Cytological Effects of Mutagenic Agents and NaCl on Mitotic Division in Two Iraqi Rice *(Oryza sativa* L.) Genotypes

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Abstract

This study was carried out to determine the effects of NaCl (0.00, 50, 100, 150, 180 or 200) mM and two different types of mutagens chemical mutagen ethyl methyl sulphonate (EMS) at 0.5% and physical mutagen UV-B irradiation (40 min time exposure), on mitotic division in two Iraqi rice genotypes Amber 33 (A33) and Amber Baghdad (AB). Different concentrations of NaCl were used. Mitotic index (MI %) was decreased significantly by increasing NaCl concentration, however the highest mitotic index was recorded in mutated genotypes with EMS and UV-B. Different abnormal chromosomes were noticed in mutated plants. Results showed high percentage of abnormal chromosomes in EMS mutated genotypes. In addition no significant differences between genotypes in mean of mitotic index with respect to all experiments. [DOI: 10.22401/JNUS.20.1.16]

Keywords: EMS, UV-B, mitotic division, NaCl, Rice (*Oryza sativa* L.) Amber genotypes.

Introduction

Iraq is one of the Asian countries which has suitable agroclimatic conditions for rice cultivation. Rice is the staple food for the greater majority of the Iraqi population. In Iraq, a number of traditional and improved genotypes have been released for cultivation in different regions. Amber genotype is the most important traditional Iraqi rice genotype [1]. Rice (*Oryza sativa* L.), belongs to the family Poaceae (Graminae), is the staple food for over two billion people over the world [2]. It is the most economic cereal crop in many parts of the world and considered as a salt sensitive crop [3]. Efforts were made to increase rice productivity, since 70% of the world's poor people depend on rice as the major source of food energy [4]. Salinity is considered an important physical factor influencing rice production. In fact, salinity can cause severe damage at any stage of rice growth and development, which leads to yield loss [5].

The mitotic index is a reliable predicator for cell proliferation in the tissue. So this assay used to identify cell proliferation and compounds that inhibit mitotic progression and resulting a decrease in the mitotic index. The MI is a cytogenetic test that used *in vivo* and *in vitro* for the examination of genotoxic effects of a compound over a short period. Considerable variations and mutagenic effect may occur at different environments [6]. The degree of cytological aberrations in either mitosis or meiosis regarded as one of the dependable criterian for estimating the effect of a mutagen. Mutagen induced chromosome aberration is the primary basis of genetic change; therefore, investigations on the mechanism of chromosome breakage, type of aberrations, and their genetic consequences form an integral part of most mutation studies. The most aberrations induced by mutagen are lagging chromosomes, bridges, translocation, and sticky chromosomes. Cytogenetical investigation is one of the best documented experimental proofs for the elucidation of the mode of speciation on different groups of plants [7]. Thus, the present work examined the possible chromosomal aberrations that may occured due to exposure to mutagens and NaCl.

Material and Methods

1. Mutagenesis

EMS concentration 0.5% (v/v) in 100 ml phosphate buffer solution at (pH7) was prepared. Seeds of the two rice genotypes Amber 33 and Amber Baghdad were presoaked in distilled water for 16-20 hrs at room temperature. The presoaked seeds were dived into two groups, the first was irradiated with UV-B (280-320 nm) for 40 min and the

second was treated with aqueous EMS 0.5% for 3 hrs under the laboratory conditions with intermittent shaking to maintain uniformity [8,9].

2. Screening rice genotypes for salinity tolerance

Seeds treated and non-treated with mutagens were germinated in Petri dishes containing 1/10 strength Murashiage and Skoog (MS) medium supplemented with 0, 50, 100, 150, 180 or 200 mM NaCl, placed in an incubator at 25 ºC, for 14 days. Seedlings were transferred to universal test tubes containing 1/2 strength MS medium supplemented with 0, 50, 100, 150, 180, or 200 mM NaCl. The test tubes were placed in a growth chamber 16/8 hrs (light/dark) photoperiod at a light intensity of 1000 lux, with ambient temperature of 25$\pm$2ºC; the medium was changed after three weeks [10].

3. Karyotype studies [11]

Root tips were dissected from both rice genotypes Amber 33 and Amber Baghdad and subjected to karyotypic studies: Excised roots from the germinated seedlings grown at 25-30ºC in dark were dissected leaving the 1-2 mm of the root tips. Root tips were treated with 2-3 drops of 1 N HCl inside vials at 55-60ºC, placed in an oven for 10 min. Washed with distilled water then transferred to another vial containing acetocarmin stain 2%, placed in an oven for10 min. Excess stain was removed carefully, then one drop of fresh stain was added on a dot-sized piece of the root tip before placing slide covers carefully, then root tips were squashed. Using a compound light microscope Genex (40×), the meristematic region of the root tip was allocated. Slides were examined at 100× magnification and chromosomes at the various stages of mitosis were seen and photographed using digital camera.

