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A Statistical Study on Chromosome Abnormalities

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Abstract

Chromosome aberrations have been used as a measure of reproductive success in plants for many years and have been correlated with morphological and taxonomical changes, fertility-sterility relationships, mutations, and other characteristics. Numerous studies have demonstrated that plant chromosomes are sensitive indicators to environmental pollutants. In this paper asetic acid organic compound with the chemical formula CH_3COOH have been used to illustrate the potential of plant systems as monitors of chromosome aberrations. The many extensively used chemicals are known to induce a wide range of chromosome aberrations. Acetic acid is the second simplest carboxylic acid (after formic acid). It is an important chemical reagent and industrial chemical, used primarily in the production of cellulose acetate for photographic film, polyvinyl acetate for wood glue, and synthetic fibres and fabrics. In households, diluted acetic acid is often used in descaling agents. In the food industry, acetic acid is controlled by the food additive code E260 as an acidity regulator and as a condiment. In biochemistry, the acetyl group, derived from acetic acid, is fundamental to all forms of life. In this study, the effect of acetic acid, which has entered our lives with different uses, on onion (Allium cepa L.) chromosomes was investigated. In order to obtain root tips, the bulbs of the plant were kept in 5, 25 and 70% solutions of acetic acid prepared with distilled water for 2 and 12 hours. In the chromosomes examined at the root tips obtained as a result of acetic acid treatment; Abnormalities such as bridge formation, adhesion, irregular distribution, fishbone and fan, ring chromosome were observed. The results were evaluated numerically and statistical analyzes were made.

Keywords: Abnormalities, acid, chromosome, plant, statistical

Kromozom Anormallikleri Üzerinde İstatistiksel Bir Çalışma

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Özet

Kromozom anormallikleri uzun yıllardır bitkilerde üreme başarısının bir ölçüsü olarak kullanılmış ve morfolojik ve taksonomik değişiklikler, doğurganlık-kısırlık ilişkileri, mutasyonlar ve diğer özellikler ile ilişkilendirilmiştir. Çok sayıda çalışma göstermiştir ki, bitki kromozomlar çevresel kirleticilere karşı hassas göstergelerdir. Bu yazıda, CH_3COOH kimyasal formülüne sahip asetik asit organik bileşiği, bitkilerdeki kromozom anormalliklerini göstermede belirleyici olarak kullanılmıştır. Yaygın olarak kullanılan birçok kimyasalın çok çeşitli kromozom sapmalarına neden olduğu bilinmektedir. Asetik asit, ikinci en basit karboksilik asittir (formik asitten sonra). Öncelikle fotoğraf filmi için selüloz asetat, ahşap tutkalı için polivinil asetat ve sentetik elyaf ve kumaş üretiminde kullanılan önemli bir kimyasal reaktif ve endüstriyel kimyasaldır. Evlerde, kireç çözücü maddelerde genellikle seyreltilmiş asetik asit kullanılır. Gıda endüstrisinde, asetik asit, asitlik düzenleyici ve gıda katkı maddesi olarak E260 kodu ile kontrol edilir. Biyokimyada, asetik asitten türetilen asetil grubu, tüm yaşam biçimlerinin temelidir. Bu çalışmada farklı kullanımları ile yaşantımıza girmiş olan asetik asitin soğan (*Allium cepa* L.) kromozomlarına olan etkisi araştırıldı. Kök uçları elde etmek için bitkinin soğanları asetik asitin saf su ile hazırlanan %5, 25 ve 70'lik çözeltilerinde 2 ve 12 saat tutularak bekletilmiştir. Asetik asit muamelesi sonucu elde edilen kök uçlarında

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incelenen kromozomlarda; köprü oluşumu, yapışma, düzensiz dağılım, balık kılçığı ve yelpaze, halka kromozom gibi anormallikler gözlenmiştir. Sonuçlar sayısal olarak değerlendirilip istatistiksel analizleri yapılmıştır.

Anahtar Kelimeler : Anormallikler, asit, kromozom, bitki, istatistik.

1. Introduction

Chromosome aberrations have been used as a measure of reproductive success in plants for many years and have been correlated with morphological and taxonomical changes, fertility-sterility relationships, mutations, and other characteristics. Numerous studies have demonstrated that plant chromosomes are sensitive indicators to environmental pollutants. [1].

