the two species. Environmental aspect, as the seedlings of the two species shared the existence of two protein bundles (the first and the second) with relative molecular weights.

There were six protein bands in each of the G50 treatments for both types and the G100 treatment for lemon seedlings. It seems that the T100 treatment had a significant effect on the process of gene expression in the kumquat seedlings and caused the disappearance of some protein bands and the survival of only three protein bands for each of the T100 and G50T100 treatments, as well The conditions of the experiment (the treatments) had a clear effect in changing the locations of the protein bundles and the difference in their molecular weights, which indicates that the treatments have caused the activation of the gene expression process and the manufacture of new proteins that may have a role in improving the growth of the seedlings, and this is what the study found in the physical and chemical characteristics of the seedlings.

As for the molecular weights of the protein bundles in Figure (2), the molecular weights of the first protein bundle for all conditions were very close and clear for all treated plants, as they ranged between (75,525 - 225,000) kDa. As for the second package, the highest value of the molecular weight was recorded in the comparison treatment of lemon seedlings and the treatment of G100T100 for kumquat seedlings, which amounted to (150,000) kilodaltons each, while the lowest value of the molecular weight of the second package of kumquat seedlings was recorded in the treatment of G50T100, which amounted to (42.241) kilodaltons.

These results indicate that treating plants with the amino acid tryptophan and the amino acid glycine may lead to the synthesis of natural proteins, as well as a change in the processes of translation and transcription, which leads to the production of new proteins through the gene expression process according to the plant's need and response to the type of treatment to ensure improved plant growth (David and Nelson, 2000).

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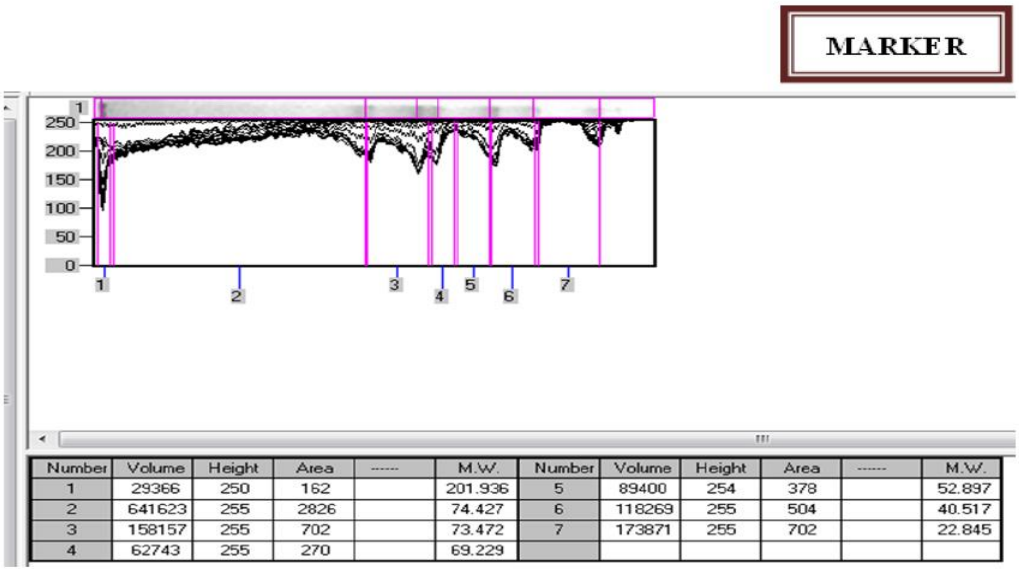
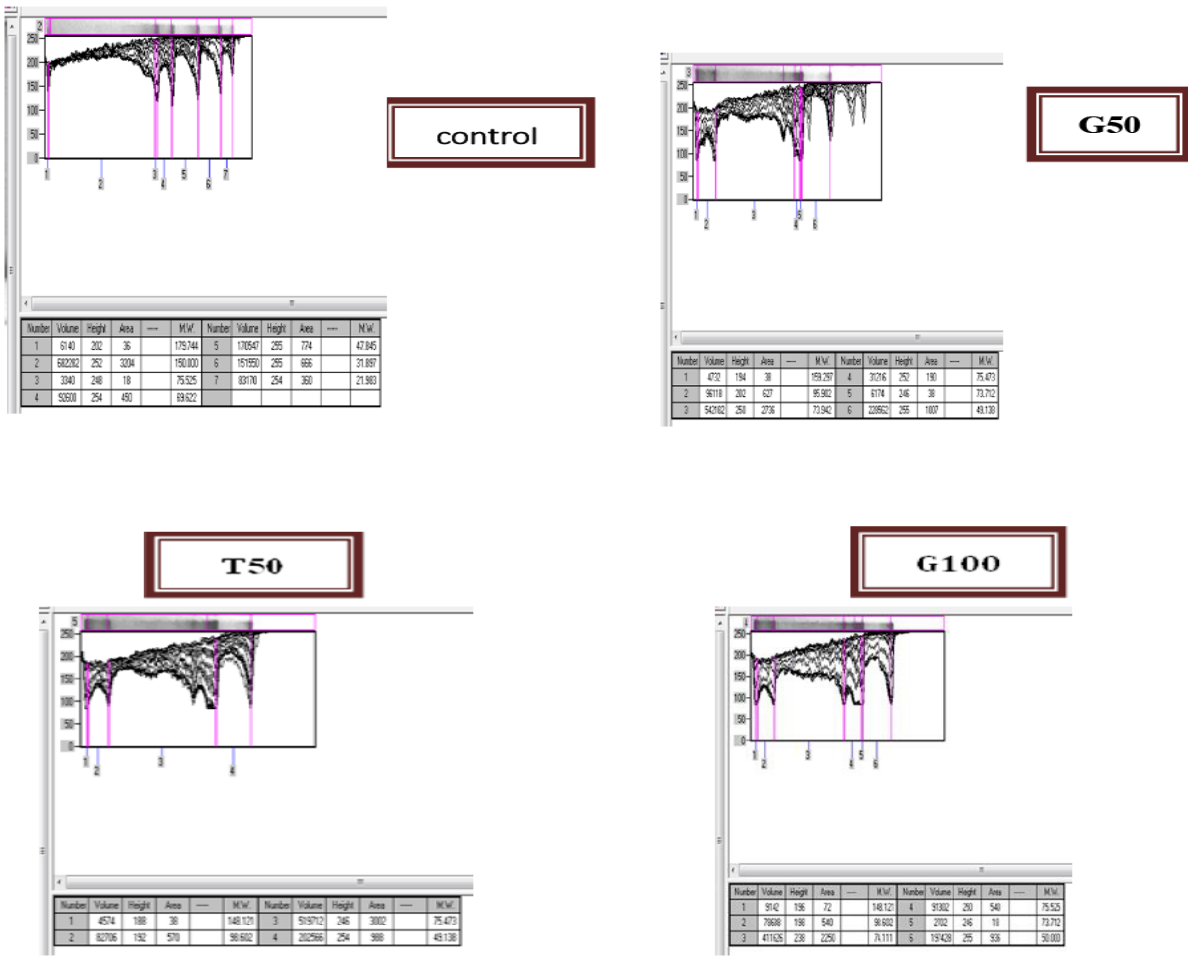



