

Helianthus annuus L.

(2012 / 1 / 9 2011/ 10 /6)

(DHFR)

Helianthus annuus L.

15 10.5

360

20

(*In Vivo*)

(*In Vivo*)

5

DHFR

DHFR

.(360 nm)

:

Measurement of Specific Activity of Dihydrofolate Reductase (DHFR) Enzyme and Determination of Folate Content Extracted from UV-Exposed Stem- Derived Callus of Sunflower (*Helianthus annuus* L.)

Sajida A. Abood

Firas H. Khathyer

*Department of Biology
College of Science
University of Mosul*

ABSTRACT

The results revealed to the increase of the specific activity of dihydrofolate reductase in stem' callus of sunflower, *Heliathus annuus* L. exposed to UV(360 nm.) for 5,10 and 15min. with the growth period. The total content of folate increased substantially, whereas 20min decreased activity of dihydrofolate reductase and folate content extracted from callus of different age.

The inhibitory effect of folate analogues aminopterin and trimethoprim, *In Vivo* and *In Vitro* on dihydrofolate reductase and folate content of sunflower callus exposed or nonexposed to UV was tested. The results revealed that the exposure of callus for five min. exhibited high level of resistance to trimethoprim and aminopterin. The specific activity of dihydrofolate reductase and the total content of folate partially reserved as a result of radiation compared with non-radiated callus.

Keyword: Dihydrofolate reductase, *Helianthus annuus*, UV 360 nm.

200-100

(1990)

.(2011 ; Logemann and Hahlbrock, 2002)

(DHFR)

(Mohammad *et al.*, 1989a) NADPH

(Basset *et al.*, 2005) DNA

.(Liu *et al.*, 2011 ; Schernhammer *et al.*, 2007 ; 1997)

.....

)

DHFR

(Vanconver, 2011 ; 2009

.(2006b ; Mohammad *et al.*, 1991)

)

.(Stakhova *et al.*, 2000 ; 2005

)

.(2008

.

-1

(*Helianthus annuus* L.)

(:) 2:1 ()

%96

Mohammad *et al.*,)

15

.(Arnon and Hoagland, 1944)

Arnon

.(1986

2000

27

8

16

27

Murashige)

-5.8 (pH)

%4

(and Skoog, 1962

/ %8

6.0

. / 0.5 NAA / 1.0 BA

20 ² / 1.02 121

.(Mohammad *et al.*, 1986)

-2

30 1

(Philip Harris

NOP 189 Scottish Science) UV

360

5 10 15 20

12

(Hepair)

-3

:

³ 3 15 50 90

1

4

(7.0)

50

(Romany PG-1545)

/ 20000

/ 3600 (Hettirh PEP1406)

.(1997)

(Osborne and Huennekus, 1958) Osborne and Huennekus

(pH6.6)

-

40

EDTA

0.1 NADPH

0.5

0.15

12 2 mercaptoethanol

10

DHFR

(Mohammad *et al.*, 1989a)

340

NADPH

.(Mathews, *et al.*, 1963) $6.2 \times 10^3 \text{ M}^{-1}$

NADPH

-4

(Association of Official Agricultural Chemist,1950)

540

Streptococcus faecalis

.....

-5

30 0.5
 / 1.0 BA MS / 0.5 NAA
 10⁻³ 10⁻⁶ 10⁻³ 10⁻⁴ *In Vivo*
 .(Mohammad *et al.*, 1986b) (*In Vitro*)
 . 30

-1

DHFR

. (Mohammad *et al.*, 1986)

-2

DHFR (1)

5 10 15

57 5 90 / / 65.7

()

/ /

41) 20

5 / /

(1)

(15 50 90)

5 10 15

.()

20

Heliathus annuus L.

:1

DHFR

(360)

(/) (SE±)			DHFR *(/ /) (SE±)			()
()			()			
90	50	15	90	50	15	
1.632 0.120±	1.321 0.021±	1.011 0.055±	57.021 0.521±	56.91 0.621±	55.32 0.213±	(0.0)
4.421 0.088±	3.891 0.872±	2.411 0.432±	65.701 0.765±	64.061 0.213±	62.211 0.832±	5
3.991 0.761±	3.012 0.871±	2.021 0.098±	62.011 0.872±	61.310 0.845±	59.231 0321±	10
1.705 0.322±	1.623 0.221±	1.420 0.562±	59.211 0.321±	58.910 0.333±	57.211 0.643±	15
1.001 0.121±	0.993 0.098±	0.920 0.009±	41.292 0.821±	40.910 0.657±	40.321 0.667±	20

/

NADPH

:

*

(In Vitro) DHFR

DHFR

(2)

(10⁻³)

(10⁻⁶)

%96 %98

(360)

In Vitro

%71 %67

5

.....

10^{-3}
 10^{-6}
:2
DHFR
(In Vitro)
 (360)
 30
Helianthus annuus L.
. 5

	DHFR SE± (/ /)	
0	43.201 0.155 ±	(0.0)
98	1.012 0.066±	+
67	14.111 0.133±	+
96	1.633 0.211±	+
71	12.555 0.321 ±	+

(In Vivo)

DHFR

(10^{-3})
 (10^{-4})

%91 %94
 DHFR

.(3)

%58

%51

%57 %90

%52 %93

(MS) (10^{-3}) (10^{-4}) :3

DHFR (/ 0.5) NAA (/ 1.0) BA *In Vivo*

Helianthus

. 5 (360) 30 *annuus L.*

	/) (SE±		DHFR / /) SE±(
0	1.721 0.123±	0	56.002 0.324±	(0.0)
93	0.120 0.067	94	3.31 0.101±	+
52	0.830 0.066	51	27.211 0.222±	+
90	0.170 0.007	91	4.710 0.121±	+
57	0.742 0.100	58	23.312 0.241±	+

Logemann and Hahlbrock, 2002)

(2007)

.(2011

;

DHFR

RNA DNA

.(2011)

.....

