

Effect of potassium polyacrylate on protein pattern changes in Canary palm (*Phoenix canariensis* L.) offshoots growing under oxidative stress conditions

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ABSTRACT: The experiment was conducted to study the effect of different levels of superabsorbent polymer (potassium polyacrylate) and drought stress levels (irrigation intervals) on changes in the protein pattern of Canary Island date palm offshoots. Twenty-seven three-year-old Canary Island date palm offshoots were selected and grown in pots. The study included a two-factor experiment. The first factor involved treating the Canary Island date palm offshoots with the polymer compound at three different concentrations (0, 100 g, and 150 g per offshoot), applied to the root zone. The second factor included treating the offshoots with different irrigation intervals (one irrigation every 2, 10, 30 days). The treatment continued for a full growing season, after which samples were taken from the offshoot leaves and the samples were dried using the freeze-dryer technique (Lyophilization technique). The protein was extracted from the samples and protein migration was performed on Polyacrylamide gel using the Slab-Electrophoresis method in the presence of SDS teratogenic agents. The molecular weights of the proteins were estimated and plotted using a special computer program, PhotoCapt Mw. The study results showed clear differences between all the study conditions, with the protein bands exhibiting varying characteristics in terms of size, area, and height depending on the treatment. The protein profile results also indicated that all the offshoots shared the same protein bands on polyacrylamide gel. The molecular weights of the proteins in the first band ranged from 221.223 to 248.864 kDa, while the molecular weights of the proteins in the second band ranged from 147.379 to 170.902 kDa. This similarity in the molecular weights of the protein bands in all the studied offshoots suggests that they originate from a single plant species. The study results also showed that the appearance of protein bundles with low molecular weights may be due to a mechanism the plant uses to cope with the stresses it is exposed to. These proteins increase with the increase in the stress period, and these proteins can be considered a type of osmotic protein whose appearance increases under stress conditions and are called osmotic protection proteins. The proteomic analysis results clearly show that the studied treatments (especially the drought treatments) significantly affected gene expression in the seedlings and caused the emergence of new protein bands. This indicates that the treatments stimulated the cells to synthesize new proteins that support growth and development indicators in plants growing under drought stress. Furthermore, the treatments had a clear effect on changing the positions of the protein bands and varying their molecular weights, suggesting that the treatments activated gene expression and the synthesis of new proteins that may play a role in improving plant growth and drought resistance.

Keywords: polymer, potassium polyacrylate, protein pattern, Canary Island date palm, oxidative stress



INTRODUCTION

Palm trees are among the beautiful trees used in landscaping and interior decoration of buildings, structures, and homes. They are distinguished by their regular shape and are planted on either side of garden entrances, either in groups in naturally designed gardens or individually in lawns (Al-Batal, 2005). They belong to the palm family *Arecaceae* and the genus *Phoenix*. The distinctive feature of this type of palm is its large, smooth, cylindrical trunk with a large, dense crown bearing fronds, sometimes numbering more than 200. The Canary Island date palm is monocotyledonous, dioecious, and unisexual, bearing the male inflorescence on a male tree and the female inflorescence on a female tree. It has spiny fronds, is slow-growing, and can reach up to two meters in length. It reproduces by seeds or offshoots, and its growth is very slow (Ghalib, 2003).

Offshoots are affected by environmental changes such as heat, drought, and salinity, as their leaves are thin and the amount of protective wax on their leaves differs from that of large offshoots. Also, their young roots are not qualified for such conditions. Drought stress causes a disruption in some plant functions and leads to oxidative stress. Consequently, the levels of reactive oxygen species increase, which in turn affect the occurrence of cytotoxicity and cause the deterioration of the lipid membranes of cell walls, thus affecting the regulation of exchange between cells. Palm offshoots are affected by abiotic stresses such as drought, salinity, and heat. Studying the effect of these stresses is one of the challenges facing the normal growth and development of the plant (Al-busaidi, 2002; Ramirez, 2020). Drought stress is considered one of the most dangerous stresses, especially in arid and semi-arid regions, because it affects plants and causes significant disruption to their vital processes. This, in turn, negatively affects the plant's anatomical tissues, which is reflected in the growth and development of plants and then their death (Yadav *et al.*, 2020; Al-Najjar, *et al.*, 2020a).

