



Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

***Molecular and enzymatic response to drought stress in wheat
(Triticum aestivum L.)***

**Haddad A. El Rabey^{1,2,*}, Fahad M. Almutairi³, Mohamed I. Sakran^{4,5}, Mazen A. Zamzami^{6,7},
Abdulbasit I. Al-Sieni⁸**

¹ Biochemistry Department, Faculty of Science, University of Tabuk, Saudi Arabia

^{2,*} Genetic Engineering and Biotechnology Institute, Sadat City University, Sadat City PO Box 79, Minufiya, Egypt

*Corresponding author: Elrabey@hotmail.com; Mobile: 00966536292730

³ Biochemistry Department, Faculty of Science, University of Tabuk, Saudi Arabia.
Email: Falrabae@ut.edu.sa, Tel. 00966500049390

⁴ Biochemistry Department, Faculty of Science, University of Tabuk, Saudi Arabia.

⁵ Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, Egypt
Email: Msakran2011@gmail.com Tel: 00966530229783

⁶ Department of Biochemistry, Cancer Metabolism and Epigenetic Unit, Faculty of Science, King Abdulaziz
University, Jeddah, Saudi Arabia.

⁷ Cancer and Mutagenesis Unit, King Fahad Center for Medical Research, King Abdulaziz University, Jeddah, Saudi
Arabia. Email: Mzamami@kau.edu.sa Tel: 00966506562400

⁸ Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
Email: Aalsieni@kau.edu.sa, Tel. 00966557085211

ABSTRACT

The activity of catalase, peroxidase and polyphenol oxidase during seedling stage under drought stress in four Egyptian wheat cultivars was studied. Giza 168 and Sakha 93 are drought tolerant, whereas Gemmiza 9 and Sakha 95 are sensitive to drought. For drought stress experiment, two-week seedling was subjected gradually to osmotic stress at three different polyethylene glycol concentrations; 6.9, 13.75 and 27.5 g/l PEG 6000. A 700 bp band was scored only in the drought tolerant wheat cultivars (Giza 168 and Sakha 93) and absent in the drought sensitive cultivars (Gemmiza 9 and Sakha 95). Enzymatic activity of catalase, peroxidase and polyphenol oxidase was assayed at 4 time points (0 control, 24 hrs, 3days, and 7 days) to evaluate their activity in the four germplasms under drought stress. The activity of the three genes was gradually increased by time and by increasing the PEG concentration. In conclusion, LEA gene is correlated to drought stress. Moreover, catalase, peroxidase and polyphenol oxidase activity in wheat seedlings of cultivars under study increased by increasing PEG concentration and the time.

Keywords: catalase, peroxidase, polyphenol oxidase, drought, polyethylene glycol, wheat.

Conflict of interest: The authors declare that they have no conflict of interest.

INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to *Poaceae* family. It occupies the world first rank for human stable food. Ninety-five percent of wheats grown today are of the hexaploid type and the other 5% is durum (*Triticum turgidum*

L., var. *durum*) tetraploid wheat [1-7]. The wheat genome is 17000 Mb [2, 8-11]. Expression of plant genes changes under drought stress [3, 12-18].

Drought is abiotic stress that affects vital biological processes and limits crop production, delay or lack of crop establishment, plant weakening or loss, susceptibility to pest or disease attack and changes in physiological and biochemical responses [4, 19-24]. Reactive oxygen species (ROS) accumulate under drought stress in plants [5, 25-27]. Adaptation to drought stress leads to generation of oxidative stress, high H₂O₂ levels and poor antioxidant enzyme response that generate enhanced membrane damage during [5, 6, 28-30]. Drought stress affected the total protein content, relative water content and photosynthetic pigments of 36 Iranian wheat landraces [7, 31-33].

High-expression levels of total glutathione S-transferase and glutathione peroxidase during grain filling in flag leaves of wheat subjected to drought stress, indicating that the gene products of these genes may play important roles in monocarpic senescence of wheat [8, 34-38]. The expression of 15 genes was up-regulated under heat, drought and combined stress condition in durum wheat by cDNA-AFLP [9, 39, 40]. Glutamine synthetase can be applied for the characterization of wheat cultivars in terms of drought stress tolerance as an indicator of drought stress [10, 41-44].

Molecular markers measure the genetic differences of the genome and may help as markers of different traits like drought tolerance [11, 45-47]. It was successively used in identifying drought tolerant cultivars in *Triticeae* [12,13, 48-51]. Identification of genes participating in drought tolerance reactions can help in understanding the biochemical and physiological basis of stress tolerance in wheat [14, 52-54]. Ali-Benali et al. [15, 55-58] stated that LEA proteins are encoded by multigenic families. Wheat seedlings contain group 3 LEA genes [16, 17, 59, 60].

LEA gene as a molecular marker was used to distinguish between the drought sensitive wheat cultivars from the drought tolerant ones. The expression of catalase, peroxidase and polyphenol oxidase genes in the four wheat cultivars was monitored under different water regimes using polyethylene glycol at three different concentrations (6.9, 13.75 and 27.5 g/l PEG 6000) at four time points (Zero point as control, 24 hours, 3 days and 7 days).

Materials and methods

Four wheat cultivars were kindly supplied by Wheat Research Center at Agricultural Research Center, Giza, Egypt and subjected to drought treatment at Biochemistry Department, Faculty of Science, King Abdulaziz University. Giza 168 and Sakha 93 wheat cultivars are drought tolerant, whereas Gemmiza 9 and Sakha 95 are drought sensitive. For drought stress experiment, seeds of the four wheat cultivars were germinated in small pots filled with organic soil for two weeks and then subjected to osmotic stress using polyethylene glycol (PEG 6000) under three different stress conditions according to Sané et al. [18], using polyethylene glycol at the following concentrations: 6.9, 13.75 and 27.5 g/l PEG 6000 for 4 time points (0 control, 24 hrs, 3days, and 7 days). Root and shoot material were harvested at each time point, frozen in liquid nitrogen, ground into a fine powder, and kept in -80°C until use.

DNA extraction

DNA of the four wheat germplasms under study was extracted from fresh growing leaves of 14-day age seedling using DNeasy Plant Mini Kit (250) from QIAGEN according to the instructions of the supplier.

LEA gene PCR amplification

Primer design:

The LEA gene (DQ663481) primer was designed by Wang et al. [19] and constructed by Metabion International AG, D-82152 Martinsried, Germany. LEA Gene primer sequence is as follows:

LEA forward primer: 50-ATGGCTCGCTGCTCTTACTC-30,

LEA reverse primer: 50-TCAGTGAGAGGATCGATTGAAC-30

PCR condition

LEA gene was amplified in a volume of 25 µl, containing 50 ng genomic DNA in 2 µl, 2.5 µl 10X PCR buffer, 1.5 µl of 25 mM MgCl₂, 1 µl of the forward and reverse primers (250 nM), 2 µl of 0.2 mM dNTPs (from Promega) and 0.3 µl *Taq* DNA polymerase (GoTaq Flexi DNA polymerase from Promega) and 14.7 µl bidistilled sterilized water. Amplification was achieved as described by Temnykh et al. (2000). Briefly, 5 min at 94°C followed by 35 cycles of 1 min 94°C, 1 min 55°C and 2 min at 72°C, with a final extension of 5 min at 72°C. The PCR product was photographed after resolving on 1.5% agarose gel against 1 kb DNA ladder.

