Annals of Agri-Bio Research **28** (2): 452-457, 2023 **Impact Score : 0.28 (Scopus)**

The Effect of Gibberellin Treatment and Bulb Weight on Protein-pattern of Narcissus Leaves *Narcissus tazetta* L.

ZAINAB A. ALI AL-TAHER*

Department of Horticulture and Landscape Engineering, College of Agriculture, University of Basrah, Iraq
**(e-mail: zainab_ali680@yahoo.com; Mobile: 00960 77056 50027)*

(Received: March 11, 2023; Accepted: May 2, 2023)

ABSTRACT

This research was carried out in the wooden canopy of the Department of Horticulture and Landscaping at the College of Agriculture on narcissus bulbs of different weights 30, 40, 50 and 60 g. After the seedlings reached the appropriate size, they were sprayed with the prepared concentrations of gibberellins 0, 50 and 100 mg/l. Protein was extracted from dried paper samples using Phenyl methane sulfonyl fluoride (PMSF), and protein movements were performed using the Slab-Electrophoresis technique on a polyacrylamide gel. Using a specialized application called PhotoCapt Mw (version17), the molecular weights of the proteins were calculated. There were variations among all of the study's circumstances, with the protein bundles' specifications varying in terms of their size, area and height. All the bulbs shared same molecular weight as the first protein bundle, which amounted to 180.732 Kilo Dalton. All bulbs of different weights also participated in the same molecular weight of the second protein package, which recorded 122.101 Kilo Dalton. The protein bundle counts varied from 4 to 6 depending on the age of the bulb and the gibberellin concentration. Five protein bundles were present in most treatments, and six protein bundles were only in two treatments (bulbs of 50 g + 100 gibberellins and bulbs of 60 g+50 (GA_3). Moreover, four protein packages in three treatments: (bulbs 30 g + 50 gibberellins, bulbs 40 g + 50 gibberellins and bulbs 50 g + 50 gibberellins.

Key words: Bulbs, gibberellin, molecular weights, narcissus, protein patterns

INTRODUCTION

Narcissus bulbs belong to the Amaryllidaceae family (Cimmino *et al.*, 2017). Its original habitat is Asia, Europe and the Mediterranean basin, and it is one of Iraq's most common winter annual bulbs (Cimmino *et al.*, 2017; Youssef *et al.*, 2017). The cluster type, *Narcissus tazetta* L., grows wild in the northern regions of Iraq. The importance of the daffodil plant comes from its early flowering at the end of winter and is suitable for harvesting, as the beautiful shape clustered flowers and their sweet scent compensate for the shortness of the flower stand by using it in low-level arrangements (Youssef *et al.*, 2017; Kayfi and Abdulrahman, 2022).

There are also medical uses for the narcissus plant, where it was discovered that it has a protective effect against some types of cancer because it contains the chemical benzaldehyde, which in the human body transforms into a substance resembling laetrile or lycorine, which then transforms into a substance resembling lycobetaine known for its ability to slow the growth of cancer cells (Ferdausi, 2017). Most flowering bulbs need treatments to prepare them for rapid production. One of these agricultural processes is growth regulators, which constitute one of the main directions of applied scientific research and play a key role in improving and producing plant flowers (Al-Hasany, 2018; Al-Hasany *et al.,* 2020). One of these growth regulators is gibberellic acid, which plays an important role in activating and encouraging the elongation of cells and the start of flower formation and development by directing metabolic products towards growing flowers (Salih, 2022) and thus increasing the size of flowers, the number of petals developing in them, and the length of the floral stem.

Plant proteins have generally high molecular weight and are characterized by their impermeability through permeable membranes. Proteins are made by plant cells through the process of photosynthesis (Shahzad *et al.*, 2016; Chen *et al.*, 2021).