The use of natural amendments improves the physical, chemical, and fertility properties of the soil, thus providing a favourable environment for plant growth in terms of increasing moisture content and providing nutrients. Treating plants with polymer compounds such as potassium polyacrylate reduces enzymatic and non-enzymatic oxidation, and is also closely related to the concentrations of osmotic protectants such as sugars, proline, and others (Yamada *et al.*, 2002).

Given the scarcity of water suitable for irrigation, new methods must be found to increase the efficiency of water use to the minimum amount of water suitable for irrigation. Given the lack of studies that have focused on studying ornamental palms and their commercial propagation, and given the high economic value of this type of ornamental palm, the people's desire to acquire them, and the increasing demand for them, and given that Iraq imports this type of ornamental palm with hard currency, and thus the possibility of producing it locally commercially and even the possibility of exporting it, this study acquires great importance because it tests the extent of the possibility of using polymer compounds in the field of cultivating and producing ornamental palms, and confirming their great role in preserving water in the root system area and not draining it into the soil and reducing the negative effects of drought stress in plant tissues.

Proteins constitute approximately 50% of the dry cell weight. They are high molecular weights and are characterized by their impermeability through permeable membranes. Alterations in gene transcription and translation processes can lead to the formation of new proteins through gene expression, depending on the plant's needs and response to the treatment. This ensures improved fruit development and plant growth (Hopkins and Muner, 2008). Most stresses to which plants are exposed cause a marked decrease in protein transformations and nucleic acid synthesis in plant tissues (Popp, 1990). Challa and Van (2004) demonstrated the direct mechanism of salinity's effect on plants by inducing certain morphological, structural, and anatomical changes. Determining stress resistance involves identifying effector molecules that trigger the adaptation mechanisms of the growing plant under stress conditions. These effector molecules are proteins and metabolites, such as the amino acid proline, which are involved in regulating vital processes within the plant in response to stress conditions, thus ensuring the plant's resistance to those conditions (Al-Najjar *et al.*, 2011).

MATERIAL AND METHODS

The experiment was conducted at the Agricultural Research Station of the College of Agriculture, University of Basra, to study the effect of different levels of superabsorbent polymer (potassium polyacrylate) and drought stress levels (irrigation intervals) on changes in the protein profile of Canary Island date palm offshoots. Twenty-seven three-year-old Canary Island date palm offshoots were selected and planted in pots. The study involved a two-factor experiment. The first factor involved treating the Canary Island date palm offshoots with the polymer compound at three different concentrations (0, 100 g, and 150 g per offshoot), which was applied to the root zone and then buried. The second factor involved treating the offshoots with different irrigation intervals (one irrigation every 2, 10, and 30 days). The treatment continued for a full growing season, after which samples were taken from the offshoot leaves and freeze-dried using the lyophilization technique at -26°C. Protein was extracted from the samples according to the method described in Al-Najjar et al. (2021). One gram of wicker was placed in a ceramic mortar with 3 ml of Tris-HCl buffer (0.1M, pH 7.5) containing phenylmethane sulfonyl fluoride (PMSF) at 4°C. Centrifugation was then performed at 4°C at 18,000 rpm for 30 minutes. Forty microliters of the filtrate were transferred to a polyacrylamide gel chromatograph. Protein chromatography on the polyacrylamide gel was performed using the Slab-Electrophoresis method with SDS denaturants, as described by Bavei et al. (2011). Broad Range Protein Molecular Weight Markers (BRPMs) from Promega were used, and the molecular weights of the proteins were determined and plotted. PhotoCapt Mw (version 17) is a special computer program.