| Number | Volume | Height | Area | ------ | M.W. | Number | Volume | Height | Area | ------ | M.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 29366 | 250 | 162 | | 201.936 | 5 | 89400 | 254 | 378 | | 52.897 |
| 2 | 641623 | 255 | 2826 | | 74.427 | 6 | 118269 | 255 | 504 | | 40.517 |
| 3 | 158157 | 255 | 702 | | 73.472 | 7 | 173871 | 255 | 702 | | 22.845 |
| 4 | 62743 | 255 | 270 | | 69.229 | | | | | | |

Figure (1-a) some specifications of the protein bands on the polyacrylamide gel for the marker




(1-b) some specifications of protein bands on polyacrylamide gel for kumquat and lemon seedlings treated with amino acids



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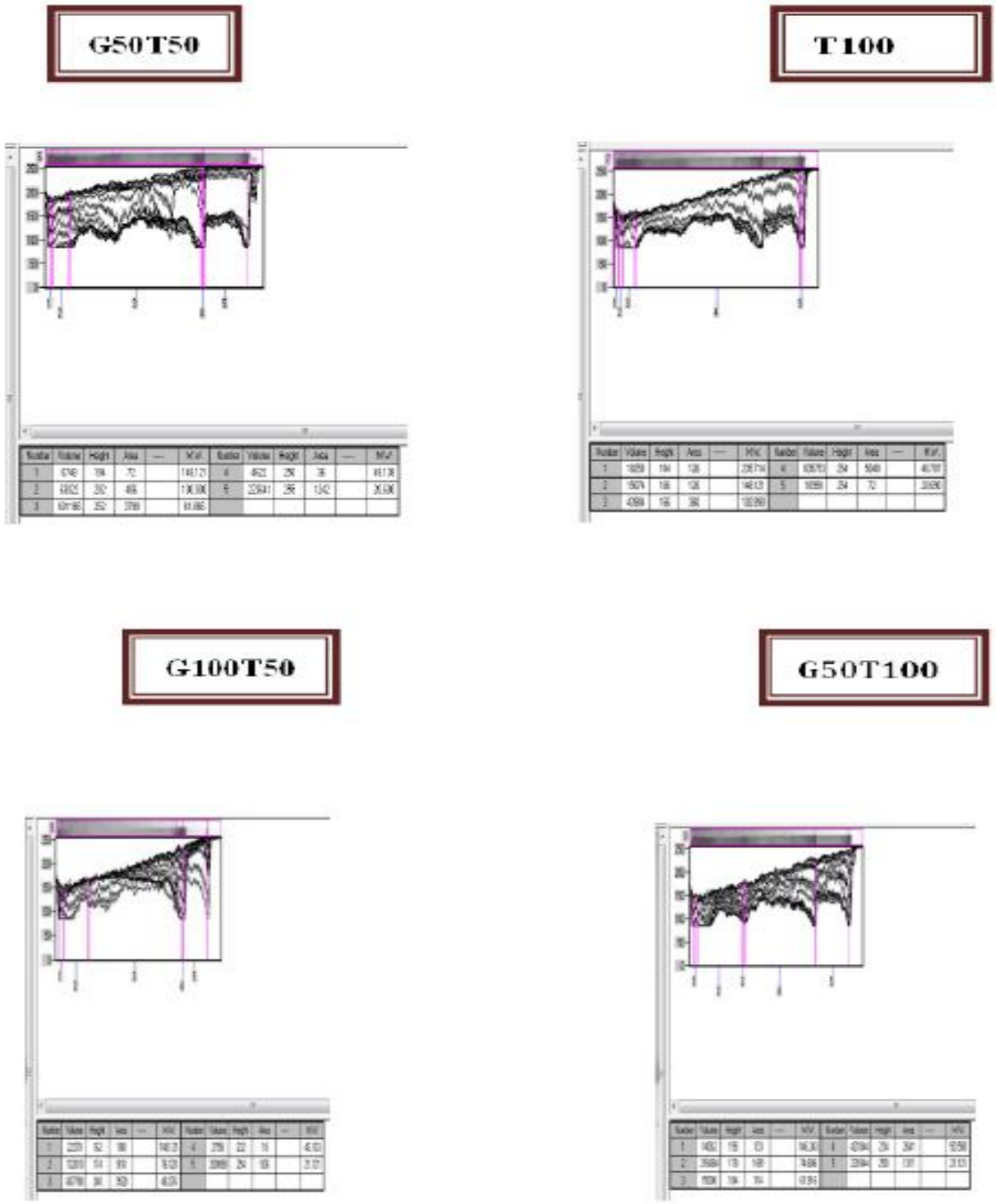


Figure (1-c) some specifications of protein bands on polyacrylamide gel for kumquat and lemon seedlings treated with amino acids