There have been studies on the technique of electrophoresis for proteins (electrophoresis technology). It means the separation of proteins and other charged particles in an

electric field using a carrier such as a polyacrylamide gel, starch or agarose at a fixed pH, where the proteins move. For example, towards the negative or positive electrode, depending on the type of charge, which carry them in the first place and on their molecular weights and the shape of their particles in the second degree (Westermeier, 2016; Al-Hasany *et al.*, 2020). Osemwota *et al.* (2021) stated that proteins contained a negative, positive or neutral charge when the protein dissolveds at a neutral pH (pH 7.0). The charge is produced through charged amino acids such as chlorine, arginine, asparagine and glutamine. The protein charge depends on the presence of types of these acids and their proportions in proteins (Sim *et al.*, 2021). Gibberellin treatment and gibberellin-regulated protein affect patterns of plant protein, and this effect has been documented in several reports (Cui *et al.*, 2020; Iizuka *et al.*, 2022; Panda *et al.*, 2022). So, in present study, the effect of gibberllin on protein patterns of Narcissus leaves (*Narcissus tazetta* L.) has been studied.

MATERIALS AND METHODS

The wooden canopy of the Department of Horticulture and Landscaping, College of Agriculture, served as the study's setting on narcissus bulbs of different weights 30, 40, 50 and 60 g treated by concentrations of gibberellin. The bulbs were planted in plastic pots (one bulb per pot) filled with a culture medium sterilized with 50% pentanol fungicide at a concentration (50 cm^3 per 100 liters water) to prevent fungal infection of the soil. Peat moss at a ratio of 1:2, respectively, at a rate of 1.5 kg per pot. After the seedlings reached the appropriate size, they were sprayed with the prepared concentrations of gibberellins 0, 50 and 100 mg/l.

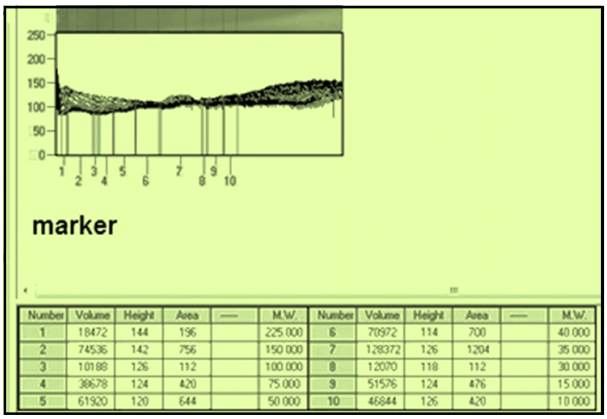
The leaf samples were dried using the Freeze-dryer (Lyophilization process) at - 26 °C following Taain *et al.* (2021). Protein was extracted from the samples by combining 1 g of leaves with 3 ml of Tris-HCl-buffer (0.1M at pH 7.5) containing (PMSF) Phenyl methane sulfonyl fluoride at a 4°C. After 30 min, centrifugation at 4°C at 18000 RPM, 40 ml of the filtrate was transferred to the migration apparatus on a polyacrylamide gel.

The protein migration was performed on a polyacrylamide gel using the Slab-Electrophoresis method in the presence of SDS denaturants. Wide Range Protein Molecular Weight Markers from Promega were employed, and a specialized computer application called PhotoCapt Mw was used to calculate and visualize the proteins' molecular weights (version17). The transactions were numbered as: Marker 2–bulbs 30 g. 3–bulbs 40 g, 4–bulbs 50 g, 5–bulbs 60 g, 6–bulbs 30 g + 50 gibberellins, 7–bulbs 30 g + 100 gibberellins, 8–bulbs 40 g + 50 gibberellins, 9–bulbs 40 g + 100 gibberellins, 10–bulbs 50 g + 50 gibberellins, 11–bulbs 50 g + 100 gibberellins, 12–bulbs 60 g + 50 gibberellins and 13–bulbs 60 g + 100 gibberellins.