The transactions are numbered on the gel image with the following numbers:

Column 1 =marker / Column 2=control(irrigation every 2 days) / Column 3=irrigation every 10 days / Column 4= irrigation every 30 days / Column 5=polymer 100gm+ irrigation every 2 days / Column 6= polymer 100gm+ irrigation every 10 days / Column 7= polymer 100gm+ irrigation every 30 days / Column 8= polymer 150gm+ irrigation every 2 days / Column 9= polymer 150gm+ irrigation every 10 days / Column 10= polymer 150gm+ irrigation every 30 days

RESULTS AND DISCUSSION

Figure 2 shows that all offshoots exhibited similarity in both the first and second protein bands on polyacrylamide gel. The molecular weights of the proteins in the first band ranged from 221.223 to 248.864 kDa, while the molecular weights of the proteins in the second band ranged from 147.379 to 170.902 kDa. This similarity in protein band molecular weights across all the studied offshoots suggests that they originate from a single plant species, as these plants share similar molecular weights. The appearance of protein bundles with low molecular weights may be due to a mechanism that the plant uses to cope with the stresses it is exposed to. These proteins increase with the increase in the stress period. These proteins can be considered a type of osmotic protein. This is what a number of researchers have confirmed, that there are proteins whose appearance increases under stress conditions and they are called osmoprotectant proteins (Hare et al., 1998; Grossman and Rhodes, 2002).

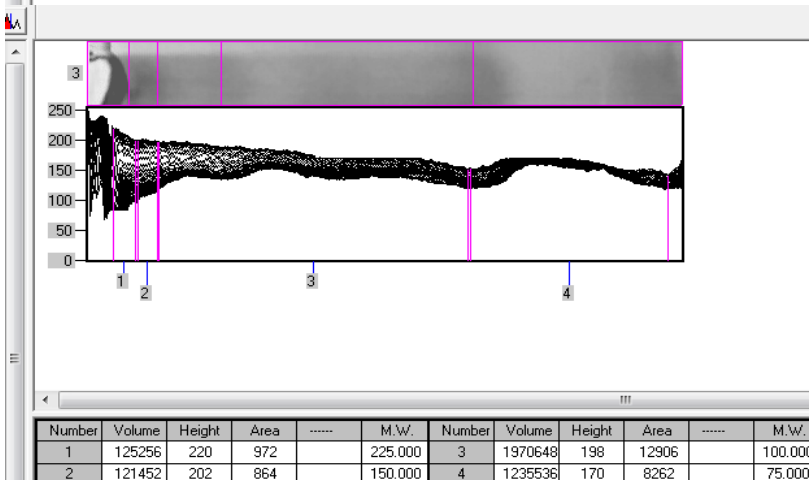
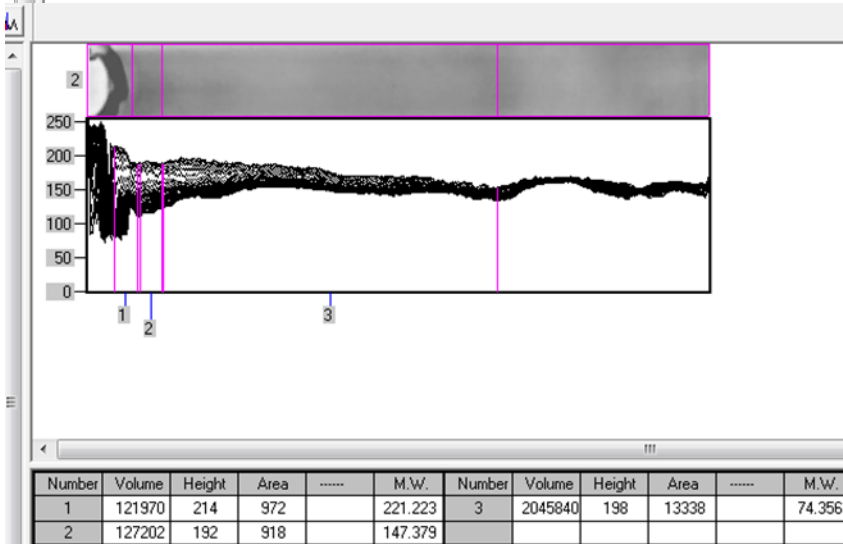
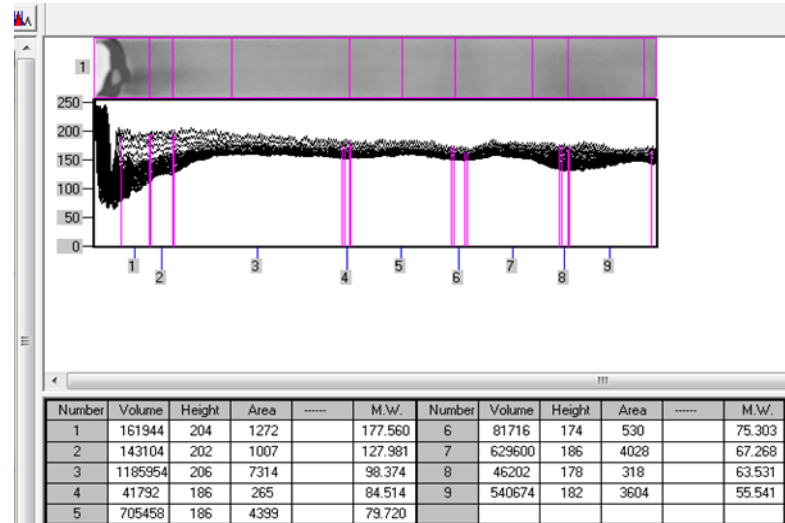
The protein profile of the Canary Island date palm offshoots under study (Figure 2) showed differences in the number, location, and characteristics of protein bands on the polyacrylamide gel. The number of protein bands ranged from three to eight, depending on the treatment.

