

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

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### 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<sub>3</sub>). 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 (Westermeyer, 2016; Al-Hasany *et al.*, 2020). Osemwota *et al.* (2021) stated that proteins contained a negative, positive or neutral charge when the protein dissolved 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 gibberellin 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<sup>3</sup> 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

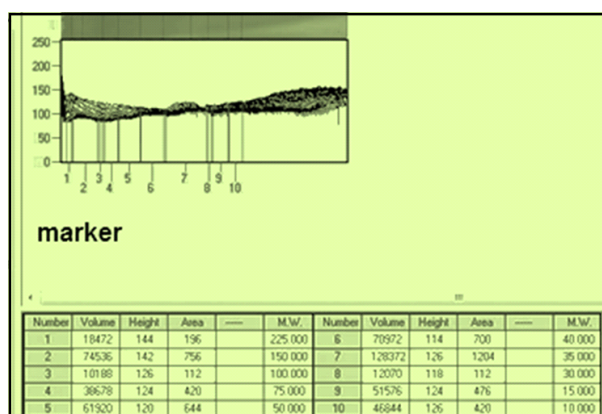


Fig. 1. Some specifications of the protein bands on the polyacrylamide gel for the marker

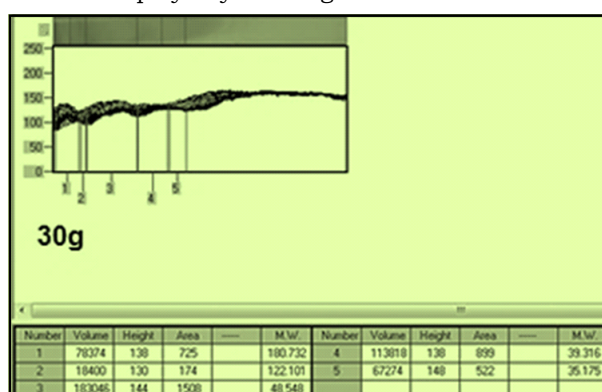


Fig. 2. Some specifications of protein bands on polyacrylamide gel for 30 g weight of narcissus bulbs.

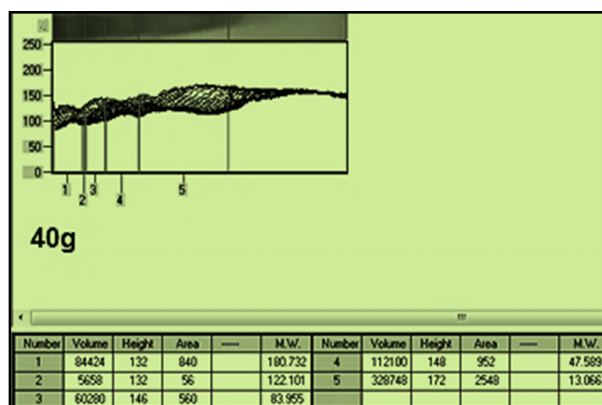


Fig. 3. Some specifications of protein bands on polyacrylamide gel for 40 g weight of narcissus bulbs.

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

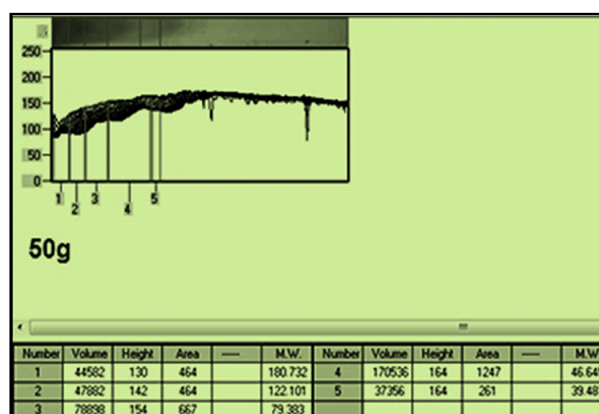


Fig. 4. Some specifications of protein bands on polyacrylamide gel for 50 g weight of narcissus bulbs.

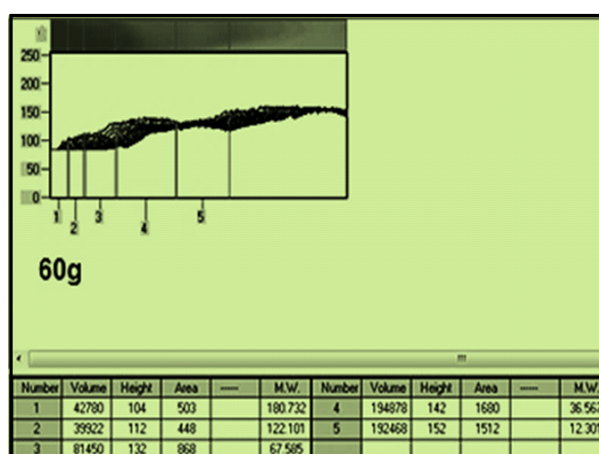


Fig. 5. Some specifications of protein bands on polyacrylamide gel for 60 g weight of narcissus bulbs.