Antioxidant enzymes measurements

Preparation of crude extract

1 g of frozen seedling was homogenized in 20 mM Tris-HCl buffer, pH 7.2 using homogenizer, and then centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant is the crude extract was then transferred and stored at -20 °C for further analysis.

Catalase assay

Catalase activity was assayed according to Bergmeyer [21].

Peroxidase assay

Peroxidase activity was assayed according to the method of Yuan and Jiang [22].

Polyphenol oxidase assay

Polyphenol oxidase assay (PPO) activity was assayed with catechol as a substrate according to the method of Jiang et al. [23].

Statistical analysis

All data were analyzed using the SPSS (Statistical Program for Sociology Scientists) Statistics Version 17.0 to calculate the mean values and the standard errors (SE). In addition, analysis of variance (ANOVA) was calculated using Duncan's Multiple Range Test [24].

Results

LEA gene

Figure (1) shows the PCR amplification of the genomic DNA of the studied wheat cultivars using the specific LEA gene primers. A 700 bp band presents only in the drought tolerant wheat cultivars (Giza168 and Sakha93) and absent in the drought sensitive ones (Gemmiza9 and Sakha95).

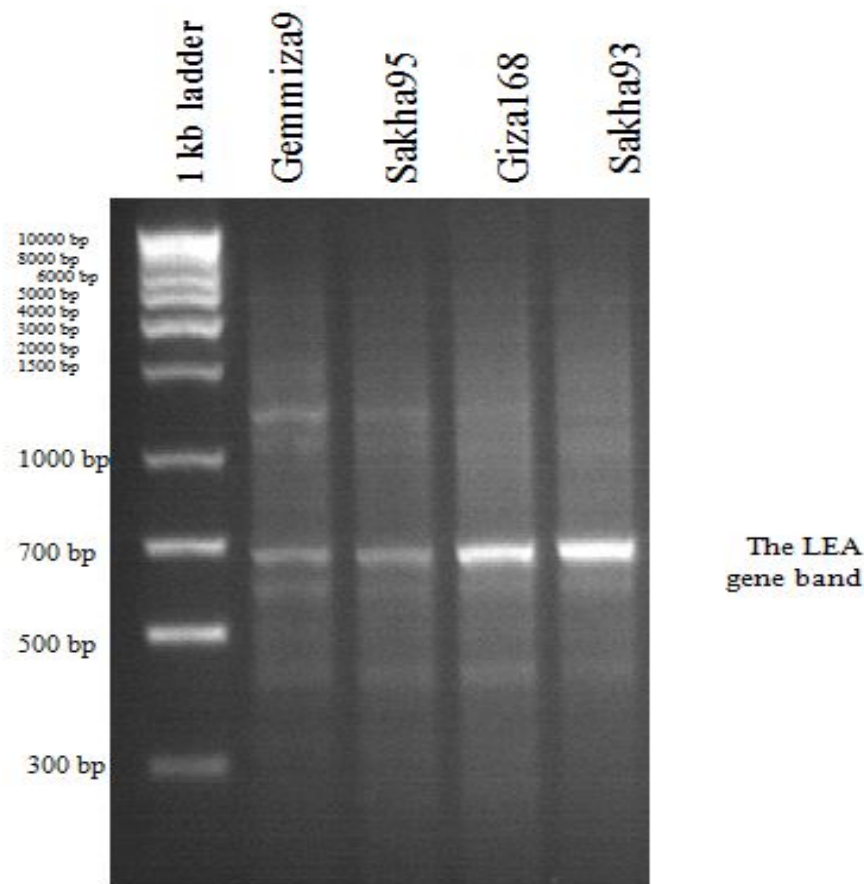


Figure (1): The amplified LEA gene in the studied wheat gemplasm.

Previous studies correlated drought tolerance in wheat to the expression of LEA gene that participate in drought adaptation and provide an integrated understanding of the biochemical and physiological basis of stress responses [14,15].

Catalase activity

Table (1) and Figure (2) show mean values of catalase activity in the studied wheat cultivars seedlings under drought stress induced by PEG treatment. At the zero time point the activity of catalase in the sensitive wheat germplasm (Gemmiza9 and Sakha95) was higher than that of the tolerant ones (Giza168 and Sakha93). After 24 hours of treating the seedlings with the lowest PEG concentration (6.9 g/l PEG), the activity of catalase was significantly increased in the drought sensitive and the drought tolerant cultivars compared with the activity of the zero-time point. Gemmiza9 showed the lowest catalase activity, whereas Sakha93 showed the highest one. After 3 days, the catalase activity was also significantly increased in all wheat cultivars. The sensitive cultivars showed higher activity than the tolerant ones. In contrast, Sakha93 showed the lowest catalase activity compared to its value after 24 hours. After seven days of PEG treatment using the lowest concentration, the sensitive cultivars showed the highest catalase activity compared with the tolerant ones. Sakha93 showed the lowest catalase activity, whereas Sakha95 showed the highest catalase activity.

Table (1): Catalase activity of the studied wheat cultivars under different drought condition induced by three concentrations of PEG for different time periods.

| Catalase U/g/min | Treatments Statistics | 6.9 g/l PEG | | | | 13.75 g/l PEG | | | 27.5 g/l PEG | | |
|-----------------------|--------------------------|-----------------------------|------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|
| | | Zero time | 24 h | 3 days | 7 days | 24 h | 3 days | 7 days | 24 h | 3 days | 7 days |
| Gemmiza9 Sensitive | Mean±SE | 86.00± 3.46 ^a | 96.00± 1.15 ^b | 120.00± 1.15 ^a | 165.33± 2.90 ^a | 97.60± 1.45 ^a | 124.00± 2.08 ^a | 174.00± 2.08 ^a | 101.00± 2.08 ^a | 132.33± 1.45 ^a | 181.67± 3.75 ^a |
| | Mean±SE | 90.00± 1.15 ^a | 100.00± 0.57 ^b | 120.00± 0.57 ^a | 186.00± 3.46 ^b | 108.00± 1.15 ^b | 130.00± 2.8 ^b | 193.00± 1.73 ^b | 110.00± 1.15 ^b | 135.33± 1.45 ^b | 200.00± 0.57 ^b |
| Giza168 Tolerant | Mean±SE | 74.00± 0.57 ^b | 100.00± 1.73 ^b | 116.00± 0.57 ^b | 153.00± 1.73 ^c | 107.00± 1.15 ^b | 118.00± 0.57 ^{bc} | 155.00± 2.88 ^c | 113.00± 0.57 ^b | 129.00± 0.5 ^{bc} | 167.00± 0.57 ^{bc} |
| | Mean±SE | 73.00± 0.57 ^b | 117.00± 1.15 ^c | 89.00± 0.57 ^c | 118.00± 1.15 ^d | 133.00± 1.73 ^c | 155.00± 2.88 ^c | 138.00± 1.73 ^d | 142.00± 1.15 ^c | 161.00± 0.57 ^c | 175.00± 2.88 ^c |
| LSD 0.05 | | 6.824 | 2.233 | 2.306 | 9.667 | 5.126 | 8.535 | 8.578 | 4.790 | 3.752 | 9.105 |

Means values within a row not sharing a common superscript letter (abcd) were significantly different at $P < 0.05$ by ANOVA. LSD= Least significant difference.

