The Effect of Environmental Stress on the Protein Pattern of *Gladiolus* Spp.

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Abstract. This study was conducted to investigate the relationship between the type of environmental stress and the protein for *Gladiolus* Spp plants grown in different stress conditions. The results showed differences in the number and location of protein bands. The number of protein bands ranged between 5 and 7 depending on the environmental stress. Hence, the protein bands. The molecular weights of the first protein band for all stress conditions converged more clearly, ranging from (248.94 to 253.12) KDa. The flooding and drought conditions affected at same level in the first bad, giving the highest molecular weight 252.08 KDa. Results revealed the efficiency of protein pattern analysis in determining the effect of nature of stress of different environmental factors on *Gladiolus* plant.

Keywords: *Gladiolus*, Protein Pattern.

1. Introduction

*Gladiolus* is an annual summer crop belongs to the Iridaceae family. The plant of this family are generally used us cutting flowers, is known by many names as dalboth , sword flower due to its sword shaped leaves or crow sword . it is one of the most important flowers of high demand and is cultivated in most countries of the word for its attractive flowers with different size and colors including red , pink , blue , white , yellow and orange , in addition to long flowery life . *Gladiolus* is the second largest producer in the European countries after the tulip and the fourth in the global trade of cut flowers as production *Gladiolus* flowers reaches 8.5% of the world production of bulb flowers [1; 2; 3]

Plant growth is influenced by many agricultural and environmental factors such as salinity, high and low temperatures and light [4; 5] Salt stress is one of the most causes plant exposure to second type of stress , which is water stress in which Salinity reduces the plant ability to water absorption. In general, most of the plant stress cause the apparent lack of protein transfer and the lack of synthesis of nucleic acids in plant tissues [6]. [7] Explained the mechanism of the direct effect of salinity on the plant by configuring of some morphological, structural and anatomical changes in the plant. The identification of resistance to stress includes influential molecules leading to the mechanism of adaptation reactions of the developing plant under stress conditions. These influencing molecules are proteins and metabolic substances such as Proline interferes with the regulation of biological processes within the plant in response to stress conditions to ensure resistance to these conditions [8]. Which can be used in genetic characterization such as protein or enzymatic markers.

The study was conducted to investigate the genetic differences of the *Gladiolus* grown in different stress conditions using Polyacrylamide gel electrophoresis.
2. Materials and methods
This study was carried out on gladiolus plants, which were healthy and homogeneous plants of volume and shape planted in plastic pots containing the beat moss produced by the German company GmpHco that treated for two months by the following treatments:

1. Salinity treatments: irrigation of plants with water containing sodium at different concentration (0, 500, 1000 and 2000) mg L⁻¹.
2. Irrigation treatments: irrigation of plants at different times (one irrigation per day, one irrigation every two days, one irrigation every five days, one irrigation every ten days).

Samples were taken from the leaves and the samples were dried with the Freeze-dryer (Lyophilization technique) and at a temperature of -26°C. Extract the protein from the samples according to the method described in [9] by taking 1 g of the leaves and placed it in a ceramic mortar with 3 ml of the Tris-HCl buffer (0.1 M, PH7.5) containing phenyl methane sulfony fluoride (PMSF) at 4°C. Centrifuge was performed at 4°C and 1800 Rm speed for half an hour and 40 microliters of leachate was used in Polyacrylamide gel electrophoresis.

Proteins electrophoresis was carried out on a polyacrylamide gel using the Slab-Electrophoresis method with the presence of SDS according to the method described by [10; 11] and the Broad Range Protein Molecular Weight Markers were used from Promega. Molecular weights of proteins were determined and drew through a special software Photo Capt Mw version 7. Treatments got the following numbers:

1. marker 2- control (0 mg L⁻¹) 3- salinity (500 mg L⁻¹) 4- salinity (1000 mg L⁻¹) 5- salinity (2000 mg L⁻¹) 6- one irrigation every two days 7-one irrigation every five days 8- one irrigation every ten days 9- one irrigation every ten day.