4. Mitotic index

The percentage of cells undergoing mitosis in each treatment was determined on the basis of a minimum of 1000 cells count. Abnormal dividing cells were calculated according to the equation:

% Mitotic Index (MI) = Total number of dividing cell/ Total number of cells examined x 100 [12].

Results and Discussion

The percentage of mitotic index was significantly decreased with increasing NaCl concentrations 50, 100, 150, 180 or 200 mM recorded 8.03, 6.75, 5.75, 5.60 and 4.65% respectively in Amber 33 compared with the –ve comtrol (8.83). Similar trend of mitotic index was also noticed in Amber Baghdad recording 8.17, 7.02, 5.77, 5.57 and 4.67% respectively Table (1). Results are also showed that mitotic index is significantly increased in treated genotypes with 0.5% EMS and those irradiated with UV-B for 40 min. Values recorded were 7.02% and 7.21% in Amber33 genotype treated with EMS and UV-B respectively compared with non- treated (5.35)%. In Amber Baghdad genotype mutagenesis caused a significant increase in the mean % of MI recording 7.30%, 7.06% and 5.52% for those treated with EMS, UV-B and non-treated respectively. The interaction between the two mutagens and NaCl caused a gradual reduction in mitotic index. The highest mitotic index was recorded at 0.5% EMS control treatment 9.23 and 9.90% in A33 and AB genotypes respectively while the lowest was in non-treated at 200 mM NaCl with mean values 3.73% and 3.93% in A33 and AB respectively. Results showed that mutagenic agents caused different types of abnormal chromosomes at different mitotic phases such as bridges, polar deviation, sticky metaphase and chromosomal degradation Figures (1,2). The percentages of abnormal chromosomes were 11.74% for A33 and 11.97% for AB respectively treated with EMS as mutagen, and 8.35%, 8.88% when treated with UV-B irradiation Table (1).

Table (1)
Effect of the interaction between genotype, mutagen agent and NaCl on the percentage of mitotic index (MI %).

Genotype	Mutagen	NaCl mM						Mean	%abnormal chromosome	Mean
		0.0	50	100	150	180	200			
A33	0.0	7.00	7.00	5.40	4.70	4.26	3.73	5.35	0.00	6.53
	0.5% EMS	9.23	8.30	7.13	6.23	6.16	5.10	7.02	11.74	
	UV-B	8.93	8.80	7.73	6.33	6.36	5.13	7.21	8.35	
Mean		8.83	8.03	6.75	5.75	5.60	4.65			
L.S.D P≤ 0.05		Mutagens=0.303				NaCl= 0.428		Interaction =0.742		
AB	0.0	7.06	7.30	5.66	4.70	4.46	3.93	5.52	0.00	6.63
	0.5% EMS	9.90	8.53	7.70	6.33	6.26	5.10	7.30	11.97	
	UV-B	8.96	8.70	7.70	6.13	6.00	4.90	7.06	8.88	
Mean		8.64	8.17	7.02	5.72	5.57	4.67			
L.S.D P≤ 0.05		Mutagens=0.305			NaCl =0.431			Interaction=0.747		
L.S.D P≤ 0.05		Genotype= 0.571			Mutagens=0.700			Interaction= 0.990		

Among the root tip cells, meristematic cells are especially important, because mitotic activity and cell division are indispensable for root growth and because meristematic cells are considered to be one of the salt-sensitive cells [13]. Environmental stress, as well as salinity stress, can induce apoptosis programmed cell death (PCD) [14]. Salt stress can induce nuclear condensation in rice roots. Results showed that mitotic index decreased significantly when NaCl concentrations increased, suggesting that the ionic block of proliferation was associated with a disproportionate synthesis of cytoplasmic components and an interference with the onset of the nuclear reproductive cycle [15]. An increase in NaCl concentration from 100 to 200 mM showed slow cell growth or cell division because may be due to mitotic activity retardation of cells in the root tip after exposure to NaCl stress. Previous results [16] suggested that in rice and maize, the central region of the root tip shows reduced mitotic activity when exposed to stress. Similar results were shown in barley seedlings salinity induced cell death of the primary root [17]. Urano *et al*. [18] suggested that NaCl at 100 mM suppresse cell division in rice B73 genotype but not in the *ct2* mutant (B73, 392 cells; *ct2*, 341 cells), this in agreement with this study which proved that cell division increased at 100, 150, 180 or 200 mM NaCl when rice genotypes were treated with 0.5% EMS and UV-B compared with non-treated rice genotypes. NaCl suppresses both cell division and cell expansion [19]. Sodium chloride is the most abundant salt in nature, damages plants through ion toxicity and high osmolality, resulting in a decline in agricultural productivity [19, 20].