RESULTS AND DISCUSSION

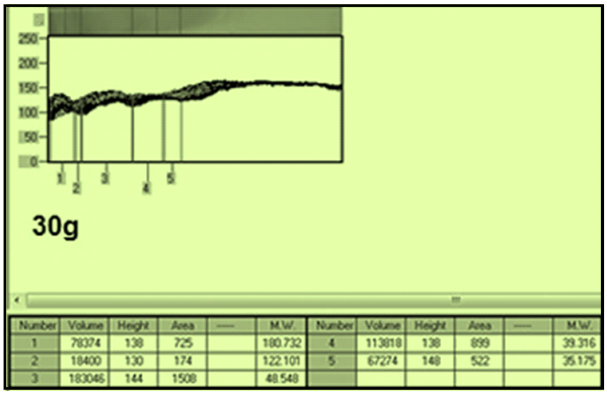
Several types of genetic indicators were used in genetic characterization or the factors affecting them, such as protein, enzymatic and cellular indicators, and DNA markers. When using proteins, the genetic fingerprint means the pattern of bundles separated by gelatinous migration resulting from the analysis of the protein content of the studied individuals (Heikal *et al.*, 2022). The genetic marker is a characteristic that is used to infer the presence of a specific locus on the chromosome or gene, and knowledge of this site helps to study the inheritance of a particular trait or gene, as genes very close to the marker are inherited with it (Hasan *et al.*, 2021).

When looking at the protein pattern of the studied narcissus leaves (Figs. 1 to 13), there were differences across all of the study circumstances since the protein bundles' specifications varied depending on the treatment in terms of size, area and height. The bulbs of all weights and without treatments participated in the presence of five protein bundles, and all the bulbs shared the same molecular weight as the first protein bundle, which amounted to 180.732 kilo-daltons (Fig. 14). Also, all bulbs of different weights participated in same weight molecular of the second protein bundle, which recorded 122.101 kilo-daltons, and this was due to being of one genetic origin. The quantity, distribution and characteristics of the protein bundles on the polyacrylamide gel varied between different treatments. Depending on the age of the bulbs and the gibberellin concentration, the number of protein bundles varied from 4 to 6. In most



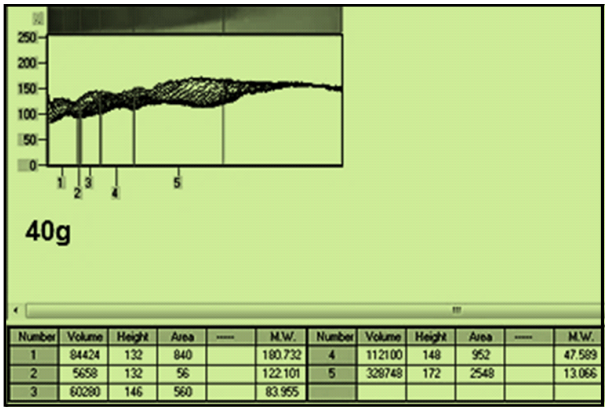
Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	18472	144	196		225.000	6	70972	114	700		40.000
2	74536	142	756		150.000	7	128372	126	1204		35.000
3	10188	126	112		100.000	8	12070	118	112		30.000
4	38678	124	420		75.000	9	51576	124	476		15.000
5	61920	120	644		50.000	10	46844	126	420		10.000

Fig. 1. Some specifications of the protein bands on the polyacrylamide gel for the marker



Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	78374	138	725		180.732	4	113818	138	899		39.316
2	18400	130	174		122.101	5	67274	148	522		35.175
3	180046	144	1508		48.548						

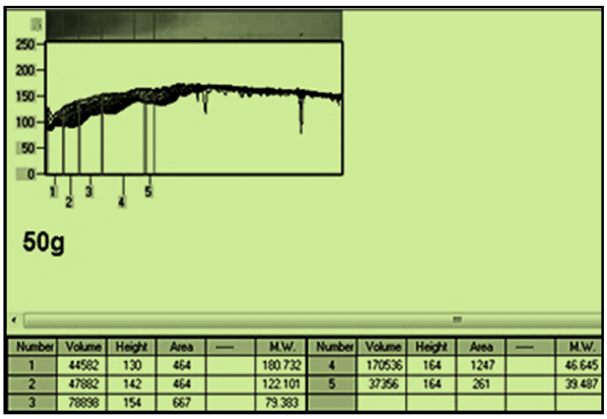
Fig. 2. Some specifications of protein bands on polyacrylamide gel for 30 g weight of narcissus bulbs.



Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	84424	132	840		180.732	4	112100	148	952		47.589
2	5658	132	56		122.101	5	328748	172	2548		13.066
3	60280	146	560		83.955						

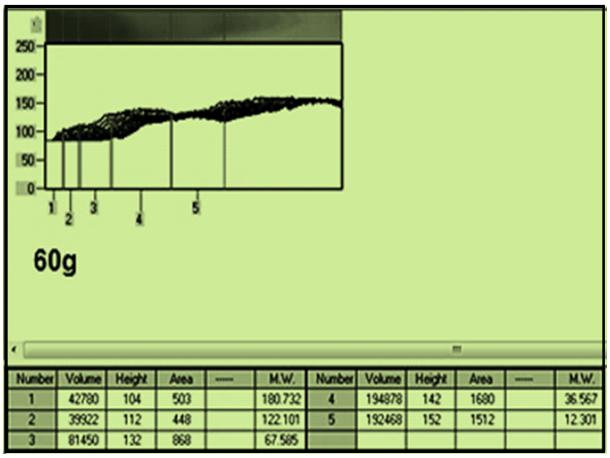
Fig. 3. Some specifications of protein bands on polyacrylamide gel for 40 g weight of narcissus bulbs.

treatments, five protein bundles and six protein bundles were present only for two treatments bulbs of 50 g + 100 (GA_3) and bulbs of 60 g + 50 (GA_3) and four protein packs for three treatments: bulbs of 30 g + 50 gibberellins, bulbs of 40 g + 50 gibberellins and bulbs of 50 g + 50 gibberellins. These three treatments seemed to significantly affect the



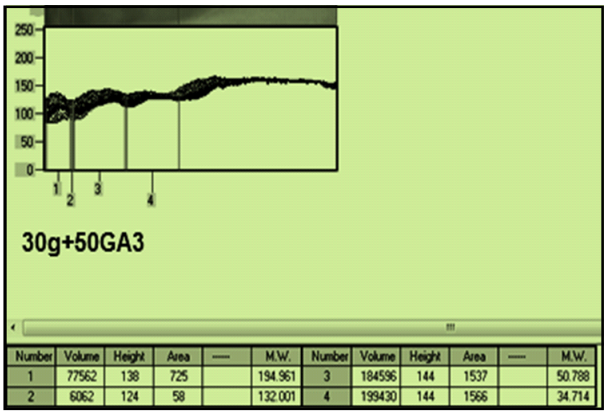
Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	44582	130	464		180.732	4	170536	164	1247		46.645
2	47882	142	464		122.101	5	37356	164	261		39.487
3	78898	154	667		79.383						

Fig. 4. Some specifications of protein bands on polyacrylamide gel for 50 g weight of narcissus bulbs.



Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	42780	104	503		180.732	4	194878	142	1680		36.567
2	39922	112	448		122.101	5	192468	152	1512		12.301
3	81450	132	868		67.585						

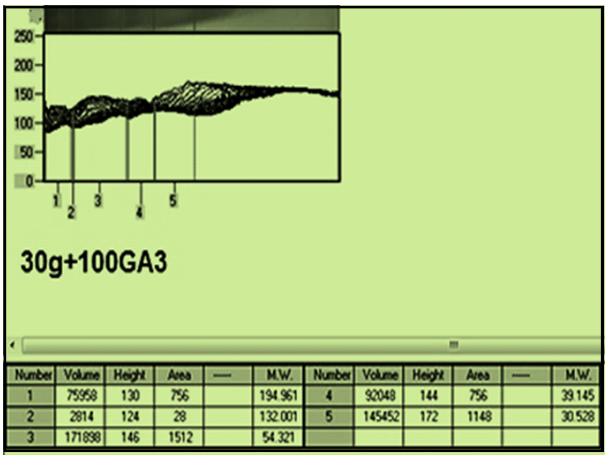
Fig. 5. Some specifications of protein bands on polyacrylamide gel for 60 g weight of narcissus bulbs.