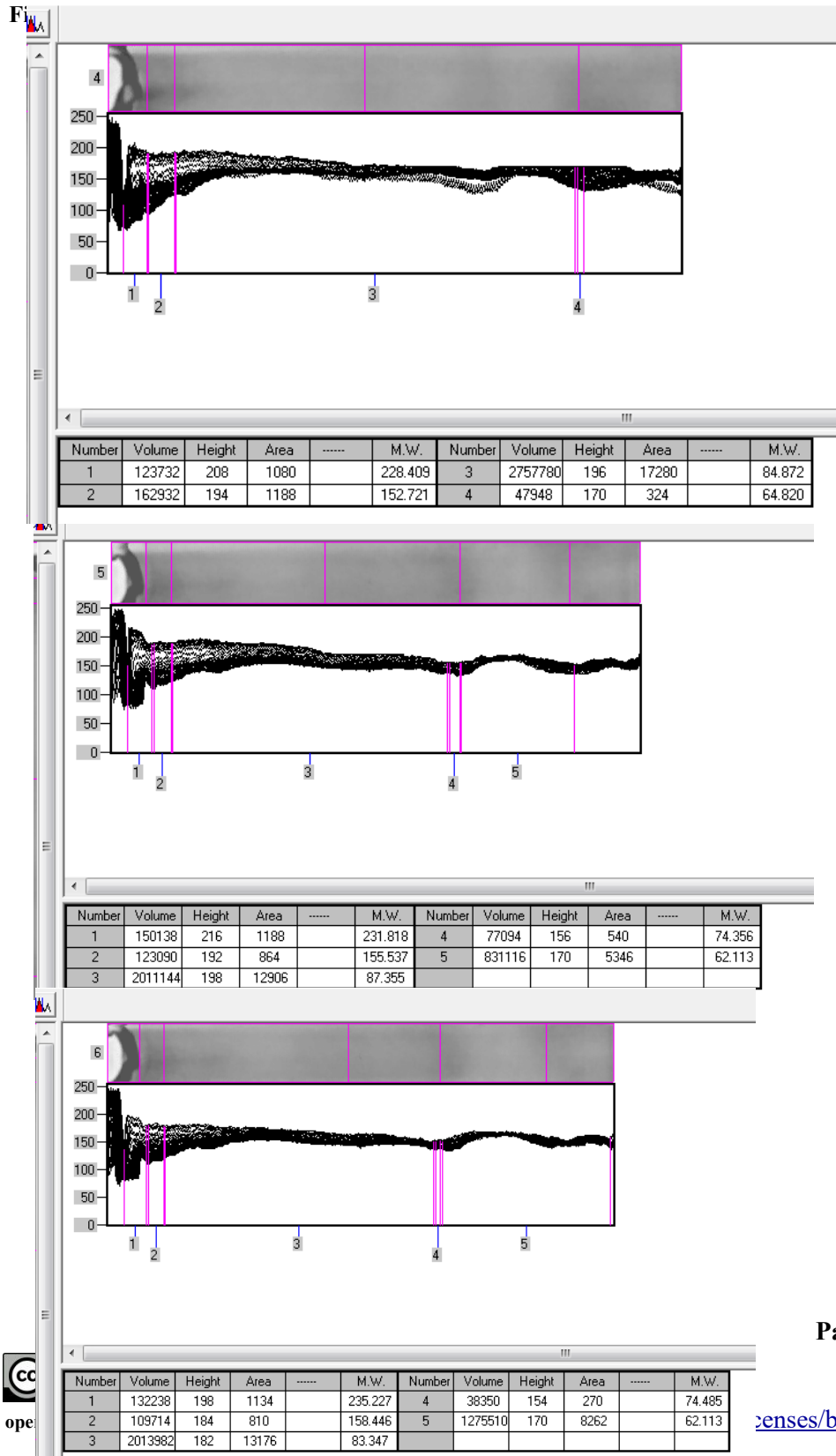


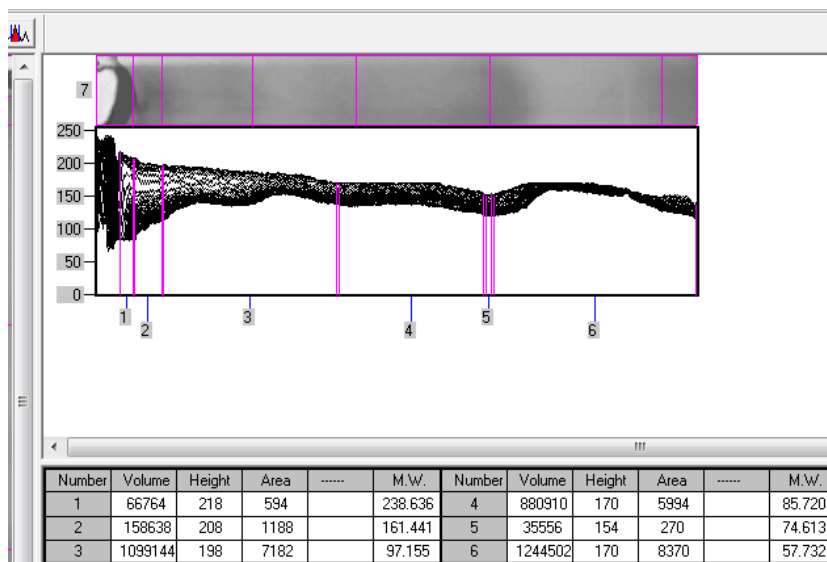
Three protein bands appeared in the two-day irrigation treatment, while four bands appeared in two other treatments (one irrigating every ten days and the other irrigating every 30 days). Five protein bands appeared in the 100g plant-1 polymer interferon treatment with two-day irrigation and the 100g plant-1 polymer interferon treatment with ten-day irrigation. Six protein bands of varying molecular weights appeared in the 100g plant-1 polymer interferon treatment with 30-day irrigation, while only eight protein bands appeared in the 150g plant-1 polymer interferon treatment with drought stress (irrigating every thirty days). When observing these interactions, we find that this compound (polymer) when exposed to periods of drought it may stimulate gene expression and the formation of new proteins that could play a significant role in enhancing plant resistance to drought stress.

The proteomic analysis results clearly show that the studied treatments (especially the drought treatments) significantly affected gene expression in the seedlings and caused the emergence of new protein bundles. This indicates that the treatments stimulated cells to synthesize new proteins that support growth and development indicators in plants growing under drought stress. The treatments also had a clear effect on changing the positions of protein bundles and varying their molecular weights, indicating that they activated gene expression and the synthesis of new proteins that may play a role in improving plant growth and drought resistance. It became clear that each cellular activity is responsible for one or more proteins, which are classified according to their function into several types, such as transport proteins, storage proteins, structural proteins, and protective proteins (Lesk, 2010).