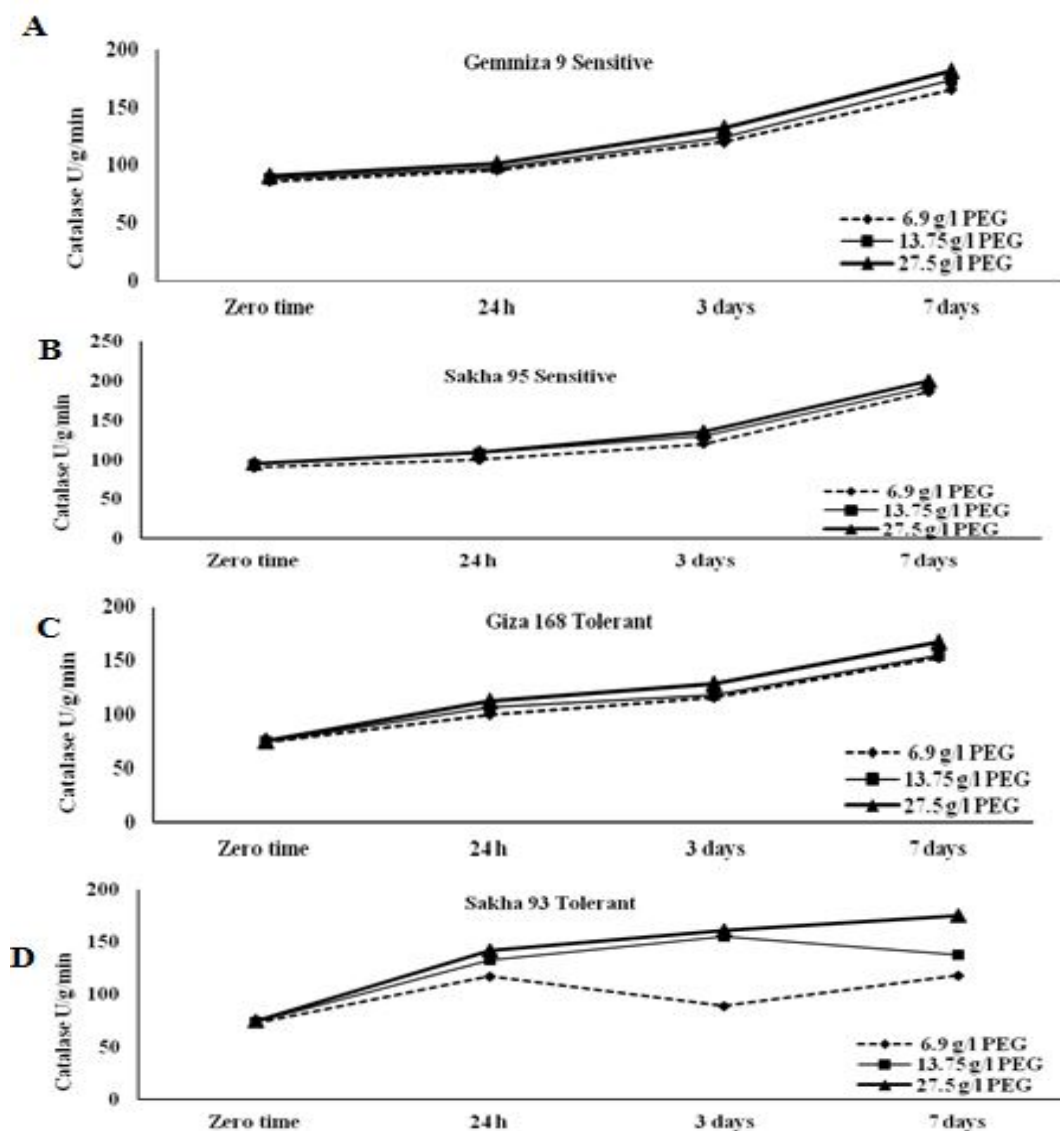


Figure (2): Catalase activity of the studied wheat cultivars as a result of drought stress using 3 different PEG concentrations for 7 days. A: Gemmiza9 (Drought sensitive cultivar), B: Sakha95 (Drought sensitive cultivar), C: Giza168 (Drought tolerant cultivar) and D: Sakha93 (Drought tolerant cultivar).

Table (1) and Figure (2) show also that, treating the seedlings with 13.75 g/l PEG concentration for 24 hours significantly increased the activity of catalase in the drought sensitive and the drought tolerant wheat cultivars compared with the activity at the zero-time point. Gemmiza9 showed the lowest catalase activity, whereas Sakha93 showed the highest one. After three days of PEG treatment, the catalase activity was also significantly increased in all wheat cultivars. Giza168 showed the lowest catalase activity, whereas Sakha 93 showed the highest catalase activity. After seven days, the sensitive cultivars showed the highest catalase activity compared with the tolerant ones. Sakha 95 showed the highest catalase activity, whereas Sakha 93 showed the highest catalase activity. Table (1) and Figure (2) show also that, using the highest PEG concentration (27.5 g/l PEG) after 24 hours, the activity of catalase was significantly increased in the drought sensitive and the drought tolerant cultivars compared with that at the zero-time point. Gemmiza9 showed the lowest catalase activity, whereas Sakha93 showed the highest one. After three days, the catalase activity was also significantly increased in all wheat cultivars as a result of drought stress induced by PEG. Giza168 showed lowest catalase activity, whereas Sakha93 showed the lowest catalase activity. In contrast, Sakha93 showed the lowest catalase activity compared to its value after 24 hours. After seven days of PEG treatment using the highest concentration, the catalase activity showed the highest values compared with other treatments. Sakha95 showed the highest catalase activity, whereas Giza168 showed the lowest activity.

This result agrees with that of Wang [25] who stated that catalase activity was increased in strawberry leaves subjected to drought stress and Huseynova [5] who stated that drought-tolerant wheat cultivars showed a significant increase in catalase, glutathione reductase and ascorbate peroxidase activity as a result of drought stress. It is also worthy to mention that the current result does not support that of Kar and Mishra [26] who noted that the activity of catalase decreased in rice leaves senescence stage as a result of drought stress.