Number	Volume	Height	Area	----	M.W.	Number	Volume	Height	Area	----	M.W.
1	77562	138	725		194.961	3	184596	144	1537		50.788
2	6062	124	58		132.001	4	199430	144	1566		34.714

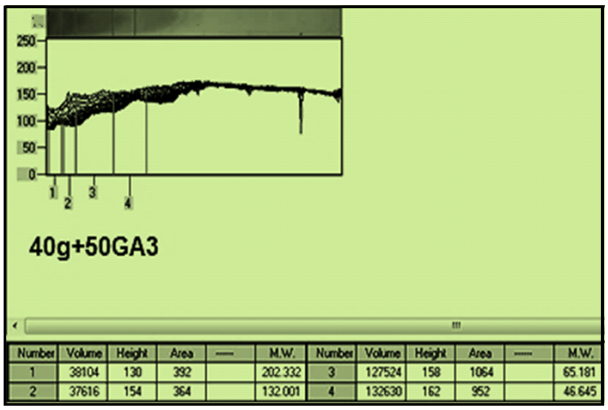
Fig. 6. Some specifications of protein bands on polyacrylamide gel for 30 g weight of narcissus bulbs with 50 mg/l gibberellin (GA_3).

gene expression process of narcissus bulbs and caused the disappearance of some protein bundles and the survival of only four protein bundles. The conditions of the experiment (the



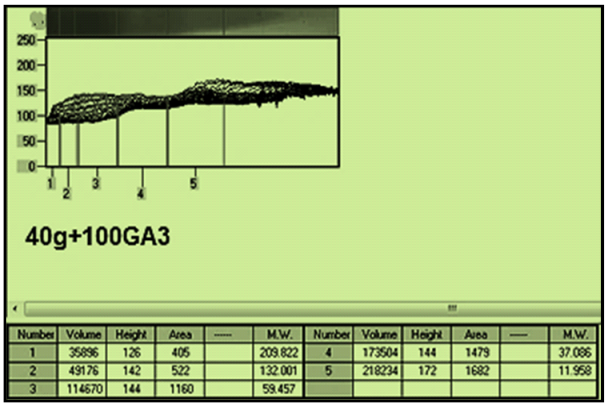
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	75958	130	756		194.961	4	92048	144	756		39.145
2	2814	124	28		132.001	5	145452	172	1148		30.528
3	171898	146	1512		54.321						

Fig. 7. Some specifications of protein bands on polyacrylamide gel for 30 g weight of narcissus bulbs with 100 mg/l gibberellin (GA_3).



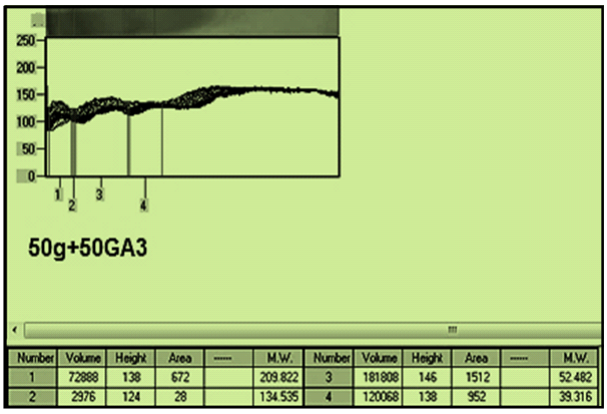
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	38104	130	392		202.332	3	127524	158	1064		65.181
2	37616	154	364		132.001	4	132630	162	952		46.645

Fig. 9. Some specifications of protein bands on polyacrylamide gel for 40 g weight of narcissus bulbs with 50 mg/l gibberellin (GA_3).



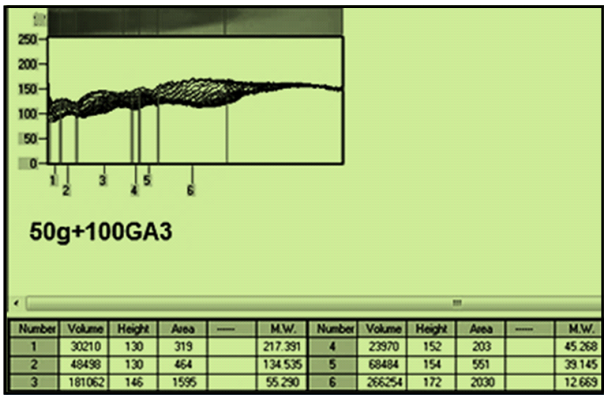
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	35896	126	405		209.822	4	173504	144	1479		37.086
2	49176	142	522		132.001	5	218234	172	1682		11.958
3	114670	144	1160		59.457						

Fig. 9. Some specifications of protein bands on polyacrylamide gel for 40 g weight of narcissus bulbs with 100 mg/l gibberellin (GA_3).