DHFR (2008) DHFR
Basset *et al.*,)

(2005

20

.(Ishibash *et al.*, 2006)

RNA DNA

(Hawser *et al.*, 2006)

.(2011 ; 2009)
5

DHFR
.(2006b 2006 a)

In Vitro
DHFR

In Vivo

DHFR

.(2008)

DHFR

Cucurbita

.(2007)

maxima

Curcurbitacin

Nigella sativa

.(2005)

L.

- .(2006a)
- .38- 26 (1) **17** . .(*Nigella sativa* L.)
- .(2006b)
- .(*Nigella sativa* L.)
- .13-1 (2) **17** .
- .(2009)
- Nigella sativa* L.
- . 23-22
- .(1990)
- .(1997)
- .(2008)
- (*Nigella sativa* L.)
- .44-34 (4) **21** .
- .(2009)
- .99-91 **22** . (*Nigella sativa* L.)
- .(2011)
- .(*Nigella sativa* L.)
- .(2011)
- .() .*Helianthus annuus* L.

Arnon, D. I. ; Hoagland, D. R. (1944). The investigation of plant nutrition by artificial culture methods. *Biol. Rev.*, **19**, 55-67.

Association of Official Agricultural Chemist (AOAC). (1950). "Official Methods of Analysis of the Association of Official Agricultural Chemist". 7th edn., Washington, D.C. 784p.

Basset, G. J. C.; Quinlivan. E. P.; Gregory, J. F. ; Hanson A. D. (2005). Folate synthesis and metabolism in plants and prospects for Biofortification., *Crop Science Society of America*, **45**, 449-453.

Hawser, S.; Lociuro, S.; Islam, K. (2006). Dihydrofolate reductase inhibitors as antibacterial agent. *Biochem. Pharmacol.* **7**(7), 941-948.

- Ishibashi, T.; Kimura S.; Furnkawa, T. ; Sakaguchi, K. (2006). DNA Repair Mechanisms in UVB Tolerant Plant . *JARO*. **40**(2), 107-113.
- Liu, J. J.; Hesson, L. B.; Rand, K.; Molloy, P. L.; Pimauda, J. ; Ward, R. L. (2011). Analysis of globa and repeat sequence DNA methylation in the blood and normal colonic mucosa of colorectal cancer patients .healthy individuals and patients with folate deficiency . *Cancer Research*, **71**, 91.
- Logemann, E. ; Hahlbrock, K. (2002). Crosstalk among stress responses in plant pathogen defense overrides UV protection through an inversely regulated ACE/ACE type of light response gene Text promoter unit –proc. *Nakl-Acad . Sci. (USA)*, 99-2428.
- Mathews, C. K.; Scrimgeour, K. G. ; Huennekens, F. M. (1963). Dihydrofolic acid reductase. Eds. Colowick, S. P. and Kaplan, N. O., *Methods in Enzymology*, **VI**, 364-368.
- Mohammad, A. M. S.; Al-Chalabi, K. A. ; Abood, S. A. (1989a). The occurrence and properties of dihydrofolate reductase isolated from sunflower callus. *J. Exp. Bot.*, **40**, 693-699.
- Mohammad, A. M. S.; Al-Chalabi, K. A. ; Abood, S. A. (1989b). Effect of folate analogues on the activity of dihydrofolate reductase and callus growth of Sunflower. *J. Exp. Bot.*, **40**, 70-76.
- Mohammad, A. M. S.; Abood, S. A.; Al-Saleh, H. S. (1991). Effect of folate analogous on protein RNA and DNA content of lettuce callus. *Iraq. J. Biol. Sci.* **11**, 5-25.
- Mohammad, A. M. S.; Al-Barhawi, R. K. ; Abood, S. A. (1986). Effect of some growth regulators on the initiation and growth of sunflower callus. *J. Univ. Kuwait (Sci)*, **13**, 199-206.
- Murashige, T. ; Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, **15**, 473-497.
- Osborn, M. J. ; Huennekens, F. M. (1958). Enzymatic reduction of dihydrofolic acid. *J. Biol. Chem.*, **233**, 969-974.
- Schernhammer, E.; Wolpin B.; Rifai, N.; Cochrane, B.; Manson, J. A. ; Fuchs C. (2007). Plasma folate, vitamin B6, and pancreatic cancer risk in four large cohorts. *American Association for Cancer Research*, **67**, 5553-5560.
- Stakhovas, L. N.; Stakhova, L. F. ; Ladygin, V. G. (2000). Effect of exogenic folic acid on the yield and amino acid composition of the seeds of *Pisum sativum* L. and *Hordeum vulgare* L., *Prikl Biokhim Mikrobiol*, **36**(1), 98-103.
- Vancouver, B. C. (2011). Trimethoprim, *Canada*. **1322**, pp. 27-29.