Peroxidase activity

Table (2) and Figure (3) show mean values of peroxidase activity as a result of treating the wheat cultivars under study with different PEG concentrations for different time periods. At the zero-time point, the activity of peroxidase in the sensitive wheat germplasms (Gemmiza9 and Sakha95) was lower than that of the tolerant one (Giza168 and Sakha93). After 24 hours, the activity of peroxidase was significantly increased in the drought sensitive and the drought tolerant wheat cultivars compared with the activity at the zero-time point. Gemmiza9 showed the lowest peroxidase activity, whereas Sakha93 showed the highest one. After three days, the peroxidase activity was also significantly increased in all wheat cultivars. Giza168 showed the lowest peroxidase activity, whereas Gemmiza9 showed the highest one. After seven days of PEG treatment using the lowest PEG concentration, Sakha93 showed the highest peroxidase activity, whereas Gemmiza9 showed the lowest one.

Table (2): Peroxidase activity of the studied wheat cultivars under different drought condition induced by three concentrations of PEG for different time periods.

| Peroxidase U/g/min | Treatments Statistics | 6.9 g/l PEG | | | | 13.75 g/l PEG | | | 27.5 g/l PEG | | |
|-----------------------|--------------------------|-------------------------------|------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|------------------------------|
| | | Zero time | 24 h | 3 days | 7 days | 24 h | 3 days | 7 days | 24 h | 3 days | 7 days |
| Gemmiza9 Sensitive | Mean±SE | 491.00± 1.73 ^a | 530.00± 2.88 ^a | 980.00± 2.88 ^a | 1038.0± 7.50 ^a | 550.00± 4.04 ^a | 1032.0± 4.04 ^a | 1211.0± 3.46 ^a | 671.00± 6.35 ^a | 1100.0± 2.88 ^a | 1392.0± 1.15 ^a |
| Sakha95 Sensitive | Mean±SE | 436.33± 27.09 ^a | 678.00± 4.04 ^b | 855.00± 1.15 ^b | 1392.0± 0.115 ^b | 737.00± 4.04 ^b | 950.00± 5.19 ^a | 1545.7± 2.02 ^b | 757.00± 1.73 ^b | 1050.0± 5.77 ^b | 1785.0± 1.73 ^b |
| Giza168 Tolerant | Mean±SE | 579.00± 4.93 ^b | 638.00± 8.08 ^c | 658.00± 6.92 ^c | 1315.0± 1.73 ^c | 690.00± 5.77 ^c | 922.00± 12.70 ^b | 1654.0± 2.30 ^c | 824.00± 15.01 ^c | 1025.0± 14.43 ^c | 1643.0± 1.73 ^b |
| Sakha93 Tolerant | Mean±SE | 592.00± 2.30 ^c | 768.00± 4.61 ^b | 918.00± 1.15 ^b | 1702.0± 4.04 ^b | 966.00± 2.30 ^d | 1056.0± 3.46 ^c | 1635.0± 2.88 ^d | 1103.3± 7.68 ^d | 1179.0± 5.19 ^c | 1641.0± 3.46 ^c |
| LSD 0.05 | | 46.922 | 21.057 | 13.088 | 15.972 | 14.556 | 24.168 | 10.236 | 33.762 | 31.631 | 7.802 |

Means values within a row not sharing a common superscript letter (abcd) were significantly different at $P < 0.05$ by ANOVA. LSD= Least significant difference.

Table (2) and Figure (3) show also that, after 24 hours of treating the wheat cultivars seedlings with 13.75g/l PEG concentration, the activity of peroxidase was significantly increased in the drought sensitive and the drought tolerant wheat cultivars compared with the activity at the zero-time point. Gemmiza9 showed the lowest peroxidase activity, whereas Sakha93 showed the highest one. After three days, the peroxidase activity was also significantly increased in all wheat cultivars. Giza168 showed the lowest peroxidase activity, whereas Sakha93 showed the highest one. After 7 days of PEG treatment, Gemmiza9 showed the highest peroxidase activity, whereas Sakha95 showed the lowest peroxidase activity.

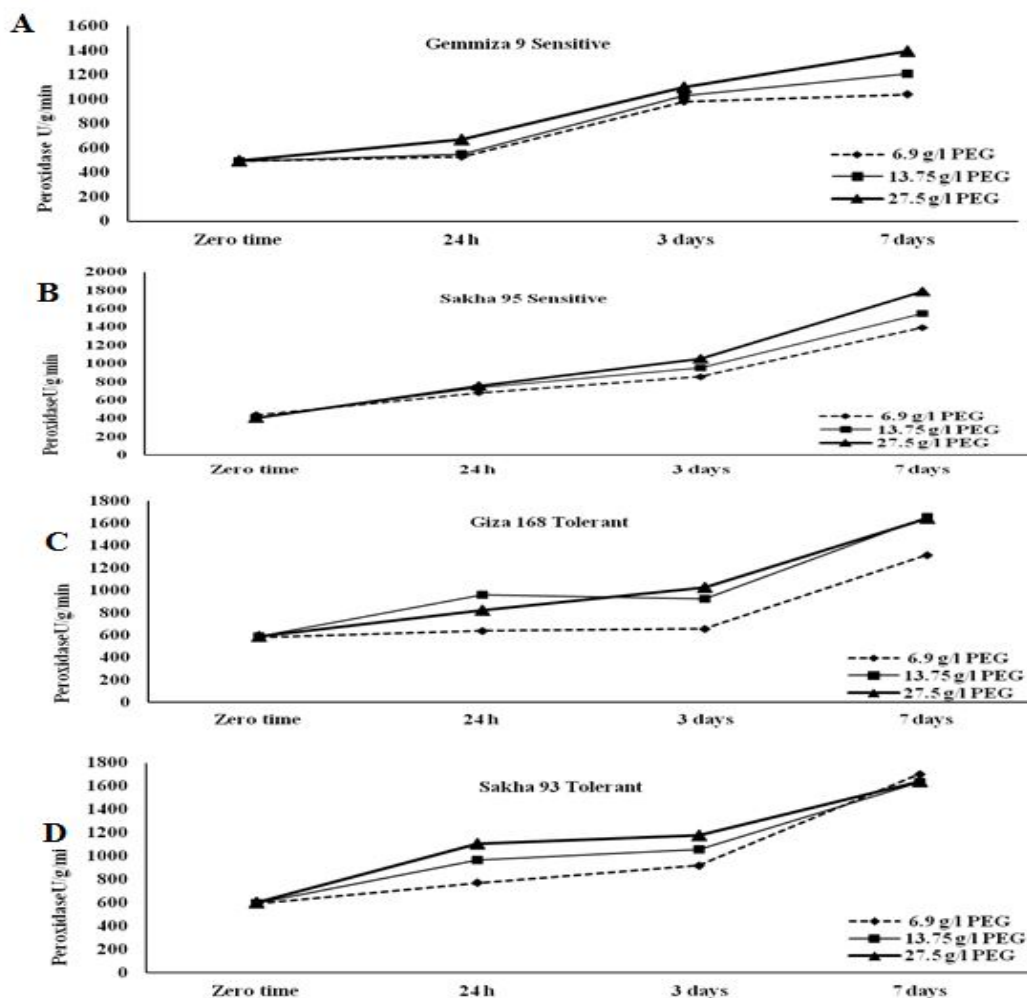


Figure (3): Peroxidase activity of the studied wheat cultivars as a result of drought stress using 3 different PEG concentrations for 7 days. A: Gemmiza9 (Drought sensitive cultivar), B: Sakha95 (Drought sensitive cultivar), C: Giza168 (Drought tolerant cultivar) and D: Sakha93 (Drought tolerant cultivar).