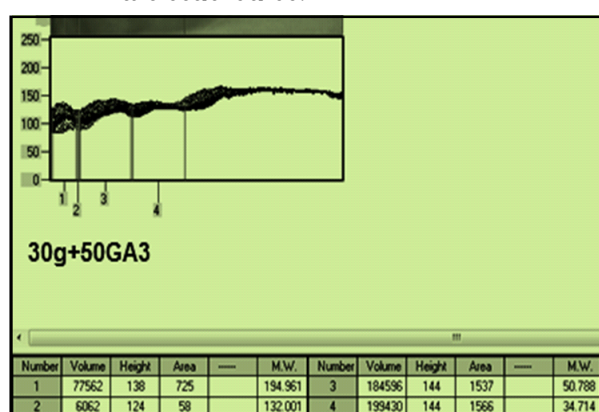


Fig. 6. Some specifications of protein bands on polyacrylamide gel for 30 g weight of narcissus bulbs with 50 mg/l gibberellin ( $GA_3$ ).

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

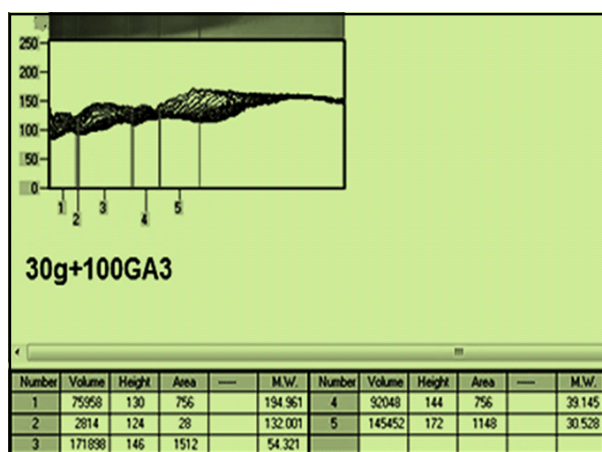


Fig. 7. Some specifications of protein bands on polyacrylamide gel for 30 g weight of narcissus bulbs with 100 mg/l gibberellin ( $GA_3$ ).

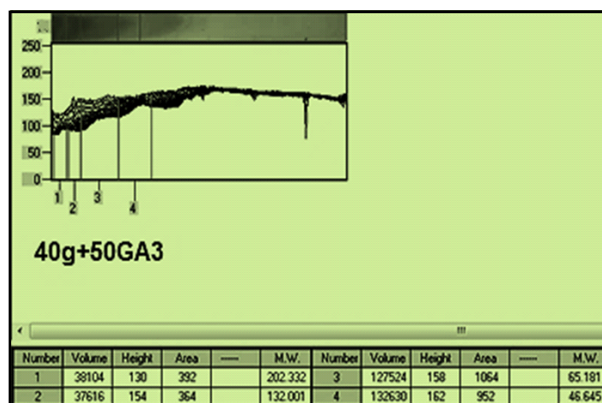


Fig. 9. Some specifications of protein bands on polyacrylamide gel for 40 g weight of narcissus bulbs with 50 mg/l gibberellin ( $GA_3$ ).

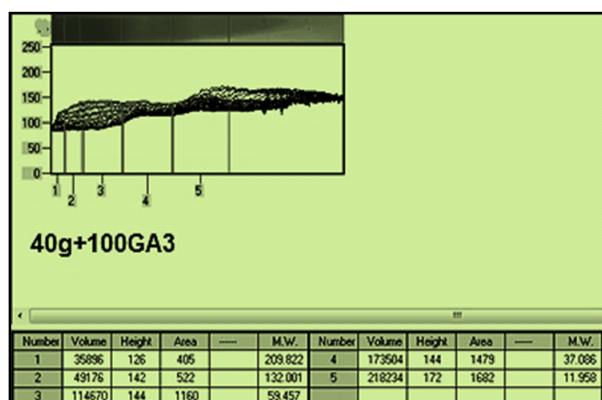


Fig. 9. Some specifications of protein bands on polyacrylamide gel for 40 g weight of narcissus bulbs with 100 mg/l gibberellin ( $GA_3$ ).

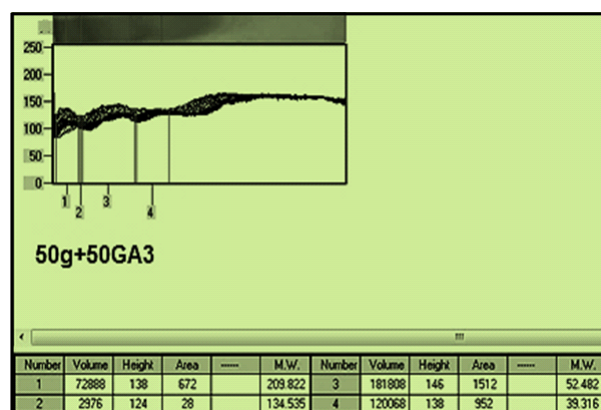


Fig. 10. Some specifications of protein bands on polyacrylamide gel for 50 g weight of narcissus bulbs with 50 mg/l gibberellin ( $GA_3$ ).

