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Respiratory Deficient Mutants Induced in Baker's Yeast by the Action of Acridine Orange and some Plant Growth Hormones

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ABCTRACT

The present study aimed to investigate the mutagenic effect of acriflavine and some plant growth hormones on mitochondrial DNA of *Saccharomyces cerevisiae.* Two yeast isolates isolated from different sources were used in this work. The results of acriflavine treatments showed significant toxicity on the viability of yeast cells via a dose – response appeared. This means that the survival of cells was decreased with the increasing in acriflavine concentrations. This is a clear evidence for the toxic action of acriflavine on yeast cells. At the concentration of 1 ppm the parent cells of isolate – A retain the normal respiratory abilities, while the isolate – B was less affected. The high rate of petite mutants appeared when the cells were exposed to 4 ppm. Isolate – B was more sensitive to acriflavine than isolate – A regarding the decline in the viability of cells and respiratory deficient mutants induced. In addition, kinetin and naphthyl acetic acid showed a dose – response for the viability of yeast cells without respiratory deficient mutants induced. This indicated that both plant growth hormones enhances the division of yeast cells leading the cells to continue growing. In addition, plant growth hormones plays several vital roles in biochemical processes related to cell cycle of yeast cells. The signaling molecules regulated cell growth must received significant attention because it is alarm sounds for the potential of carcinogenicity.

Keywords : Respiratory deficient mutants , *Saccharomyces cerevisiae* , viability , acriflavine , kinetin , naphthyl acetic acid.

INTRODUCTION

Studies on respiratory deficient mutants in yeast has opened a new field in microbial genetics so – called biochemical mutations. Respiration deficient mutants shows a clear – cut variation from respirationally wild type yeast with respect morphological, physiological and enzymatic traits. Respiration – deficient mutants in yeast were less efficient in the utilization of sugars than the wild type strains and is, therefore, slower in growth (Ephrussi 1953). This outstanding trait leading the colonies of mutants are much smaller than the wild type colonies when the concentration of the sugar was low in the nutrient medium. In contrast to baker's yeast, most fungi cannot survive when mtDNA deleted function even when they are grown in the presence of a fermentable carbon source (Chen and Clark – Walker, 1999).These mutants are the designatied petite colonies.

These mutants were induced by a certain factor which resides in the mitochondria, as well as, by a nuclear gene mutation. The cytoplasmic element controls the formation of a series of respiratory enzymes which is autonomous in its replication but it was dependent on a dominant nuclear gene in its function (Yanagishima, 1958).

The baker's yeast has been used to characterize a wide variety of chemical agents that affect mtDNA. These agents induce respiratory deficient mutants that induce a complete or partial loss of their mitochondrial DNA (Berlin *et al*., 2005). The petite mutants lacked a mitochondrial function which exhibited extensive deletion of mt DNA (*p-*) or no mt DNA (p^0) (Contamine and Picard, 2000). Petite – negative mutants are not able to form small colonies. Instead, they form micro colonies that are die before becoming visible to the naked eye. In addition, the " petite positive " mutants were defined as a species able to form small respiration – deficient colonies after mutagenic treatment (Fekete *et al*., 2007).

Petite mutants in yeast are produced by the variety of chemicals. Most of them such as acriflavine are active only in dividing cells while others such as ethidium bromide are active in both dividing and non – dividing cells. Induction of respiratory deficient mutants in dividing cells was attributed to the inhibition of mitochondrial DNA replication, which results from the deletion of mitochondrial DNA in the progeny of cells. In addition, petite mutants induced in the non – dividing cells has been attributed to the degradation of mitochondrial DNA by DNase stimulated by the mutagenic agents (Iwamoto and Mifuchi, 1984). Acriflavine inhibits the formation of some cytochrome components of the respiratory system in yeast cells which are adapted to oxygen, this inhibition proceeds mutagenesis.

Kinetin was first cytokinin isolated and identified in 1955 (Miller et al., 1956). Cytokinin is the general name used to describe a plant-growth substance that stimulate cell division and It may play a role in cell differentiation. Most commonly, cytokinins comprise a group of N^6-substituted adenine derivatives that produce division and organogenesis in plant cultures which affect other physiological and developmental processes (Barciszewski et al., 1999).