Table (2) and Figure (3) show also that, after 24 hours of treating the wheat cultivars seedlings with 27.5 g/l PEG concentration, the activity of peroxidase was significantly increased in the drought sensitive and the drought tolerant wheat cultivars compared with the activity at the zero-time point. Gemmiza9 showed the lowest peroxidase activity, whereas Sakha93 showed the highest one. After three days, the peroxidase activity was also significantly increased in all wheat cultivars. Giza 168 showed the lowest peroxidase activity, whereas Sakha93 showed the highest one. After seven days of PEG treatment, Sakha95 showed the highest peroxidase activity, whereas Gemmiza9 showed the lowest peroxidase activity.

The current result is supported by that of Kar and Mishra [26], Wang [25] and Huseynova [5]. On the other hand, Csiszár et al. [27] stated that peroxidase activity decreased under osmotic stress in the tolerant wheat cultivars than that of the sensitive cultivars.

Polyphenol oxidase activity

Table (3) and Figure (4) show the mean values of polyphenol oxidase activity under drought stress condition resulted from treating the wheat cultivars under study with PEG. At the zero-time point, the activity of polyphenol oxidase in Giza168 seedlings was the lowest value, whereas Sakha 93 was the highest one. After 24 hours of treatment with the lowest PEG concentration (6.9 g/l PEG), the activity of polyphenol oxidase was significantly increased in the drought sensitive and the drought tolerant wheat cultivars compared with the activity at the zero-time point. Gemmiza9 showed the lowest polyphenol oxidase activity, whereas Sakha93 showed the highest one. After three days of PEG treatment, the polyphenol oxidase activity was also significantly increased in all wheat cultivars. Gemmiza9 showed the lowest polyphenol oxidase activity, whereas Sakha93 showed the highest one.

After seven days of PEG treatment using the lowest PEG concentration, Giza168 showed the highest polyphenol oxidase activity, whereas Gemmiza9 and Sakha95 showed the lowest ones.

Table (3): Polyphenol oxidase activity of the studied wheat cultivars under different drought condition induced by three concentrations of PEG for different time periods.

| Polyphenol oxidase U/g/min | Treatments Statistics | 6.9 g/l PEG | | | | 13.75 g/l PEG | | | 27.5 g/l PEG | | |
|----------------------------|-----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|
| | | Zero time | 24 h | 3 days | 7 days | 24 h | 3 days | 7 days | 24 h | 3 days | 7 days |
| Gemmiza9 Sensitive | Mean±SE | 19.40± | 21.25± | 27.10± | 40.40± | 21.70± | 35.00± | 48.00± | 40.60± | 39.50± | 75.00± |
| | | 0.11 ^a | 0.01 ^a | 0.05 ^a | 0.23 ^a | 0.05 ^a | 0.57 ^a | 0.57 ^a | 6.30 ^a | 0.28 ^a | 1.15 ^a |
| Sakha95 Sensitive | Mean±SE | 18.20± | 27.100± | 33.60± | 40.00± | 31.25± | 35.00± | 45.80± | 32.50± | 48.40± | 62.90± |
| | | 0.05 ^b | 0.05 ^b | 0.11 ^b | 0.57 ^b | 0.14 ^b | 1.15 ^a | 0.17 ^a | 0.11 ^a | 0.11 ^b | 0.23 ^b |
| Giza168 Tolerant | Mean±SE | 12.40± | 33.30± | 45.00± | 71.00± | 33.53± | 48.40± | 74.20± | 50.40± | 79.60± | 79.60± |
| | | 0.05 ^c | 0.05 ^c | 0.11 ^c | 0.28 ^c | 0.17 ^c | 0.23 ^b | 0.46 ^b | 0.23 ^{ab} | 0.11 ^c | 0.34 ^c |
| Sakha93 Tolerant | Mean±SE | 21.50± | 40.00± | 48.00± | 58.40± | 41.00± | 50.30± | 73.75± | 44.20± | 73.00± | 100.00± |
| | | 0.05 ^d | 0.11 ^d | 0.23 ^d | 5.90 ^c | 0.17 ^d | 0.17 ^b | 0.43 ^c | 0.11 ^b | 0.05 ^d | 0.63 ^d |
| LSD 0.05 | | 0.251 | 0.276 | 0.450 | 9.815 | 0.541 | 2.118 | 1.967 | 10.741 | 0.623 | 2.705 |

Means values within a row not sharing a common superscript letter (abcd) were significantly different at $P < 0.05$ by ANOVA. LSD= Least significant difference.

Table (3) and Figure (4) show also the mean values of polyphenol oxidase activity under drought stress condition resulted from treating the wheat cultivars under study with 13.75 g/l concentration of PEG. After 24 hours of PEG treatment, the activity of polyphenol oxidase was significantly increased in the drought sensitive and the drought tolerant wheat cultivars compared with the activity at the zero-time point. Gemmiza9 showed the lowest polyphenol oxidase activity, whereas Sakha93 showed the highest one. After three days of PEG treatment, the polyphenol oxidase activity was also significantly increased in all wheat cultivars. Gemmiza9 showed also the lowest polyphenol oxidase activity, whereas Sakha93 showed the highest one. After seven days of PEG treatment, Giza168 showed the highest polyphenol oxidase activity, whereas Sakha95 showed the lowest one.

Table (3) and Figure (4) show also the mean values of polyphenol oxidase activity under drought stress condition resulted from treating the wheat cultivars under study with 27.5 g/l concentration of PEG. After 24 hours of PEG treatment, the activity of polyphenol oxidase was significantly increased in the drought tolerant cultivars than the drought sensitive ones and both of them was higher than that of the zero-time point. Sakha95 showed the lowest polyphenol oxidase activity, whereas Giza168 showed the highest one. After three days of PEG treatment, the polyphenol oxidase activity was also significantly increased in all wheat cultivars. Gemmiza9 showed also the lowest polyphenol oxidase activity, whereas Sakha93 showed the highest one. After seven days of PEG treatment, Sakha93 showed the highest polyphenol oxidase activity, whereas Sakha95 showed the lowest one.