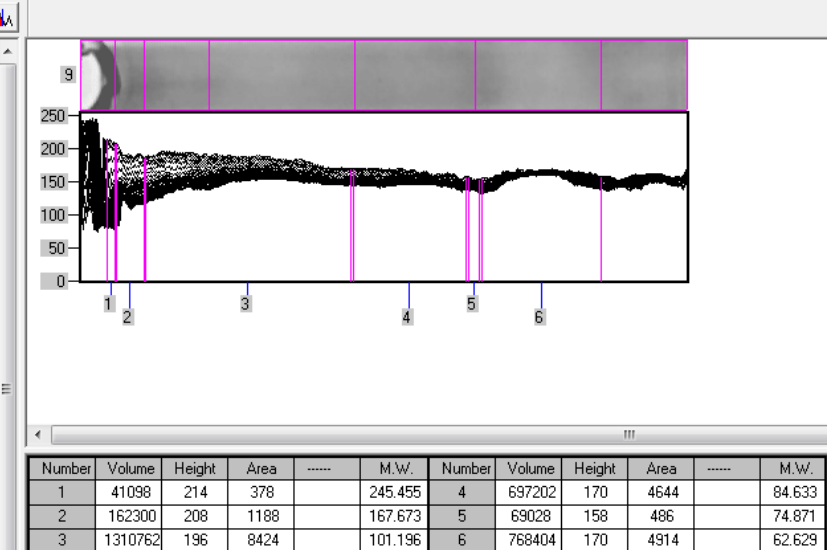
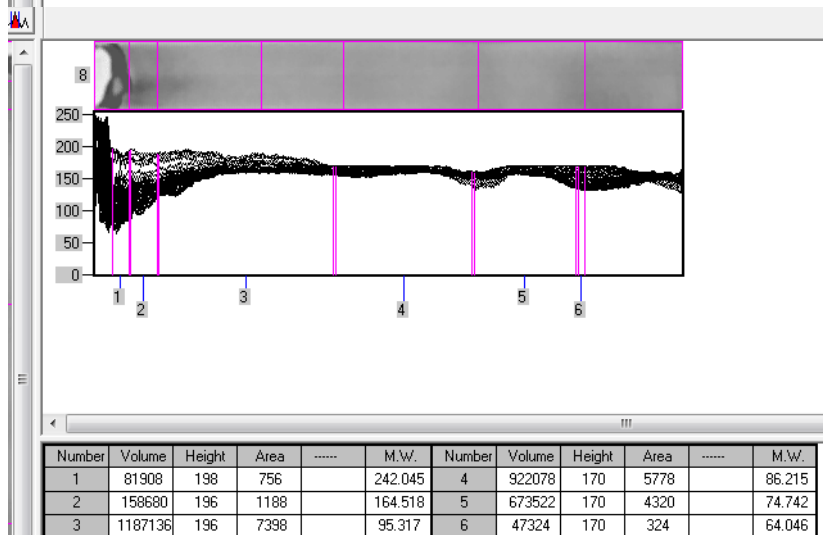
These results indicate that the studied treatments led to protein synthesis and may also cause changes in the processes of translation and translation, leading to the production of new proteins through gene expression according to the plant's needs and in response to the type of treatment, thus ensuring improved plant growth (David and Nilson, 2000). (Khairallah, 2009). They are involved in the structure of the nucleus and cytoplasm, and some of them are enzymes that have important functional roles in various metabolic processes during plant and fruit growth and ripening (Cooper and Hausman, 2007). Drought stress causes the generation of new proteins with low molecular weights, which play an important role in repairing and regulating damaged proteins related to various vital activities in the plant. These new proteins may be shock proteins resulting from drought stress, which play an essential role in the plant's response to physiological changes caused by drought and are usually at low levels under normal conditions (Polenta et al., 2020; Ghirardo et al., 2021).







ylamide gel.



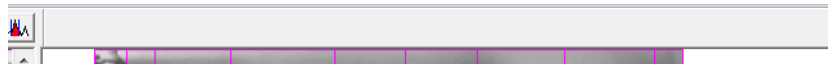


Figure (1-c) shows some specifications of protein bundles on polyacrylamide gel.