Kinetin affected to delay the reduction of chlorophyll, RNA and protein, as well as, enhances the formation of RNA and protein in leaf tissue of several

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plant species. Actinomycin – D inhibit the effect of kinetin due to the influences on regulating DNA – dependent RNA synthesis (Osborne, 1965). Further, Von abrams and Pratt (1968). demonstrated that kinetin indirectly increases chlorophyll concentration as a result of directly stimulation RNA synthesis. Kinetin was isolated from autoclaved herring sperm DNA and it is an artificial DNA rearrangement product (Miller *et al*., 1956). It is dissolved in strong acids such as glacial acetic acid, although it is slightly soluble in ethanol, butanol, acetone and ether, but it is insoluble in distilled water (Barciszewski *et al*. 1999). Thus, the purpose of this study was to investigate the effect of acridine orange and Kinetin NAA on cell viability and respiration in the yeast *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Yeast strains : Two isolates of *Saccharomyces cerevisiae* were used in this study. They are designated A and B, isolate – A was isolated from a dry commercial baker's yeast produced by Holw El Sham Company for Food Industries and Agriculture Investment , 6 October City , Cairo , Egypt . Isolate – B was isolated from a fresh commercial baker's yeast produced by Lesaffre Egypt for Yeast Industry, Yeast Factory, New Nubaria City, Beheira Governorate, Egypt.

Growth medium : Yeast extract peptone dextrose medium (YEPD) consisted of yeast extract 0.2 % , glucose 2 % , peptone 2 % . When solid medium was required 2 % agar was added. Yeast extract peptone glycerol (YEPG) medium was used for detection of respiration deficient colonies, it was as similar to YEPD but 2 % glycerol was added instead of glucose. YEPD medium was used to maintain stock cultures (Millbank and Hough, 1961).

Sodium acetate medium : It was consisted of sodium acetate , 20 g yeast extract , 2 g peptone , 4 g $MgSo_4.7H_2O$, 1 g KH_2PO_4 , 1 g NaCl , 1 g agar 20 g and water up to 1000 ml (Watkins *et al*., 1974).

Lactic acid medium : It was the same as sodium acetate medium but lactic acid 10ml/L was added instead of sodium acetate, pH was adjusted using sodium hydroxide 1N.

Mutagenic agents : Acridine orange was used in this study as a solution prepared in distilled water at the concentrations of 1 ppm , 2 ppm , 3 ppm and 4 ppm in addition to the control. The concentrations were sterilized by steaming for three successive days (Millbank and Hough, 1961). Acridine orange was a molecular formula $C_{14}H_{14}$ CIN_3, and having a molecular weight 259.74 $g.mol^{-1}$. It was produced by Fluka Chemie, Buchs baked in Switzerland. Acriflavine was discovered by the great German scientist (Paul Ehrlich in 1912). Since that date, it was used as a fungicide and as an effective drug against parasitic infections. It also induced petite mutations in baker's yeast (Avers *et al*. 1965). In addition, kinetin 6 – furfural amino purine $C_{10}H_9N_5O$ was used for testing its mutagenicity. It 's a molecular weight is 215.21 g mol and produced by Roth Company, Germany. The appropriate concentrations were 00 , 2 , 4 , 6 and 8 ppm. They were filter sterilized and added after the media were autoclave sterilized. The cultures were incubated at 30° C without shaking. Petite colonies were assessed by their lack of growth on YEPG agar plates (Rickwood *et al*., 1988). Meanwhile, 1 –naphthyl acetic acid 99% was used with the concentrations of 20 , 40 , 60 and 80 ppm.

Estimation of viability : One ml suspension of yeast cells was inoculated in 100 ml YEPD medium over a period of 24 hr at 30° C with each concentration of acridine orange and the control. One drop of 0.1 ml of each dilution was transferred to solid YEPD medium in petri dishes. After 3 days of incubation at 30° C , the preparation was examined by counting the total number of colonies grown. The appropriate dilution was used for estimating survival and some colonies were pickedup to test for the formation of petite colonies.