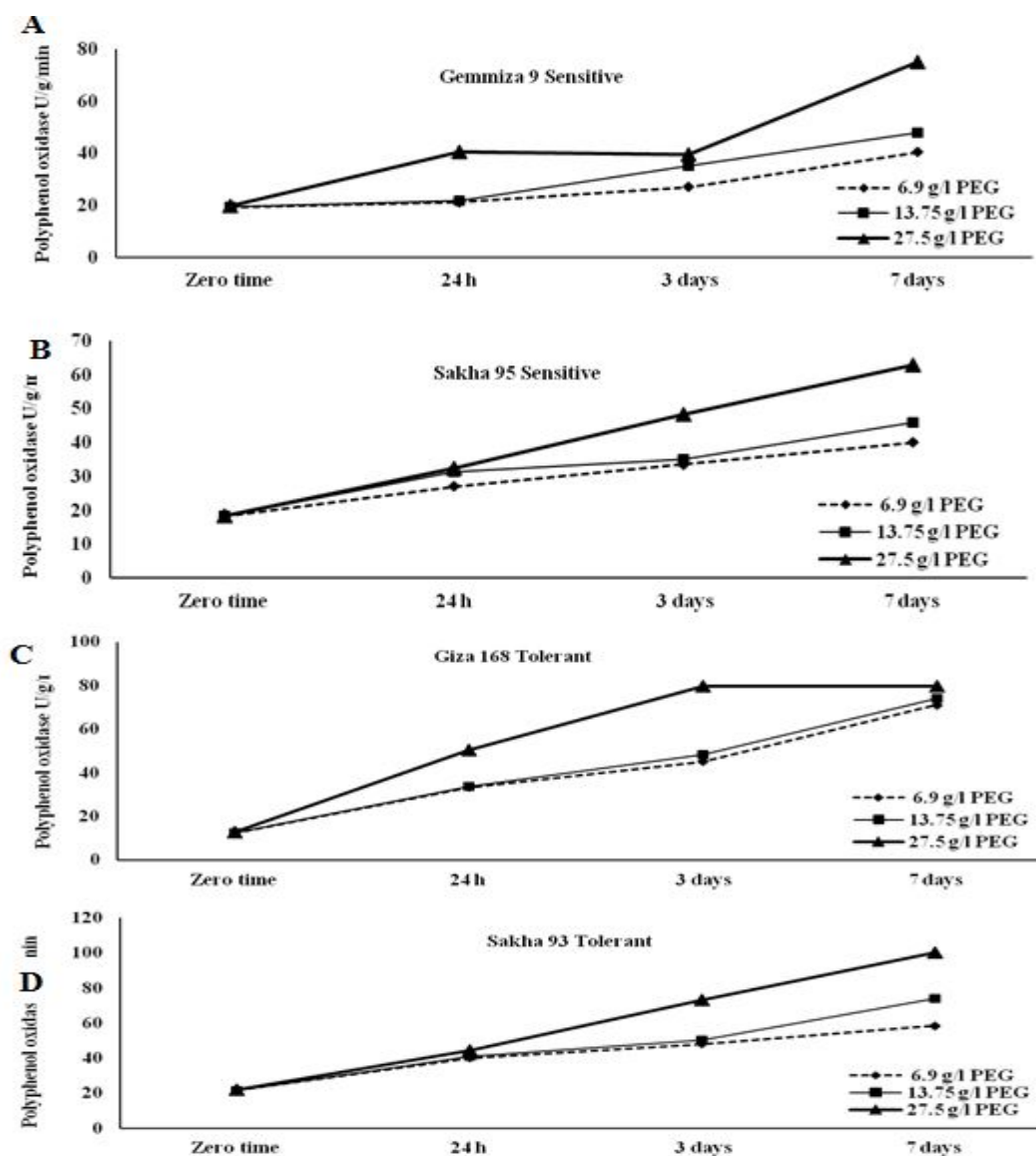


Figure (4): Polyphenol oxidase activity of the studied wheat cultivars as a result of drought stress using 3 different PEG concentrations for 7 days. A: Gemmiza 9 (Drought sensitive cultivar), B: Sakha95 (Drought sensitive cultivar), C: Giza 168 (Drought tolerant cultivar) and D: Sakha 93 (Drought tolerant cultivar).

The activity of polyphenol oxidase in all wheat cultivars under study was also increased as a result of increasing the concentration of PEG and also by increasing the time of treatment. The polyphenol oxidase activity was increased 3-5 folds more than that of the control value (zero point). This result is supported by that of Kar and Mishra [26] who noted that the activity of polyphenol oxidase increased in rice leaves senescence stage as a result of drought stress.

The enzymatic activity of the current study is in agreement with the theory that, plants produce a variety of antioxidants that counteract the generation of ROS in response to drought stress [28]. These include nonenzymatic antioxidants such as tocopherols, carotenoids, ascorbic acid, glutathione, and phenolics, as well as enzymatic antioxidants such as superoxide dismutase, catalase, and enzymes of the ascorbate/glutathione cycle [29] and various responses and adaptations are evolved that enable plants sustain growth and development under water-limited condition (Ashraf et al., 2011).

Conclusion

In conclusion, LEA gene is correlated to drought stress. Moreover, the activity of catalase, peroxidase and polyphenol oxidase in wheat seedlings of cultivars under study increased by increasing PEG concentration and the time.

Reference

- [1] Heun, M., Schäfer-Pregl, R., Klawan, D., Castagna, R., Accerbi, M., Borghi, B., Salamini, F., Site of Einkorn Wheat Domestication Identified by DNA Fingerprinting, *Science*, **1997**, 278 (5341), 1312-1314.
- [2] Hartl, D.L., Jones, E.W, Genome evolution in the grass family. In, genetics and analysis of genes and genomes. Fifth edition, Jones and Bartlett publishers/Sudbury, Massachusetts, USA, **2001**.
- [3] Way, H., Chapman, S., McIntyre, L., Casu, R., Xue, G.P., Manners, G., Shorter, R., Identification of differentially expressed genes in wheat undergoing gradual water deficit stress using a subtractive hybridization approach, *Plant Sci.*, **2005**, 168, 661–670.
- [4] Mohammadi, R., Haghparast, R., Aghaee-sarbarze, M., Abdollahi, A.V., An evaluation of drought tolerance in advanced durum wheat genotypes based on physiologic characteristics and other related indices, *J. Agric. Sci.*, **2006**, 37, 561-567.
- [5] Huseynova, I.M., Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought, *Biochim Biophys Acta*, **2012**, 1817, 1516-1523.
- [6] Khanna-Chopra, R., Selote, D.S., Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than -susceptible wheat cultivar under field conditions, *Environ. Exp. Bot.*, **2006**, 60, 276–283.
- [7] Dorostkar, S., Dadkhodaie, A., Heidari, B., Effects of drought stress on protein, photosynthetic pigments and relative water content of some Iranian wheat landraces, *Adv. Crop. Sci.*, **2013**, 13 (9), 646–656.
- [8] Galle, A., Csiszar, J., Secenji, M., Guóth A, Cseuz L, Tari I, Gyorgyey J and Erdei E., Glutathione transferase activity and expression patterns during grain filling in flag leaves of wheat genotypes differing in drought tolerance: Response to water deficit, *J. Plant Physiol.*, **2009**, 166, 1878-1891.
- [9] Rampino, P., Mita, G., Fasano, P., Borrelli, G.M, Aprile, A., Dalessandro, G., De Bellis L., Perrotta, C., Novel durum wheat genes up-regulated in response to a combination of heat and drought stress, *Plant Physiol. Biochem.*, **2012**, 56, 72-78.
- [10] Nagy, Z., Németh, E., Guóth, A., Bona, L., Wodala, B., Pécsváradi, A., Metabolic indicators of drought stress tolerance in wheat: Glutamine synthetase isoenzymes and Rubisco. *Plant Physiology and Biochemistry*, *Plant Physiol. Biochem.*, **2013**, 67, 48-54.
- [11] Teulat, B., This, D., Hhairallah, M., Borries, C., Ragot, C., Sourdille, P., Leroy, P., Monneveux, P., Charrier, A., Several QTLs involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.), *Theor. Appl. Genet.*, **1998**, 96, 688-698.
- [12] Bibi, S., Dahot, M.O., Nizamani, G.S., Khan, I.A., Khatri, A., Naqvi, M.H., Oad, F.C., Burio, U.A., Molecular marker assisted selection for drought tolerant wheat genotypes, *Pak. J. Bot.*, **2010**, 42(4), 2443-2452.
- [13] El Rabey, H.A., Khan, J.A., Abulnaja1, K.O., Al-Malki, A.L., Molecular characterization of barley (*Hordeum vulgare* L.) genome for drought tolerant cultivars selection, *Afr. J. Biotech*, **2012**, 11(40), 9527-9533.
- [14] Chang-Xing, Z, Ling-Yu, G, Abdul Jaleel, C, Hong-Bo, S, Hong-Bing, Y., Prospectives for applying molecular and genetic methodology to improve wheat cultivars in drought environments. *C. R. Biologies*, **2008**, 331, 579-586.
- [15] Ali-Benali, M.A., Alary, R., Joudrier P., Franc, M. and Gautier, O., Comparative expression of five Lea Genes during wheat seed development and in response to abiotic stresses by real-time quantitative RT-PCR, *Biochim. Biophys. Acta.*, **2005**, 1730, 56-65.