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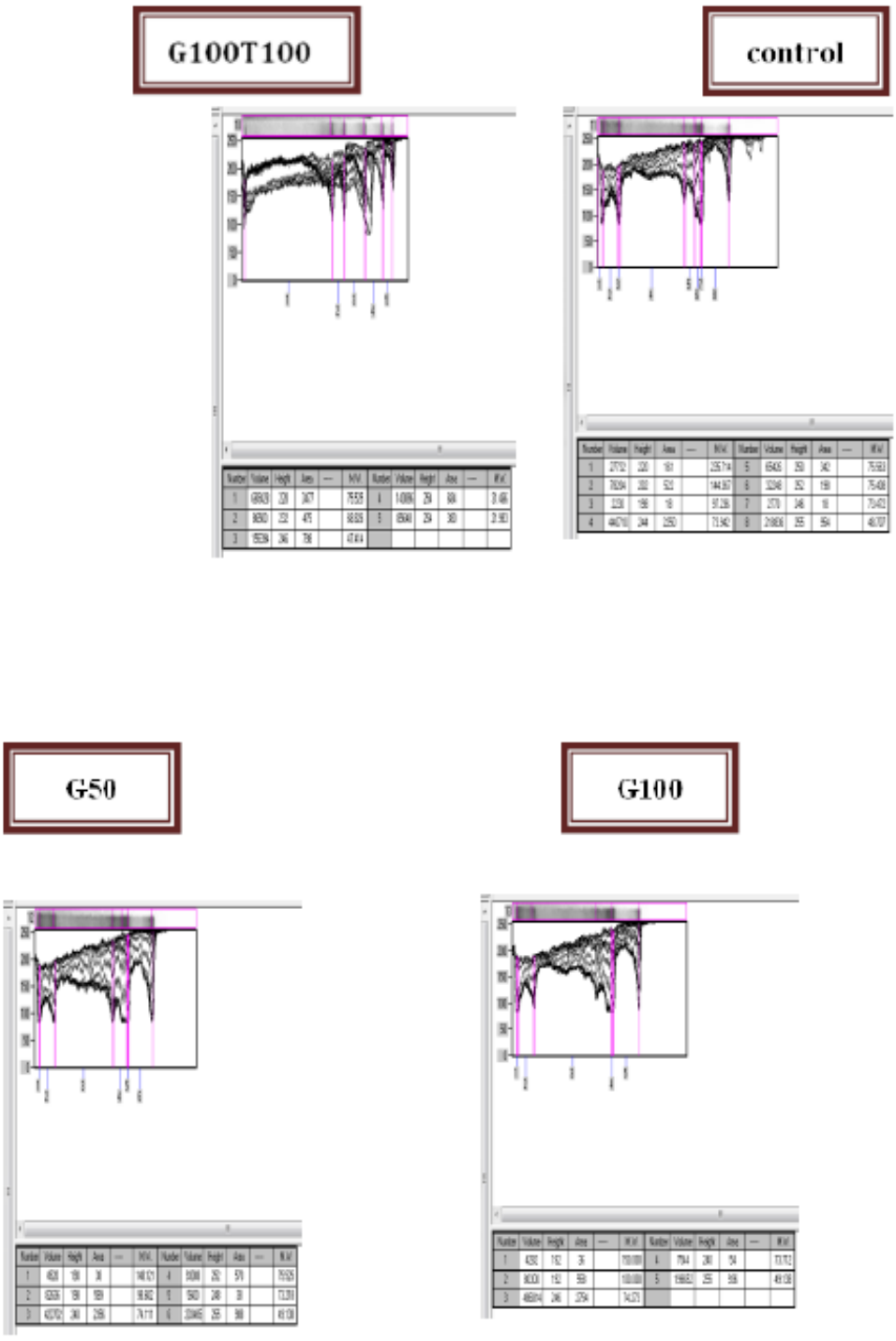


Figure (1-d) some specifications of protein bands on polyacrylamide gel for kumquat and lemon seedlings treated with amino acids.