Detection of respiration deficient mutants : Cell populations were plated on YEPG medium and incubated at 30° C for three days. The colonies which were not grown were considered as mutants of the respiratory deficient, or petite type, they are smaller, less opaque and more glistening than the wild type colonies. Further evidence of the similarity to petite mutants was obtained from the inability of the respiratory deficient mutants to grow in media with acetate or lactate as a carbon sources (Millbank and Hough 1961).

Petite frequency : The number of colonies on YEPD and YEPG agar plates was counted to be used for calculating the frequency of the petite mutants as given below (Anuradha *et al*. 2013).

$$\text{Petite frequency (\%)} = \frac{\text{No.of colonies which not grow on carbon sources}}{\text{Total colonies grown on YEPD}} \times 100$$

Statistical analysis : The data were subjected to statistical analysis variance using a completely randomized design. LSD values were calculated to compare between means if the F- test was significant (Snedecor and Cochran 1956).

RESULTS AND DISCUSSION

Acriflavine effect

Induction of petite mutation in yeast is attributed to the selective inhibition of mitochondrial DNA replication, which resulted from the deletion of mitochondrial DNA in the progeny of cells. Petite induction has been attributed to the degradation of mitochondrial DNA by a specific DNase stimulated by the mutagens (Iwamoto and Mifuchi 1984).

Table 1. Effect of acriflavine on the survival of cells and respiratory deficient mutants induced in yeast isolate – A

Acriflavine concentrations (ppm)		Total number of colonies × 10^8	Survival percent	Petite mutants induced		
				Glycerol	Sodium acetate	Lactic acid
0		22	1.0	0	0	0
1		20	0.90	0	0	0
2		13	0.61	1	0.67	0.67
3		11	0.50	0.67	0.67	1
4		10	0.44	1.67	1.33	1
F-test		NS	*	*	*	NS
L.S.D	0.05	10.28	0.31	1.03	0.92	1.06
	0.01	14.94	0.46	1.50	1.33	1.55

NS , * : Insignificant and significant differences at 0.05 level of probability , respectively.

As shown from the results presented in (Table 1) acriflavine showed a dose – response for reducing the cell survival of isolate – A which was significantly reduced at 2 ppm , 3 ppm and 4 ppm compared to the control. This indicated that the yeast cells exposed to acriflavine were sensitive to the toxic effect of this mutagen. This agreed with Millbank and Hough (1961), who found that acriflavine exerted a pronounced toxic effect. However, in the present study this is a clear evidence for direct genotoxic action of acriflavine on the yeast cells. Survival of the cells was decreased with increasing acriflavine concentrations.

Respiration deficiency mutants was also produced from 2 , 3 and 4 ppm As it was not grown in glycerol, sodium acetate and lactic acid media. At the concentration of 1 ppm the parent cells appear not to be affected by the mutagen and maintain their normal respiratory abilities. It is evident that a high rate of petite mutants appeared when the cells were exposed to 4 ppm, where the viability of cells were declined significantly. The genotoxic actions of acriflavine were associated with the rates of concentration adsorbed and penetrated the cytoplasmic membranes. The results obtained herein are in harmony with Millbank and Hough (1961), who found that the production of respiratory deficient mutants was rapid in *Saccharomyces cerevisiae* exposed to acriflavine. Acriflavine inhibits the synthesis of some cytochrome components of the respiratory system in cells which are adapted to oxygen, the inhibition of this synthesis precedes mutagenesis (Slonimski, 1953).

The results summarized in (Table 2) showed the same trend on isolate – B of *Saccharomyces cerevisiae* concerning the genotoxic effect of acriflavine. Isolate – B showed more decline in survival percent which decreased significantly beginning from 1 ppm – 4 ppm. The toxicity of acriflavine showed a dose – response. The different responses towards acriflavine give additional evidence that both isolates were genotype dependent. Thus a high proportion of mutants appeared in isolate – B than in isolate – A. Differences between both isolates of *S. cerevisiae* with regard to the toxic and mutagenic actions of acriflavine are associated with the rates of acriflavine adsorbed and the differences between both isolates in the rate of acriflavine penetrates the cytoplasmic membranes. The results obtained herein were in partial agreent with Millbank and Hough (1961), who found that the absorption of acriflavine by strains of *Saccharomyces carlsbergensis* and *S. cerevisiae* occurred at the same rate and was greatest in the range pH 4 - 5. Under alkaline conditions the some authors found that acriflavine exerted a pronounced toxic effect at pH 4 - 5, the mutant cells produced was about 50 times greater in *S. cerevisiae* than in *S. carlsbergensis*. The higher sensitivity of isolate – B than isolate – A to acriflavine may be due to higher resistance of cell wall material of isolate – A to acriflavine penetration to cause the lower proportion of petite mutants obtained from isolate – A, as compared to isolate – B. However, higher lethality was obtained in isolate B than was more sensitive to mutation.