- [16] Curry, J., Morris, C.F., Walker-Simmons, M.K., Sequence analysis of a cDNA encoding a group 3 LEA mRNA inducible by ABA or dehydration stress in wheat, *Plant Mol. Biol.*, **1991**, 16, 1073–1076.
- [17] Ried, J.L., Walker-Simmons, M.K., Group 3 late embryogenesis abundant proteins in desiccation-tolerant seedlings of wheat (*Triticum aestivum* L.), *Plant Physiol.*, **1993**, 102, 125–131.
- [18] Sané, D., Kneyta, M.O., Diouf, D., Diouf, D., Badiane, F.A., Sagna, M., Borgel, A., Growth and development of date palm (*Phoenix dactylifera* L.) seedlings under drought and salinity stresses, *Afr. J. Biotechnol.*, **2005**, 4 (9), 968-972.
- [19] Wang, Y., Jiang, J., Zhao, X., Liu, G., Yang, C., Zhan, L., A novel LEA gene from *Tamarix androssowii* confers drought tolerance in transgenic tobacco, *Plant Sci*, **2006**, 171, 655–662.
- [20] Temnykh, S., DeClerck, G., Lukashova, A., Lipovich, L., Cartinhour, S., McCouch, S., Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.), *Theor Appl Genet.*, **2000**, 100, 697.
- [21] Bergmeyer, H.U., *Methods of Enzymatic Analysis*, Volume 1, 2nd edition, Edited by Bergmeyer, H.U., Academic press, New York, **1974**, pp. 438.
- [22] Yuan, Z.Y., Jiang, T.J., *Horseradish peroxidase*. In Handbook of Food Enzymology. Edited by Whitaker JR, Voragen A, Wong DWS. Marcel Dekker Inc., New York, pp. 403-411, **2003**.
- [23] Jiang, Y.M., Zhang, Z.Q., Joyce, D.C., Ketsa, S, Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.), *Postharvest Biol. Technol.*, **2002**, 26, 241-252.
- [24] SAS, Statistical Analysis System, *SAS User's Guide: Statistics*, version 5 ed. SAS Inst. Inc., Cary, NC, USA, **1986**.
- [25] Wang, S.Y., Methyl jasmonate reduces water stress in strawberry, *J. Plant Growth Regul.*, **1999**, 18, 127–134.
- [26] Kar, M., Mishra, D., Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence, *Plant physiol.*, **1976**, 57, 315-319.
- [27] Csiszár, J, Gallé, A, Horváth, E, Dancsó, P, Gombos, M, Váry, Z, Erdei, L, Györgyey, J, Tari I, Different peroxidase activities and expression of abiotic stress-related peroxidases in apical root segments of wheat genotypes with different drought stress tolerance under osmotic stress, *Plant. Physiol. Biochem.*, **2012**, 52, 119-129.
- [28] Wang, W.B., Kim Y.H., Lee, H.S., Kim, K.Y., Deng, X.P., Kwak, S.S, Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses, *Plant Physiol. Biochem.*, **2009**, 47, 570-577.
- [29] Jaleel, A.C., Sankar, B., Murali, P.V., Gomathinayagam, M., Lakshmanan, G.A., Panneerselvam, R., Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation, *Colloids Surf. B*, **2008**, 62, 105–111.
- [30] Ashraf, M., Akram, N.A., Al-Qurainy, F., Foolad, M.R., *Adv. Agron.*, **2011**, 111, ISSN 0065-2113, DOI: 10.1016/B978-0-12-387689-8.00002-3©2011 Elsevier Inc.
- [31] Ghaderi N, Taymoori P, Yousefi F, Nouri B. The prevalence of Cigarette Smoking among adolescents in Marivan City- Iran: Based on health belief model (HBM). *International Journal of Pediatrics*. 2016. 4(9), pp. 3405-3414.
- [32] Sharifi G, Bakhtevvari MH, Samadian M, Alavi E, Rezaei O. Endoscopic Surgery in Nonhydrocephalous Third Ventricular Colloid Cysts: A Feasibility Study. *World Neurosurg*. 2015 Aug;84(2):398-404. doi: 10.1016/j.wneu.2015.03.033. Epub 2015 Mar 28.