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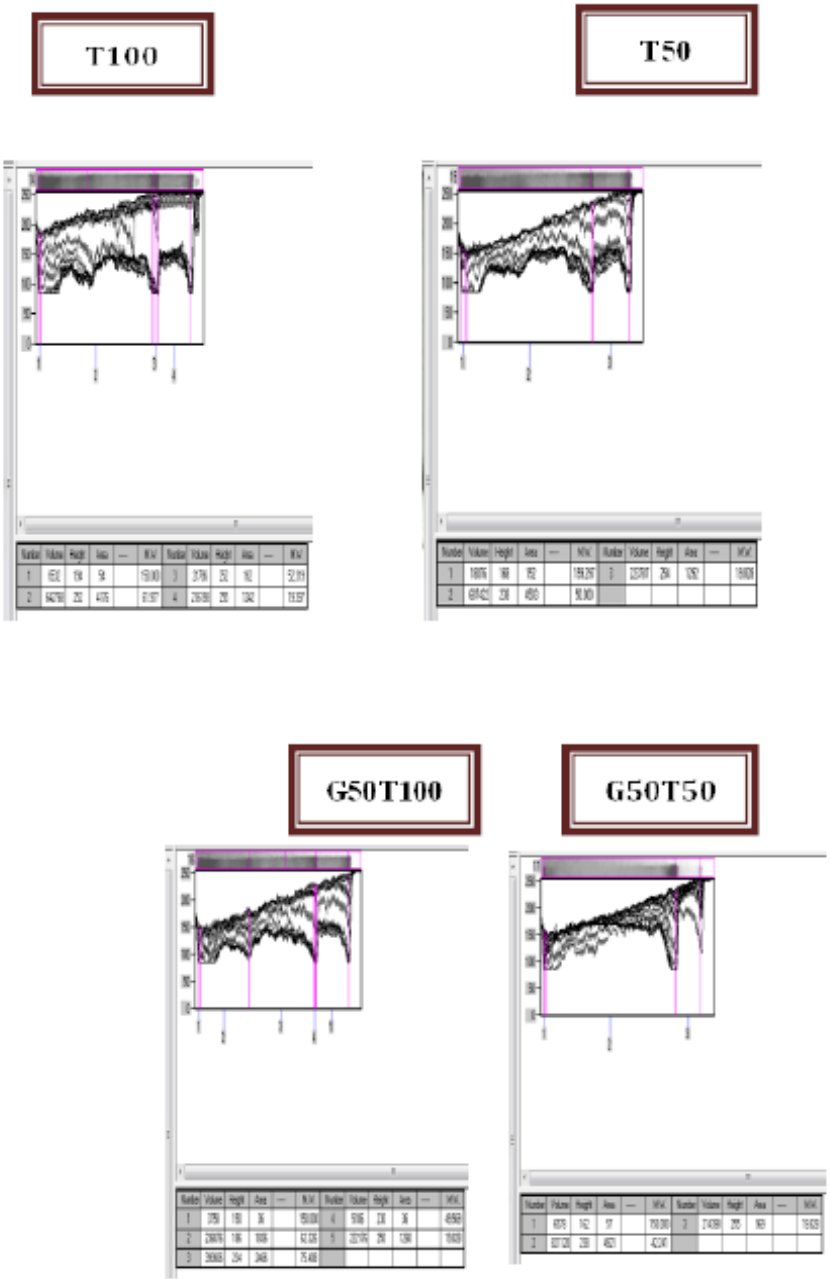


Figure (1-e) some specifications of protein bands on polyacrylamide gel for kumquat