Table 2. Effect of acriflavine on the survival of cells and respiratory deficient mutants induced in yeast isolate – B

Acriflavine concentrations (ppm)		Total number of colonies × 10^8	Survival percent	Petite mutants induced		
				Glycerol	Sodium acetate	Lactic acid
0		25	1.0	0	0	0
1		11	0.45	0.33	0.33	0.33
2		9	0.36	2	0.67	1
3		7	0.27	2.33	2.33	2.33
4		5	0.19	3.67	3.67	3.67
F-test		*	**	**	**	**
L.S.D	0.05	12.61	0.15	1.56	1.31	1.44
	0.01	18.31	0.21	2.27	1.90	2.09

*,** **: Significant at 0.05 and 0.01 levels of probability , respectively.**

The yeast colonies of different sizes after exposure to different acriflavine concentrations were tested for respiratory deficient mutants (Table 3). The results shown herein indicated that petite mutants isolated from isolate – A were obtained from 2 , 3 and 4 ppm, however acriflavine showed no mutagenic effect at 1 ppm. It is evident that lower concentration of acriflavine do not affect mitochondrial DNA of yeast cells. Petite mutants can not ferment glycerol, sodium acetate and lactic acid. The rate of fermentation of different carbon sources by the treated cells was taken as a measure of the toxicity of acriflavine since it was possible to estimate the viability of cells. On the other hand, the grand and medium colony sizes showed sufficient respiratory rates, while petite mutants were obtained from the small colony size were largely unaffected by acriflavine

Table 3. Respiratory deficient mutants induced in *Saccharomyces cerevisiae* isolate – A by acriflavine .

Colony Size	Number of colonies tested for each concentration	Acriflavine concentrations (ppm)															Total number of colonies		Petite frequency %
		0			1			2			3			4					
		G	S	L	G	S	L	G	S	L	G	S	L	G	S	L	Tested	Petite	
Grand	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0
Medium	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0
Small	6	0	0	0	0	0	0	3	2	2	2	2	3	5	4	3	30	11	36.67
Total	18	0	0	0	0	0	0	3	2	2	2	2	3	5	4	3	90	11	12.22

G : Medium containing glycerol as a sole carbon source.
S : Sodium acetate medium.
L : Medium containing lactic acid as a sole carbon source.

From the results presented in (Table 4) it is evident that isolate – B was more sensitive to acriflavine than isolate – A. This may be due to either (1) a higher rate of acriflavine penetration through the cytoplasmic membranes, or (2)

higher susceptibility of the receptor substances. The lower concentration of acriflavine (1 ppm) damage the respiratory mechanisms of the yeast cells. Thus, the populations of *S. cerevisiae* were converted into petite form. The cell wall or associated membrane may be responsible for the susceptibility of isolate – B. Petite mutants were obtained from the medium and small colony size but not from the grand size. Isolate – B produced more number of petite mutants than that obtained from isolate – A. This work is therefore concerned with the action of acriflavine on yeast isolated from different sources from which the wall differed in penetration of mutagenic agents.

Considerable interest was shown in medium size of colony appeared cytoplasmically mutation which exhibit multiple deficiencies in mitochondrial function because of the lesion in mitochondrial DNA. The primary lesion in mitochondrial DNA was not associated with any apparent damage to the nuclear genome.

The results obtained in this study agreed with Wallis and Whittaker (1974), who found that starvation of baker's yeast in 2% glycerol induced high levels of petite mutants. Hamada *et al.* (1984) reported that 50 – 100 mitochondrial DNA molecules was present per yeast cell, and if mt DNA molecules in daughter cells are totally damaged by acriflavine, the petite mutants in the population may exceed 99% after six to seven generations.