Ionic toxicity is primarily due to the sodium ion rather than the chloride ion. Plants cope with high salinity by three mechanisms: tolerance to osmotic stress; exclusion of sodium from roots or secretion from leaves [19]. Inhibition of cell division by NaCl is due to its osmotic effect rather than sodium-specific toxicity Table (1). The root, a sensory organ of osmolality, is spatially separated from the shoot apical meristem. High salinity increases or decreases some hormones and sugar metabolites [21, 22]. The percentage of abnormal chromosomes was higher in treated genotypes with EMS rather than UV-B. Bhat *et al*. [23] showed that chemical mutagens such as EMS produce higher mutation rates more than physical mutagens, and have higher efficiency and relatively greater specificity of mutation. EMS mutagenesis gives high point mutation densities by base substitution with a low level of chromosome breaks and thus less of aneuploidy, sterility and dominant lethality

[24, 25]. Akhtar *et al*. [26] suggested that EMS could be more effective in inducing additional variability and different types of chromosomes abnormalities such as laggard, 3-nucleate conditions, unsynchronized movement at metaphase and bridges at anaphase and telophase this in line with current study.

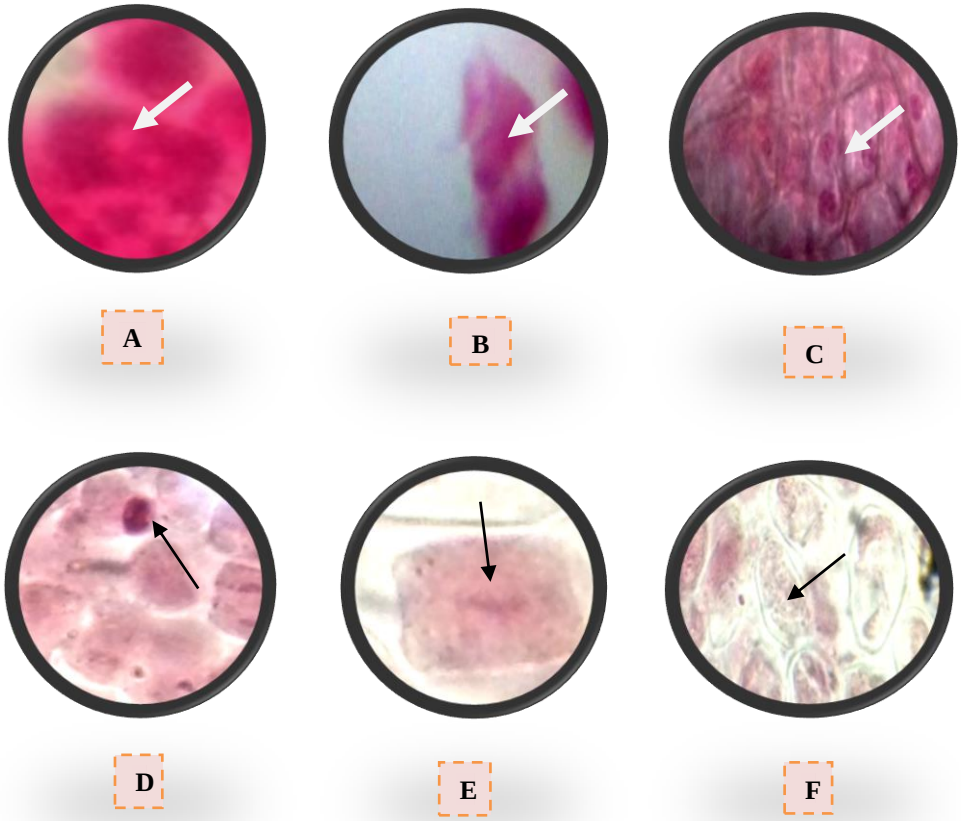

Fig.(1): Cytological aberrations in root tips merstematic cells of Amber 33 rice genotype mutated with 0.5% EMS, A: bridges in telophase, B: bridges in anaphase, C: lagging chromosomes, D: abnormal telophase, E: sticky metaphase, F: abnormal prophase. X= 1000.