and lemon seedlings treated with amino acids

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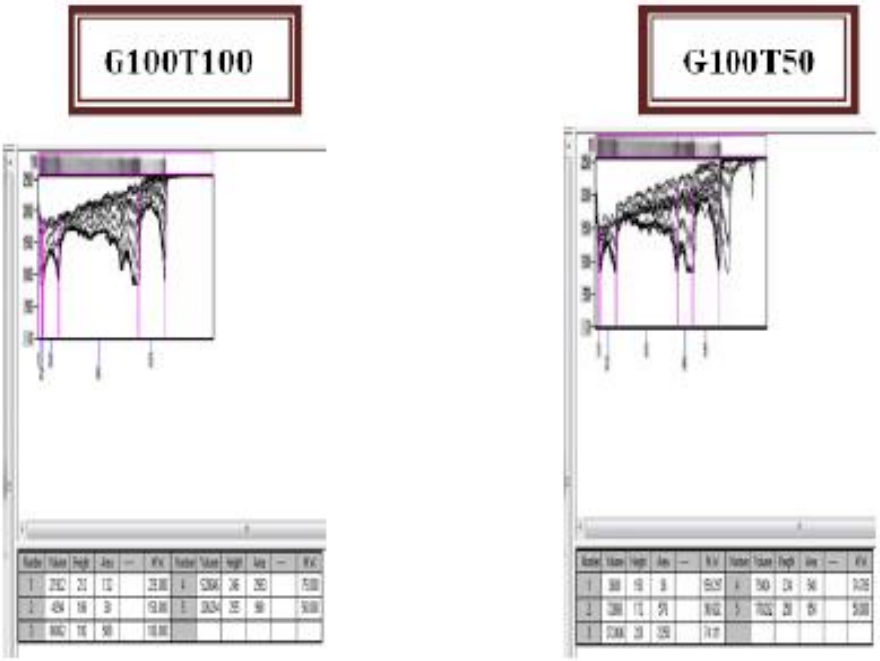
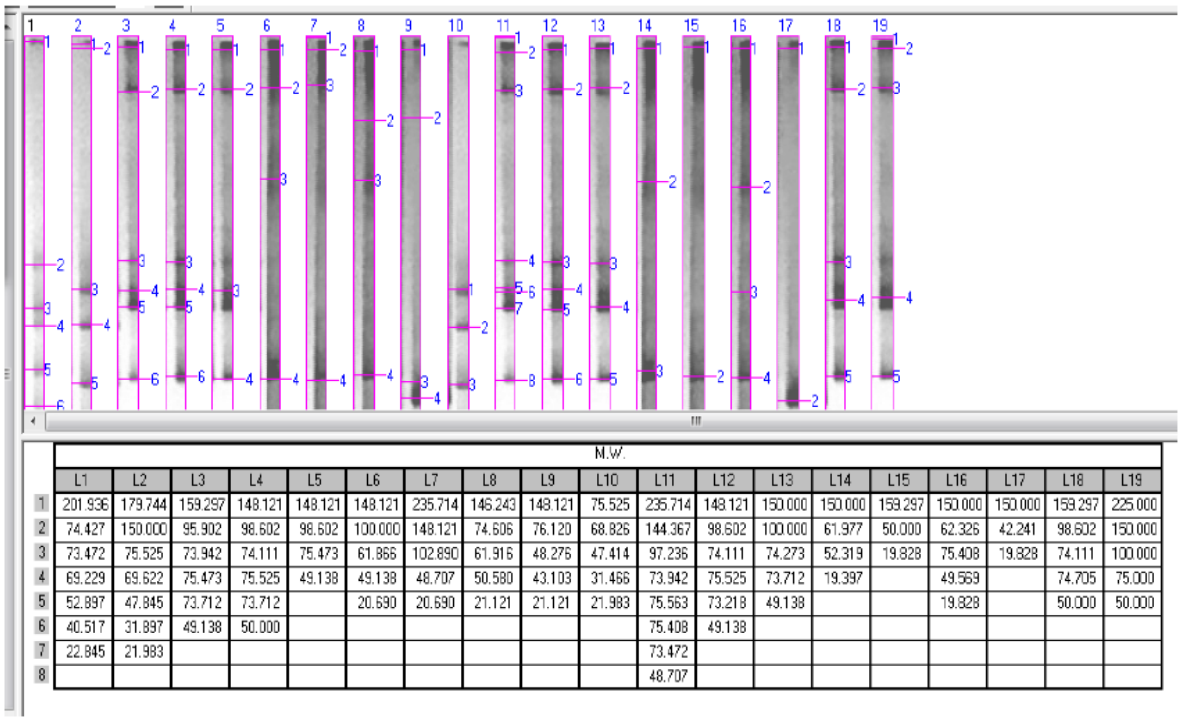