Table 4. Respiratory deficient mutants induced in *Saccharomyces cerevisiae* isolate – B by acriflavine

Colony Size	Number of colonies tested for each concentration	Acriflavine concentrations (ppm)															Total number of colonies		Petite frequency
		0			1			2			3			4					
		G	S	L	G	S	L	G	S	L	G	S	L	G	S	L	Tested	Petite	%
Grand	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	0
Medium	6	0	0	0	0	0	0	3	0	0	3	3	3	6	6	6	30	12	40
Small	6	0	0	0	1	1	1	3	2	3	4	4	4	5	5	5	30	14	46.67
Total	18	0	0	0	1	1	1	6	2	3	7	7	7	11	11	11	90	26	28.89

G : Medium containing glycerol as a sole carbon source.
S : Sodium acetate medium.
L : Medium containing lactic acid as a sole carbon sourc.

Mutagenic properties of kinetin

Kinetin promotes cell division and may play a role in cell differentiation. It was stimulated transcription initiation (Gaudino and Pikkard, 1997). As shown from the results presented in (Table 5), kinetin showed a dose – response for increasing the survival of yeast cells with a significant rates compared with the control. Gaudino and Pikkard (1997) suggested that kinetin action is not tissue specific. It may be the main molecular regulator of transcription and hence the growth in plant cells. On the other hand, kinetin enhances the incorporation of adenine into DNA and RNA, thus increases the germinating capacity of the seeds (Morales *et al*., 1987). Furthermore, kinetin increased cell dividsion rate leading to significant increase the survival percent compared to the control. These results agreed with Murai *et al.* (1977), who found a close relationship between DNA and RNA biosynthesis and the ability of cells to continue growing. In addition, Nudel and Bamberger (1971) reported that kinetin inhibits incorporation of uracil by tobacco cells suspension culture. Furthermore, Barciszewski *et al.* (1992) showed that radioactive kinetin was incorporated site – specifically into prokaryotic tRNAs. Meanwhile, Zhang *et al*. (1996) reported that cells lacking kinetin were arrested in G_2 phase. The significant survival of cells obtained in this study agreed partially with Zhang *et al*. (1996), who found that kinetin stimulated the removal of phosphate, activation of the enzyme and rapid synchronous entry into mitosis. This means that yeast cells can not control cell division due to hormonal signal leading to survival increase. On the other hand, kinetin, did not induce any petite mutants in yeast. This indicated that kinetin do not affect mitochondrial DNA and can affect cell division. It means that kinetin promotes an increased cell division without genotoxicity effect. Although, kinetin promotes an increased formation of haploid ascosporec in yeast, it may affect many genes and their products involved in cell cycle control. These include mostly adenyl cyclase and cAMP dependent and independent kinases (Laten, 1995).

The results obtained in this study agreed with Schumaker and Gizinski (1993), who reported that the plant hormone applied to moss cells causes profuse premature bud formation and localized increase in Ca (II) from 250 to 750 nM, as well as, it takes place after addition of kinetin the cytokinin – induced cell division.

Table 5. Effect of kinetin on the survival of yeast cells isolate – A

Kinetin concentrations (ppm)		Total number of colonies × 10^8	Survival percent	Petite mutants induced		
				Glycerol	Sodium acetate	Lactic acid
0		6	1.0	0	0	0
2		14	2.34	0	0	0
4		21	3.44	0	0	0
6		31	5.11	0	0	0
8		33	5.56	0	0	0
F-test		**	**			
L.S.D	0.05	10.09	1.60			
	0.01	14.67	2.33			

**** : Significant at 0.01 probability level.**

The results presented in (Table 6) demonstrated the same trend of a dose – response significant survival increases compared to the control. This suggested that kinetin functions as an anti – stress agent (Chaloupkova

and Smart 1994). Kinetin may stimulate other defence pathways such as DNA repair. In addition, kinetins do not induce respiratory deficient mutants in isolate – B of yeast cells. The cells of isolate – B were more stimulated for cell division than the cells of isolate – A. The survival of isolate – B cells treated with kinetin ranged between 2.67 – 7.17%, meanwhile the survival of isolate – A cells was ranged between 2.34 – 5.56%. The results indicated that kinetin plays several vital roles in physiological processes related to cell cycle in yeast cells. This agreed with Rao *et al*. (2010), who showed that indole 3 – acetic acid is a signaling molecule that regulates microbial growth.