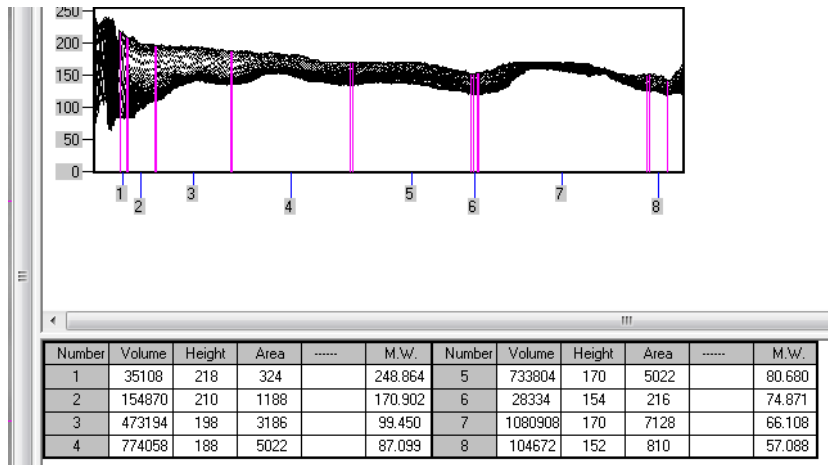
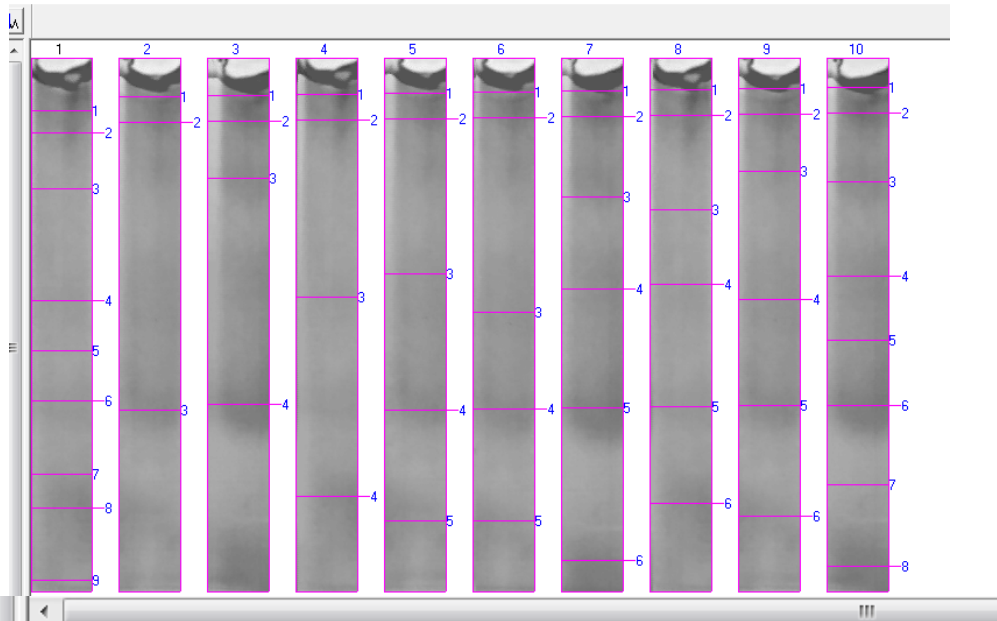


Figure (1-d) shows some specifications of protein bundles on polyacrylamide gel.



		M.W.									
		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
1	177.560	221.223	225.000	228.409	231.818	235.227	238.636	242.045	245.455	248.864	
2	127.981	147.379	150.000	152.721	155.537	158.446	161.441	164.518	167.673	170.902	
3	98.374	74.356	100.000	84.872	87.355	83.347	97.155	95.317	101.196	99.450	
4	84.514		75.000	64.820	74.356	74.485	85.720	86.215	84.633	87.099	
5	79.720				62.113	62.113	74.613	74.742	74.871	80.680	
6	75.303						57.732	64.046	62.629	74.871	
7	67.268									66.108	
8	63.531									57.088	
9	55.541										

Colour
4= irr
irriga
irrigation every 2 days / Column 9= polymer 150gm+ irrigation every 10 days / Column 10= polymer 150gm+ irrigation every 30 days

**Figure (2) Number and locations of protein bundles and their molecular weights
(a side view of the Photocapt program)**

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