Figure (1-f) some specifications of protein bands on polyacrylamide gel for kumquat and lemon seedlings treated with amino acids



M.W.

| | L1 | L2 | L3 | L4 | L5 | L6 | L7 | L8 | L9 | L10 | L11 | L12 | L13 | L14 | L15 | L16 | L17 | L18 | L19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 201.936 | 179.744 | 159.297 | 148.121 | 148.121 | 148.121 | 235.714 | 146.243 | 148.121 | 75.525 | 235.714 | 148.121 | 150.000 | 150.000 | 159.297 | 150.000 | 150.000 | 159.297 | 225.000 |
| 2 | 74.427 | 150.000 | 95.902 | 98.602 | 98.602 | 100.000 | 148.121 | 74.606 | 76.120 | 68.826 | 144.367 | 98.602 | 100.000 | 61.977 | 50.000 | 62.326 | 42.241 | 98.602 | 150.000 |
| 3 | 73.472 | 75.525 | 73.942 | 74.111 | 75.473 | 61.866 | 102.890 | 61.916 | 48.276 | 47.414 | 97.236 | 74.111 | 74.273 | 52.319 | 19.828 | 75.408 | 19.828 | 74.111 | 100.000 |
| 4 | 69.229 | 69.622 | 75.473 | 75.525 | 49.138 | 49.138 | 48.707 | 50.580 | 43.103 | 31.466 | 73.942 | 75.525 | 73.712 | 19.397 | | 49.569 | | 74.705 | 75.000 |
| 5 | 52.897 | 47.845 | 73.712 | 73.712 | | 20.690 | 20.690 | 21.121 | 21.121 | 21.983 | 75.563 | 73.218 | 49.138 | | | 19.828 | | 50.000 | 50.000 |
| 6 | 40.517 | 31.897 | 49.138 | 50.000 | | | | | | | 75.408 | 49.138 | | | | | | | |
| 7 | 22.845 | 21.983 | | | | | | | | | 73.472 | | | | | | | | |
| 8 | | | | | | | | | | | 48.707 | | | | | | | | |

Figure (2) the number and locations of protein bands and their molecular weights for the leaves of kumquat and lemon seedlings treated with amino acids (a side of the Photocapt program)



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