3. Results and discussion
From the observation of the results of the protein pattern of the leaves of the gladiolus plant (Fig. 1-a,b,c and d), there are differences between the plants for all the stress conditions, in which Protein bands varied in size, area and height. As shown in Fig.2, there are differences between these plants in the number, location and characteristics of Protein bands on Polyacrylamide gel, the number of protein bands varies between 4-7 depending on the stress condition. There were 6 protein bands in each of the salinity conditions of 0 mg L⁻¹ and 5 protein bands for drought conditions (irrigation every 10 days). Stress conditions had a significant effect in changing protein location.
Figure 1-a. Some specifications of protein bands on protein bands on polyacrylamides gel for grown gladiolus plants under different stress conditions.

Figure 1-b. Some specifications of protein bands on protein bands on polyacrylamides gel for grown gladiolus plants under different stress conditions.
Figure 1-c. Some specifications of protein bands on protein bands on polyacrylamides gel for grown gladiolus plants under different stress conditions.
Figure 2. Number and location of protein bands and their molecular weights of the gladiolus plants grown under different stress conditions.

The molecular weights of the first protein band ranging from 248.94 to 253.12 kDa (Table 1), and converged significantly and clearly for all conditions for all treated plants, the second batch recorded the highest molecular weight value (220.08) kDa for all, in the drought and salt stress conditions. In the third group we found that the conditions of flood stress (daily irrigation) and drought stress conditions (irrigation every 10 days) did not cause changes in the molecular weight of protein bundles compared with control treatment, which amounted to (146.99) kDa. Flood stress had a similar effect on the molecular weight of the fourth protein band (daily irrigation and irrigation every two days) with
144.51 Kd. In the fifth band, all conditions varied in terms of the molecular weight of the protein band. The drought stress conditions (irrigation every 10 days) caused the disappearance of the sixth protein band and gave the lowest molecular weight of the sixth protein band under drought stress (irrigation every 5 days) amounted 3.57kDa. Conditions of salt stress led to appearance a new protein band (2000 mg.L⁻¹) which surprised 94.04 kDa compared with control treatment.

These results indicated that the exposure of plants to different stress condition may result in a lack of synthesis of natural proteins and may occur changes in the translation and transcription, which leads to the production of new proteins by Gene expression according to the need of the plant and response to the type of stress that is exposed to ensure the control of these conditions [12]. This study concludes that Proteins electrophoresis on a polyacrylamide gel can be used to find genetic differences between plants that develop under different environmental conditions and recommend to be used as a accurate easy chemical method.

| Table 1. Protein bands and their molecular weights for gladiolus plants grown under different stress condition (KDa). |
| Protein bands | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| marker | 254.16 | 146.68 | 139.38 | 133.24 | 96.42 | 65.47 | 19.04 |
| salinity(0mg.L⁻¹) | 253.12 | 143.66 | 132.54 | 84.52 | 40.47 | 16.66 | 0 |
| one irrigation every day | 252.08 | 219.10 | 146.78 | 144.11 | 140.22 | 90.47 | 0 |
| salinity(500mg.L⁻¹) | 251.04 | 219.10 | 143.66 | 140.99 | 133.24 | 124.01 | 94.04 |
| one irrigation every five days | 252.08 | 220.08 | 146.99 | 146.78 | 86.90 | 3.57 | 0 |
| one irrigation every two days | 248.94 | 217.15 | 146.78 | 144.51 | 140.22 | 90.47 | 0 |
| salinity(1000mg.L⁻¹) | 252.08 | 148.03 | 146.78 | 143.66 | 139.81 | 91.66 | 0 |
| salinity(2000 mg.L⁻¹) | 251.04 | 220.08 | 216.17 | 146.78 | 144.69 | 140.22 | 94.04 |
| one irrigation every ten days | 252.08 | 216.17 | 146.78 | 140.62 | 92.85 | 0 | 0 |

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