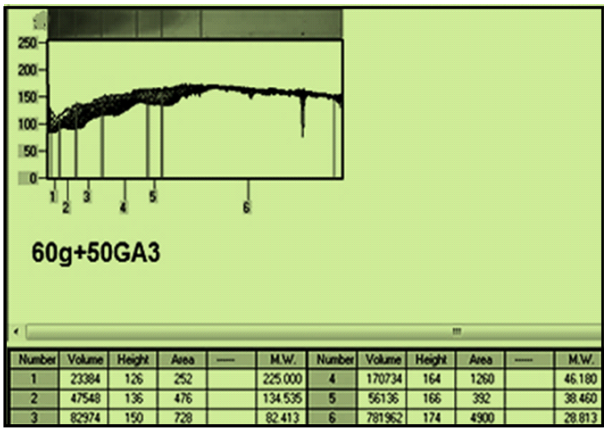
Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	72888	138	672		209.822	3	181808	146	1512		52.482
2	2976	124	28		134.535	4	120068	138	952		39.316

Fig. 10. Some specifications of protein bands on polyacrylamide gel for 50 g weight of narcissus bulbs with 50 mg/l gibberellin (GA_3).



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	30210	130	319		217.391	4	23970	152	203		45.268
2	48498	130	464		134.535	5	68484	154	551		39.145
3	181062	146	1595		55.290	6	266254	172	2030		12.669

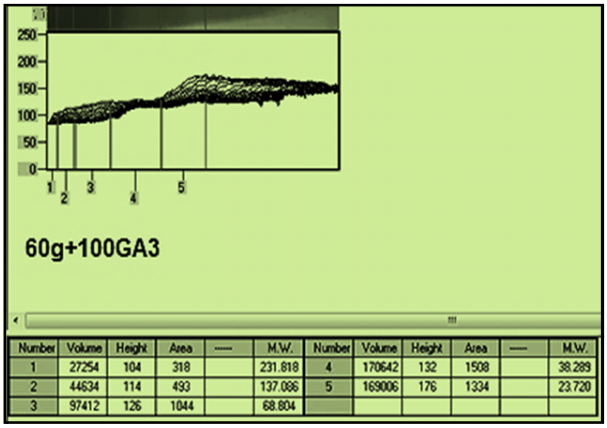
Fig. 11. Some specifications of protein bands on polyacrylamide gel for 50 g weight of narcissus bulbs with 100 mg/l gibberellin (GA_3).



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	23384	126	252		225.000	4	170734	164	1260		46.180
2	47548	136	476		134.535	5	56136	166	392		38.460
3	82974	150	728		82.413	6	781962	174	4900		28.813

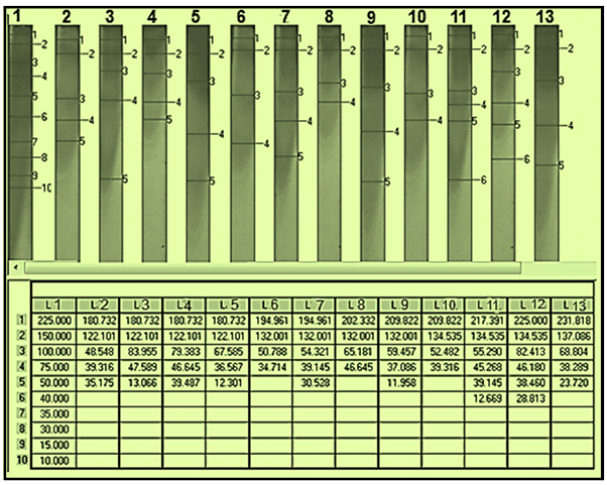
Fig. 12. Some specifications of protein bands on polyacrylamide gel for 60 g weight of narcissus bulbs with 50 mg/l gibberellin (GA_3).

treatments) had a definite impact on the protein bundles' positions and various molecular weights which indicated that the



Number	Volume	Height	Area	-----	M.W.	Number	Volume	Height	Area	-----	M.W.
1	27254	104	318		231.818	4	170642	132	1508		38.289
2	44634	114	493		137.086	5	169006	176	1334		23.720
3	97412	126	1044		68.804						

Fig. 13. Some specifications of protein bands on polyacrylamide gel for 60 g weight of narcissus bulbs with 100 mg/l gibberellin (GA_3).