Table 6. Effect of kinetin on the survival of yeast cells isolate – B

Kinetin concentrations (ppm)		Total number of colonies × 10^8	Survival percent	Petite mutants induced		
				Glycerol	Sodium acetate	Lactic acid
0		4	1.0	0	0	0
2		11	2.67	0	0	0
4		16	3.92	0	0	0
6		21	5.17	0	0	0
8		29	7.17	0	0	0
F-test		**	**			
L.S.D	0.05	4.97	1.23			
	0.01	7.22	1.79			

**** : Significant at 0.01 probability level.**

Effect of naphthyl acetic acid

Table (7) showed that survival percent was significantly increased in relation to the control. Naphthyl acetic acid showed a dose – response for the viability of yeast cells without respiratory deficient mutants induced. The viability of cells ranged between 1.89 – 4.89%. This agreed with Doi *et al.* (1973), who reported that high concentrations of auxin induced callus formation. However, Yanagishima and Shimoda (1967) found that in diploid yeast auxin causes production of genetic variants which differed from the original strains in cell form. Auxin showed higher DNA content in yeast, suggesting that auxin – induced variation in yeast involves changes in genome structure (Yanagishima *et al*., 1968). Meanwhile, Doi *et al*. (1973) reported that auxin increased in DNA and other macromolecules and cell division. The fact that exogenous cytokinins stimulated cell division and nucleotide synthesis related to increase DNA. This leading to promote cell division and activate DNA replication, transcription and protein synthesis in eukaryotic cells (Farrow *et al*., 1976).

Table 7. Effect of naphthyl acetic acid on the survival of yeast cells isolate – A

Naphthyl acetic acid concentrations (ppm)		Total number of colonies × 10^8	Survival percent	Petite mutants induced		
				Glycerol	Sodium acetate	Lactic acid
0		3	1.0	0	0	0
20		6	1.89	0	0	0
40		9	2.89	0	0	0
60		11	3.78	0	0	0
80		15	4.89	0	0	0
F-test		**	**			
L.S.D	0.05	2.08	1.02			
	0.01	3.02	1.48			

**** : Significant at 0.01 level of probability.**

The results summarized in (Table 8) showed the same trend for significant increase in cell survival above the control. The viability of cells showed a dose – response. It ranged between 1.33 – 3.0. % Furthermore, the cell survival of isolate – A was higher than that of isolate – B. This indicated that isolate – A was more affected by naphthyl acetic acid (NAA) than the isolate – B. In addition, no petite mutants were obtained in response to naphthyl acetic acid treatment. These results revealed that NAA stimulates the growth of yeast cells.

Table 8. Effect of naphthyl acetic acid on the survival of yeast cells isolate – B

Naphthyl acetic acid concentrations (ppm)		Total number of colonies × 10^8	Survival percent	Petite mutants induced		
				Glycerol	Sodium acetate	Lactic acid
0		6	1.0	0	0	0
20		8	1.33	0	0	0
40		14	2.28	0	0	0
60		16	2.61	0	0	0
80		18	3.0	0	0	0
F-test		**	**			
L.S.D	0.05	3.06	0.51			
	0.01	4.44	0.74			