- [33] Samadian M, Bakhtevvari MH, Nosari MA, Babadi AJ, Razaeei O. Trigeminal Neuralgia Caused by Venous Angioma: A Case Report and Review of the Literature. *World Neurosurg.* 2015 Sep;84(3):860-4. doi: 10.1016/j.wneu.2015.04.063. Epub 2015 May 9.
- [34] Bakhtevvari MH, Sharifi G, Jabbari R, Shafizad M, Rezaei M, Samadian M, Rezaei O. Giant leaking colloid cyst presenting with aseptic meningitis: Review of the literature and report of a case. *World Neurosurg.* 2015 Dec;84(6):1997-2001. doi: 10.1016/j.wneu.2015.06.064. Epub 2015 Jul 2.
- [35] Farhadifar F, Molina Y, Taymoori P, Akhavan S. Mediators of repeat mammography in two tailored interventions for Iranian women Health behavior, health promotion and society. *BMC Public Health.* 2016. 16(1)
- [36] Macooie AA, Nikibakhsh AA, Vatan AE, Rasmi Y. Comparing the serum total antioxidant capacity in children suffering from Henoch-Schönlein purpura in both active and remissive phase of the illness. *Acta Medica Mediterranea.* 2015. 31(7), pp. 1405-1409.
- [37] Anbari K, Sahraei N, Ahmadi SAY, Baharvand P. Barriers of breast cancer screening from the viewpoint of women in Khorramabad (West of Iran). *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 2016. 7(6), pp. 2044-2049
- [38] Nejad DB, Azandeh S, Gholami MR, Gharravi AM, Zhaleh M. Superficial palmar arch with Persistent median artery. *Journal of the Anatomical Society of India.* 2016. 65(2), pp. 175-176
- [39] Sane S. The Effect of intrathecal fentanyl on Bi-spectral index during spinal anesthesia in patients with lower limb orthopedic surgery. *Journal of Global Pharma Technology.* 2016. 8(12), pp. 435-440
- [40] Sane S, Khoshkbari M, Abbasyvash R, Mahoori AR. Evaluation of the effect of preoperative oral Tizanid in on the rate of anesthetic consumption & hemodynamic changes in Tiva (total intravenous anesthesia). *Journal of Global Pharma Technology.* 2016. 8(12), pp. 441-446
- [41] Rabieepur S, Ebrahimi M, Sadeghi E. Relationship between sexual health and contraception methods in women. *Journal of Mazandaran University of Medical Sciences.* 2015. 25(130), pp. 30-39
- [42] Ghazavi A, Abbasi E, Nikibakhsh A, Sadeghi E, Sadeghimanesh J. Comparison of prophylactic effect of clobazam and diazepam in children with simple febrile convulsion (SFC). *International Journal of Tropical Medicine.* 2016. 11(2), pp. 21-23
- [43] Valizadeh R, Taymoori P, Yousefi F, Rahimi L, Ghaderi N. The effect of puberty health education based on health belief model on health behaviors and preventive among teen boys in Marivan, North West of Iran. *International Journal of Pediatrics.* 2016. 4(8), pp. 3271-3281
- [44] Farhidnia N, Memarian A. Congenital anomalies following use of isotretinoin: Emphasis on its legal aspects. *Med Leg J.* 2017 Mar;85(1):33-34. doi: 10.1177/0025817216668720. Epub 2016 Sep 25.
- [45] Mehrpisheh S, Mosayebi Z, Memarian A, Kadivar M, Nariman S, Ostadrahimi P, Dalili H. Evaluation of specificity and sensitivity of gastric aspirate shake test to predict surfactant deficiency in Iranian premature infants. *Pregnancy Hypertens.* 2015 Apr;5(2):182-6. doi: 10.1016/j.preghy.2015.01.006. Epub 2015 Feb 21.
- [46] Ameri M, Memarian A, Behtash N, Karimi Zarchi M. The importance of re-examination with deep biopsies in diagnosing cervical malignancies despite multiple negative pathology reports: A case report. *Int J Surg Case Rep.* 2015;14:48-9. doi: 10.1016/j.ijscr.2015.07.010. Epub 2015 Jul 21.
- [47] Memarian A, Ameri E, Aghakhani K, Mehrpisheh S, Ameri M. The epidemiology of lower extremities injuries in Iranian population. *Iran J Public Health.* 2016 Jul;45(7):960-1.
- [48] Asgary MR, Hemmati H. A study on benign breast disease. *Der Pharmacia Lettre.* 2016. 8(20), pp. 137-139.

- [49]Kazemi S, Khayati G, Faezi-Ghasemi M. β -galactosidase production by *Aspergillus niger* ATCC 9142 using inexpensive substrates in solid-state fermentation: Optimization by orthogonal arrays design. *Iranian Biomedical Journal*. 2016. 20(5), pp. 287-294
- [50]Rastegarian A, Jahromi MJ, Sanie MS, Kalani N. Comparing the anxiety of children when entering and leaving the operating room with and without the presence of parents. *Journal of Global Pharma Technology*. 2016. 8(6), pp. 42-46
- [51]Arazpour M, Samadian M, Bahramizadeh M, Joghtaei M, Maleki M, Ahmadi Bani M, Hutchins SW. The efficiency of orthotic interventions on energy consumption in paraplegic patients: A literature review. *Spinal Cord*. 2015 Mar;53(3):168-175. doi: 10.1038/sc.2014.227. Epub 2015 Jan 20.
- [52]Sotoudeh A, Jahanshahi A, Zareiy S, Darvishi M, Roodbari N, Bazzazan A. The influence of low-level laser irradiation on spinal cord injuries following ischemia-reperfusion in rats. *Acta Cir Bras*. 2015 Sep;30(9):611-6.
- [53]Asgary MR, Hemmati H, Rafiei E. The management of large perforations of duodenal ulcers. *Der Pharmacia Lettre*. 2016. 8(15), pp. 241-244.
- [54]Rashidpanah M, Abolghasemi J, Toosi MN, Salehi M. Investigating the different stages in the progress of cirrhosis using the Markov model. *Govaresh*. 2016. 21(3), pp. 157-166
- [55]Taymoori P, Molina Y, Roshani D. Effects of a randomized controlled trial to increase repeat mammography screening in Iranian women. *Cancer Nursing*. 2015. 38(4), pp. 289-297
- [56]Behnam Soboti , Shima Javadinia , Samileh Noorbaksh , Ramin Asgarian , Nastaran Khosravi , Azardokht Tabatabaee . Diagnostic value of the level of interleukins in cerebrospinal fluid in children meningitis. *Tehran Univ Med J* 2015, 72(12): 847-853.
- [67]Poorgholami F, Mansoori P, Montaseri Z, Najafi K. Effect of self care education with and without telephone follow-up on the level of hope in renal dialysis patients: A single-blind randomized controlled clinical trial. *International Journal of Community Based Nursing and Midwifery*. 2016. 4(3), pp. 256-264
- [58]Taheri L, Kargar Jahromi M, Hojat M. Comparison Patients and Staffs Satisfaction in General Versus Special Wards of Hospitals of Jahrom. *Glob J Health Sci*. 2015 Apr 2;7(6):95-100. doi: 10.5539/gjhs.v7n6p95.
- [59]Aghamohammadzadeh N, Niafar M, Dalir Abdolahinia E, Najafipour F, Gharebaghi SM, Adabi K, Abdolahinia ED, Ahadi, H. The effect of pioglitazone on weight, lipid profile and liver enzymes in type 2 diabetic patients. 2015. *Therapeutic Advances in Endocrinology and Metabolism*. 6(2), pp. 56-60.
- [60]Darvishi M. Antibiotic resistance pattern of uropathogenic methicillin-resistant staphylococcus aureus isolated from immunosuppressive patients with pyelonephritis. (2016) *Journal of Pure and Applied Microbiology*, 10 (4), pp. 2663-2667.