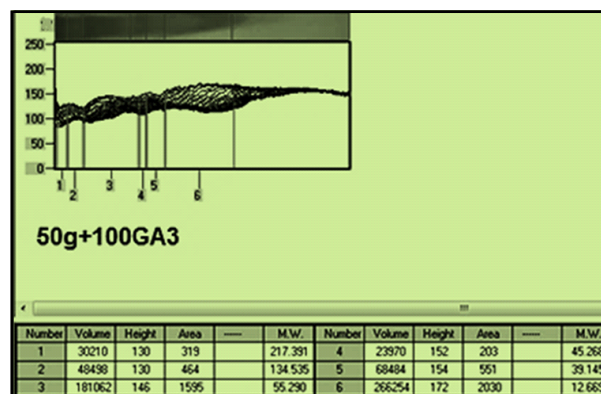


Fig. 11. Some specifications of protein bands on polyacrylamide gel for 50 g weight of narcissus bulbs with 100 mg/l gibberellin ( $GA_3$ ).

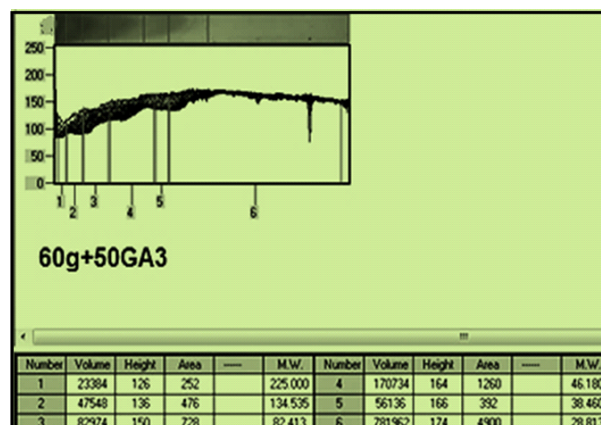


Fig. 12. Some specifications of protein bands on polyacrylamide gel for 60 g weight of narcissus bulbs with 50 mg/l gibberellin ( $GA_3$ ).

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

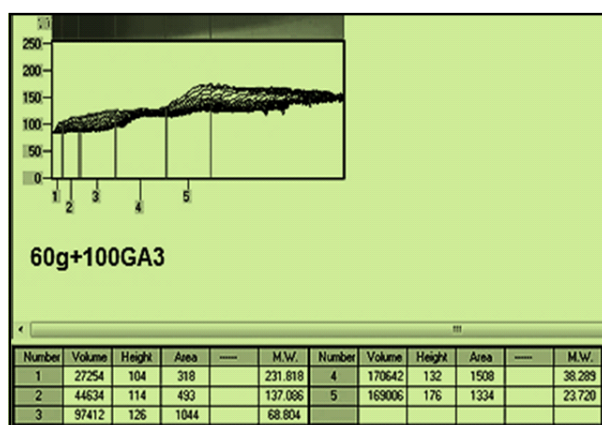


Fig. 13. Some specifications of protein bands on polyacrylamide gel for 60 g weight of narcissus bulbs with 100 mg/l gibberellin ( $GA_3$ ).

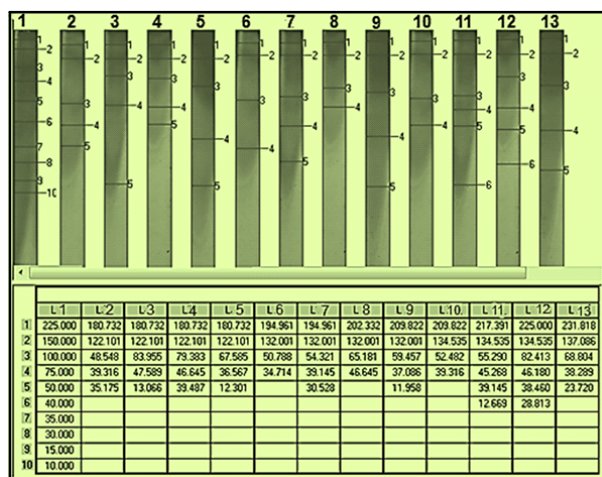


Fig. 14. The number and locations of protein bundles and their molecular weights for different weights of narcissus bulbs treated with different concentrations of gibberellins (a side of the photocapt program).

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 ( $GA_4+7$ ) 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](https://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](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 Jardín Botánico de Madrid* **74**: 07-15. <https://dialnet.unirioja.es/servlet/articulo?codigo=6088487>.