	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
1	225.000	180.732	180.732	180.732	180.732	194.961	194.961	202.332	209.822	209.822	217.391	225.000	231.818
2	150.000	122.101	122.101	122.101	122.101	132.001	132.001	132.001	132.001	134.535	134.535	134.535	137.086
3	100.000	48.548	83.955	79.383	67.585	50.788	54.321	65.181	59.457	52.492	55.290	82.413	68.804
4	75.000	39.316	47.589	46.645	36.567	34.714	39.145	46.645	37.086	39.316	45.268	46.180	38.289
5	50.000	35.175	13.066	39.487	12.301		30.528		11.958		39.145	38.460	23.720
6	40.000										12.669	28.813	
7	35.000												
8	30.000												
9	15.000												
10	10.000												

Fig. 14. The number and locations of protein bundles and their molecular weights for different weights of narcissus bulbs treated with different concentrations of gibberellins (a side of the photocapt program).

treatments had caused activating gene expression and manufacturing new proteins that may play a role in improving bulb growth. These findings suggest that gibberellin treatment of bulbs may result in the synthesis of natural proteins as well as a change in the translation and transcription processes, which results in the production of new proteins through the gene expression process in response to the type of treatment and in accordance with the needs of the plant to ensure improved plant growth (Marthandan *et al.*, 2020). In addition, it helps to activate a certain type of genes to build RNA that is important for building proteins; as the water potential becomes more negative, thus reducing the swelling pressure, the wall's tensile resistance decreases, and water and nutrients permeate, thus increasing cell size, and the important role of gibberellins in the formation of proteins and nucleic acids (Sun *et al.*, 2022).

CONCLUSION

According to the study, protein bundle counts varied from 4 to 6 depending on the age of the bulb and the gibberellin concentration. Five protein bundles were present in most treatments, and six protein bundles were only for two treatments bulbs of 50 g + 100 (GA_3) and bulbs of 60 g + 50 (GA_3). Moreover, four protein packages for three treatments: bulbs 30 g + 50 gibberellins, bulbs 40 g + 50 gibberellins and bulbs 50 g + 50 gibberellins.

REFERENCES

Al-Hasany, A. R., Alhilfi, S. K. and Alfarjawi, T. M. (2020). Effect of foliar feeding with nano-boron on the growth and yield of two cultivars of faba bean crop (*Vicia faba* L.). *Int. J. Agri. Stat. Sci.* **16**: 237-241.

Al-Hasany, A. R. K. (2018). Effect of foliar nutrition of proline and mixture of nutrients on growth and yield of different varieties of broad bean (*Vicia faba* L.). Ph. D. thesis, College of Agriculture, Al-Muthanna University, Iraq.

Chen, D., Jones, O. G. and Campanella, O. H. (2021). Plant protein-based fibers: Fabrication, characterization and potential food applications. *Crit. Rev. Food Sci. Nut.* **2021**: 01-25. *https://doi.org/10.1080/10408398.2021.2004991.*

Cimmino, A., Masi, M., Evidente, M., Superchi, S. and Evidente, A. (2017). Amaryllidaceae alkaloids: Absolute configuration and biological activity. *Chirality* **29**: 486-499.