**** : Significant at 0.01 level of probability.**

The results obtained in this study agreed with Singh *et al*. (2011), who reported that indole – 3 – acetic acid promote plant growth, as well as, regulates cell differentiation and affects gene expression in microbial cells. This finding supports the results obtained in this study about the role of NAA as a signaling molecule that regulates the growth of microbial cells. On the other hand, Yanagishima (1962) found that both IAA and NAA promote cell elongation in petite mutants but not so in wild type strains. It make the cell liable to elongation without

auxin addition. Yanagishima (1953) found that the addition of NAA increases the length of yeast cells. Furthermore, Yanagishima and Shimoda (1967) found that auxin induced genetic variants which differed from the original yeast strains in cell form and responsiveness to cell – expanding action of auxin. Most of these variants showed higher DNA content, suggesting that auxin – induced variation in yeast involving changes in genome structure. In addition, Doi *et al.* (1973) found that haploid cells of yeast after auxin treatment had DNA content per cell roughly equivalent to that of diploid strain. Auxin caused unbalance between increase in DNA and other macromolecules and cell division. It is demonstrated that there is a common machinery between induction of mutants in yeast and callus induced in higher plants (Doi *et al*., 1973). Biochemical and genetic analysis indicates that most variants induced by auxin in yeast involve changes in genome structure (Doi *et al*., 1973). John *et al*. (1993) reported that cytokinins are necessary for the activation of cell cycle specific protein kinase in tobacco tissue. However, Laten (1995) demonstrated that synthetic cytokinin promoted the increase of sporulation frequencies in yeast. Matsubara (1990) found that cytokinins induce dose – dependent responses. The present study clearly demonstrate that NAA showed a dose – dependent responses of cell division without possibly mutants of respiration deficient induced.

In conclusion, differences were shown between isolate – A and isolate – B of yeast cells with regard to the mutagenic and toxic actions of acriflavine which are associated with the rates of adsorption and penetrates of the acriflavine into the cytoplasmic membranes. In contrast, kinetin and naphthyl acetic acid showed stimulation in cell division with a higher rates of significance in relation to the control without any effect on mitochondrial DNA. The observations described above indicated that the yeast cells affords us a promising tool for investigating the mutagenicity and carcinogenicity of some compounds.

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طفرات نقص التنفس المستحدثة في خميرة الخباز كنتيجة للمعاملة بالأكريدين وهرمونات النمو النباتية

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تهدف هذه الدراسة إلى تقييم التأثير الطفري للأكريفلافين وبعض هرمونات النمو على المادة الوراثية DNA في خلايا الخميرة. إستخدم في هذا البحث عزلتين معزولتين من مصادر مختلفة. أوضحت النتائج وجود سمية بدرجة معنوية للأكريفلافين على حيوية الخلايا من خلال وجود إستجابة مع زيادة الجرعة المستخدمة من المادة المطفرة أكريفلافين. تعكس هذه العلاقة إستجابة حيوية الخلايا للإنخفاض بدرجة معنوية مع زيادة تركيزات الأكريفلافين. وهذا دليل واضح على طبيعة التأثير السمي للأكريفلافين على خلايا الخميرة. إستمرت خلايا العزلة A بطبيعتها التنفسية المعتادة بعد المعاملة بتركيز واحد جزء في المليون من الأكريفلافين ، بينما تأثرت خلايا العزلة B بدرجة طفيفة حيث نتج عنها معدل منخفض من طفرات نقص التنفس. ظهر أعلى معدل من طفرات نقص التنفس عندما تمت معاملة الخلايا بتركيز 4 جزء في المليون. عكست النتائج أن خلايا العزلة B كانت أكثر حساسية للأكريفلافين مقارنة بخلايا العزلة A وذلك فيما يتعلق بإنخفاض حيوية الخلايا وإستحداث طفرات نقص التنفس. هذا بالإضافة إلى أن الكينتين و نفثالين حمض الخليك قد أظهرا إستجابة في حيوية الخلايا مع زيادة الجرعة المستخدمة في المعاملة وذلك بدون إستحداث أي طفرات تنفسية. تعكس هذه النتائج أن كلا من هرموني النمو قد أدوا إلى زيادة في معدل إنقسام خلايا الخميرة مؤدية بالخلايا إلى الإستمرار في النمو. لذا تعلب هذه الهرمونات دور جوهري وحيوي في العمليات البيوكيميائية المتعلقة بدورة حياة الخلية في الخميرة. حيث تعمل الإشارات الجزيئية لهذه الهرمونات على حث المسارات الكيموحيوية المتعلقة بإنقسام الخلية لذا يجب أن تنال هذه الهرمونات إهتمام كبير لأنها تدق ناقوس الخطر فيما يتعلق بإمكانية قدرتها السرطانية.