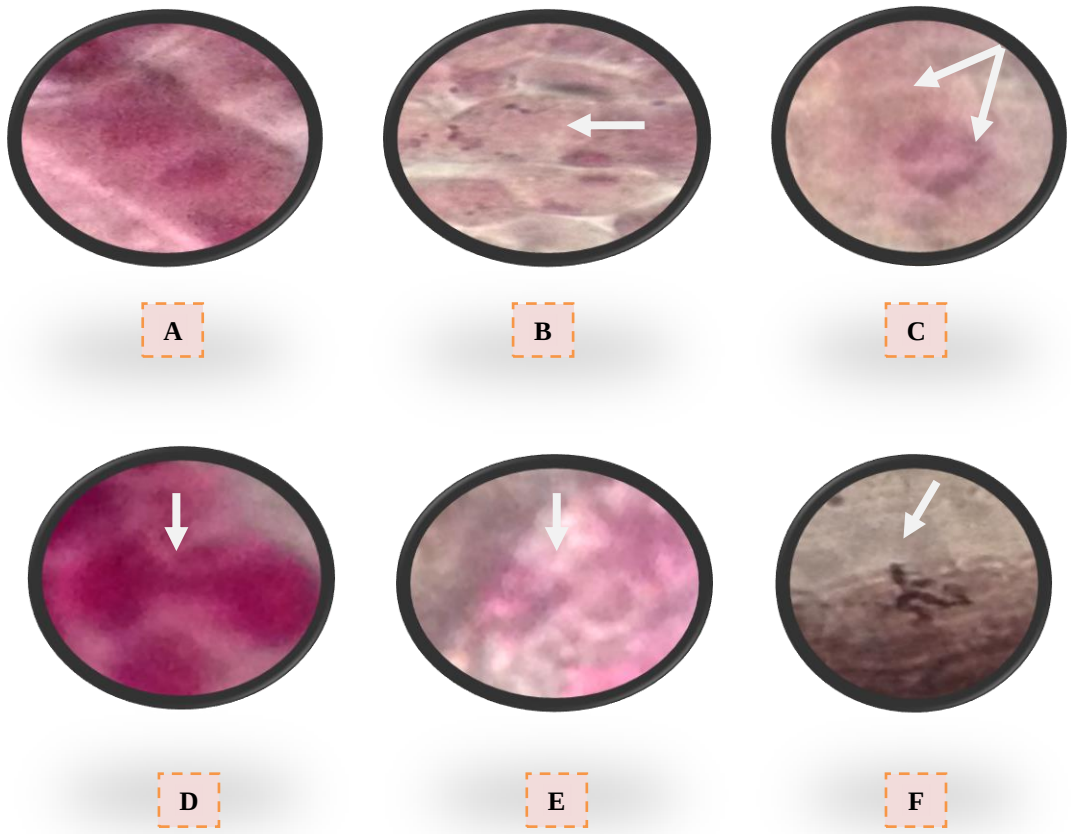

Fig.(2): Cytological aberrations in root tips of merstematic cells in Amber Baghdad rice genotype mutated with UV-B irradiation, A: unequal division in Telophase, B: polar deviation in Telophase, C: Bridges in anaphase, D: bridges in telophase, E: sticky metaphase, F: chromosomes degradation in metaphase. X= 1000.

It is concluded that sodium chloride exhibited toxic effects on mitotic index, but Ethyle methane sulphonate (EMS) and UV-B radiation caused an increase in mitotic index.

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الخلاصة

أجريت هذه الدراسة لتحديد تاثير ملح كلوريد الصوديوم NaCl (٠.٠٠، ٥٠، ١٠٠، ١٥٠، ١٨٠ أو ٢٠٠) ملمولر ونوعين مختلفين من المطفرات، المطفر الكيميائي إيثيل ميثان سلفونيت بتركيز ٠.٥% والفيزيائي الأشعة فوق البنفسجية (٤٠ دقيقة زمن تعرض) على معدل الانقسام الخلوي في تركيبين وراثيين من الأرز العراقي عنبر ٣٣ (A33) وعنبر بغداد (AB). اظهرت النتائج انخفاض دليل الانقسام الخلوي (MI) معنوياً بازدياد تركيز ملح كلوريد الصوديوم، بينما سجل اعلى لنسبة مئوية لدليل الانقسام الخلوي في التراكيب الوراثية الطافرة والمعاملة بكلا المطفريين. ظهرت حالات مختلفة من الزيغ الكروموسومي في النباتات الطافرة. كما أظهرت النتائج نسبة عالية من الكروموسومات غير الطبيعية في ألاصناف المعاملة ب المطفر EMS. اضافةً الى ذلك عدم وجود اختلافات معنوية بين الأصناف فيما يتعلق بمعدل الانقسام الخلوي بالنسبة الى جميع التجارب.