Cui, W., Song, Q., Zuo, B., Han, Q. and Jia, Z. (2020). Effects of gibberellin (GA4+ 7) in grain filling, hormonal behaviour, and antioxidants in high-density maize (Zea mays L.). *Plants* **9**: 978. *https://doi.org/10.3390/plants9080978.*

Ferdausi, A. (2017). A metabolomics and transcriptomics comparison of Narcissus pseudonarcissus cv. Carlton field and in vitro tissues in relation to alkaloid production. The Publication of University of Liverpool (United Kingdom), Liverpool. *http://doi.org/10.17638/03007178.*

Hasan, N., Choudhary, S., Naaz, N., Sharma, N., Laskar, R.A. (2021). Recent advancements

in molecular marker-assisted selection and applications in plant breeding programmes. *J. Genet. Eng. Biotechnol.* **19**: 128 *https://doi.org/10.1186/s43141-021-00231-1.*

Heikal, Y. M., El-Esawi, M. A. and Galilah, D. A. (2022). Morpho-anatomical, biochemical and molecular genetic responses of canola (Brassica napus L.) to sulphur application. *Environ. Experimental Bot.* **194**: 104739. *https://www.sciencedirect.com/science/article/pii/S0098847221003695.*

Iizuka, T., Barre, A., Rougé, P., Charpin, D., Scala, E., Baudin, B. and Poncet, P. (2022). Gibberellin-regulated proteins. *Front. Allergy* **2022**: 01-11. *https://doi.org/10.3389/falgy.2022.877553.*

Kayfi, S. and Abdulrahman, M. D. (2021). Ethnopharmacology of plants in Choman, The Kurdistan Region of Iraq. *Appl. Biol. Res.* **23**: 322-330. *http://dx.doi.org/10.5958/0974-4517.2021.00042.2.*

Marthandan, V., Geetha, R., Kumutha, K., Renganathan, V. G., Karthikeyan, A. and Ramalingam, J. (2020). Seed priming: A feasible strategy to enhance drought tolerance in crop plants. *Int. J. Mol. Sci.* **21**: 8258. *doi.org/10.3390/ijms21218258.*

Osemwota, E. C., Alashi, A. M. and Aluko, R. E. (2021). Comparative study of the structural and functional properties of membrane-isolated and isoelectric pH precipitated green lentil seed protein isolates. *Membranes* **11**: 694. *https://doi.org/10.3390/membranes11090694.*

Panda, S., Jozwiak, A., Sonawane, P. D., Szymanski, J., Kazachkova, Y., Vainer, A. and Aharoni, A. (2022). Steroidal alkaloids defence metabolism and plant growth are modulated by the joint action of gibberellin and jasmonate signalling. *New Phytologist* **233**: 1220-1237.

Salih, L. D. S. M. (2022). Effects of spraying GA3 and KT-30 on the vegetative and glowering growth of Ranunculus asiaticus. *Nasaq* **35**: 698-714. *https://www.iasj.net/iasj/download/92614f6ebbdd29b5.*

Shahzad, M. A., Khan, A., Khalid, M. and Gul, A. (2016). Plant proteomics: An overview. In: Plant Omics: Trends and Applications, Hakeem, K., Tombuloglu, H., Tombuloglu, G. (eds.). Springer, Cham. *https://doi.org/10.1007/978-3-319-31703-8_12.*

Sim, S. Y. J., Srv, A., Chiang, J. H. and Henry, C. J. (2021). Plant proteins for future foods: A roadmap. *Foods* **10**: 1967. *https://doi.org/10.3390/foods10081967.*

Sun, H., Cao, Y., Kim, D. and Marelli, B. (2022). Biomaterials technology for agrofood resilience. *Adv. Fun.. Mat.* **32**: 2201930.

Taain, D. A., AL-Najjar, M. A. H. and El-qatrani, N. A. (2021). Investigation the protein pattern of leaves and roots of barhi and khalas date palm (Phoenix dactylifera L.) cultivars propagated by offshoots and tissue culture techniques. Plant Cell Biotech. *Mol. Biol.* **22**: 09-17. *https://ikppress.org/index.php/PCBMB/article/view/5829.*

Westermeier, R. (2016). Electrophoresis in practice: A guide to methods and applications of DNA and protein separations. John Wiley and Sons. https://analyticalscience.wiley.com/do/10.1002/sepspec.9783527338801/.

Youssef, S., Mahmoud, A. M. and Vela, E. (2017). On the genus Sternbergia (Amaryllidaceae) in Iraq. *Anales del Jardin Botanico de Madrid* **74**: 07-15. *https://dialnet.unirioja.es/servlet/articulo?codigo=6088487.*