The chemical name of acetic acid, also known as 'vinegar acid' among the people, is 'ethanoic acid'. Acetic acid is used in plastic production and plastic shaping with its solvent feature. Terephthalic acid is used in the production of pet bottles that we all know very well, which we use frequently in daily life, and acetic acid is needed to produce this terephthalic acid. Acetic acid is used as an important solvent in the compositions made to obtain this chemical. Likewise, we see that acetic acid is an important area of use in the food industry. With its acetic acid buffer feature, it finds use as an additive in the food industry. It is used as an additive with the code E260 in the food industry [2]. Dense acetic acids are acetic acids with a concentration of 25% or more. Although non-concentrated dilute acetic acids are not as dangerous as concentrated acetic acids, they can cause irreversible damage to the human body. The fatal change in blood acidity is one of these damages. For this reason, it is important to pay close attention to the usage patterns of acetic acids and to avoid excessive use, drinking or skin contact [3].

In this study, the effect of acetic acid on the chromosomes of *Allium cepa*, which we witnessed as a kitchen onion, was investigated. The bulbs of the plant, which were kept in solutions of 5, 25 and 70% of acetic acid for 2 and 12 hours, were ensured to root in suitable conditions. Some chromosomal abnormalities were observed in the chromosomes at the root tips formed as a result of rooting. In the numerical evaluation of the results obtained, statistically significant relationships were found between the percentage of solution, the application time and chromosomal abnormalities. As a result of the literature search, different studies have been seen on the cytogenetic effects of various chemical substances on plant chromosomes [4]- [6]. It has been determined that the studies in the literature are mostly studies on the effects of heavy metals and pesticides on chromosomes [6]- [9]. In addition, studies on the chromosomes of acetic acid are very limited [10]. In the literature, no study has been found on the effect of acetic acid, which is the subject of our study, on *Allium cepa* chromosomes.

2. Materials and Methods

Healthy and similarly sized bulbs of Allium cepa, which were used as research material in obtaining root tips, were selected for root tip extraction. On the other hand, 5, 25 and 70% solutions of acetic acid with distilled water were prepared. After the bulbs were kept in these solutions for 2 and 12 hours, the processes were continued with tap water to obtain the root tips. In addition, the bulbs were rooted by using distilled water without using acetic acid solution for control purposes. Root tips treated with control and acetic acid were fixed in a 3:1 (3 alcohol: 1 glacial acetic acid) mixture and then transferred to 70% ethanol. Then, root tips were softened by keeping them in 1N HCl solution for 7-10 minutes, and the preparations were prepared by staining with the feulgen method [12]. These preparations, prepared for cytogenetic examinations, were examined under a Leica DM 3000 camera microscope, chromosome abnormalities were detected, and their photographs were taken. Statistical evaluations were made using the Pearson correlation test method. The results were interpreted with significance values of $P < 0.05$ and $P < 0.01$. Chromosomal abnormalities were coded as A, B, C, D, E, and F for statistical evaluations. Acetic acid %

amounts and application times are coded as 1-6 (5/2 hours): 1, (5/12 hours): 2, (25%/2 hours): 3, (25/12 hours): 4 (70%/2 hours): 5, (70%/12 hours): 6 (Table1,2).
In the study, by using different percentage solutions of acetic acid for different durations, we determined that the root tips obtained from onions, and at the end of microscopic observations, the percentage of chromosomes in mitosis varies depending on the acetic acid type and application time. At different stages of mitosis, chromosomal aberrations such as bridge formation, adhesion, irregular distribution, ring chromosome and fan chromosome were observed in chromosomes by applying different ratios and durations of acetic acid to obtain onion root tips (Figure 1-6).
As seen in Table 1 regarding the mitotic index and chromosomal abnormality diversity due to acetic acid application in root tip cells, the application with the highest mitotic index belongs to the period coded 6 and the highest value in percentage (70/12 hours).
Similarly, according to the same table, the highest chromosomal abnormality belongs to the application coded with 6, as expected. The lowest level of total chromosomal abnormalities was observed in the applications coded with 2 (5 / 12 hours) (Table 1). Starting from Table 2, when we compare the different chromosomal abnormalities observed in the study, it is seen that the highest rate of 9.40 percent is in the form of bridging chromosome abnormalities. This abnormality is followed by adhesion, ring chromosome, fan chromosome, scattered chromosome and fish bone abnormalities, respectively, with different rates. Bridging and adhesion chromosomal abnormalities were seen in all acetic acid applications, while other abnormalities were not observed in some applications (Table 2). As a result of statistical studies, according to Table 3, based on the comparison of chromosomal abnormalities due to the effect of acetic acid with Pearson's correlation test, B-C; B-D; B-F; Similarities were found between the chromosomal abnormalities encoded by C-F at 0.01 and 0.05 degrees of significance (Table 3). On the other hand, according to Table 4, as a result of Pearson correlation test, 1-2; It was observed that there was a significant correlation between the acetic acid administration groups coded with 1-3 and 2-5 at a significance level of 0.05 and 0.01 (Table 4).

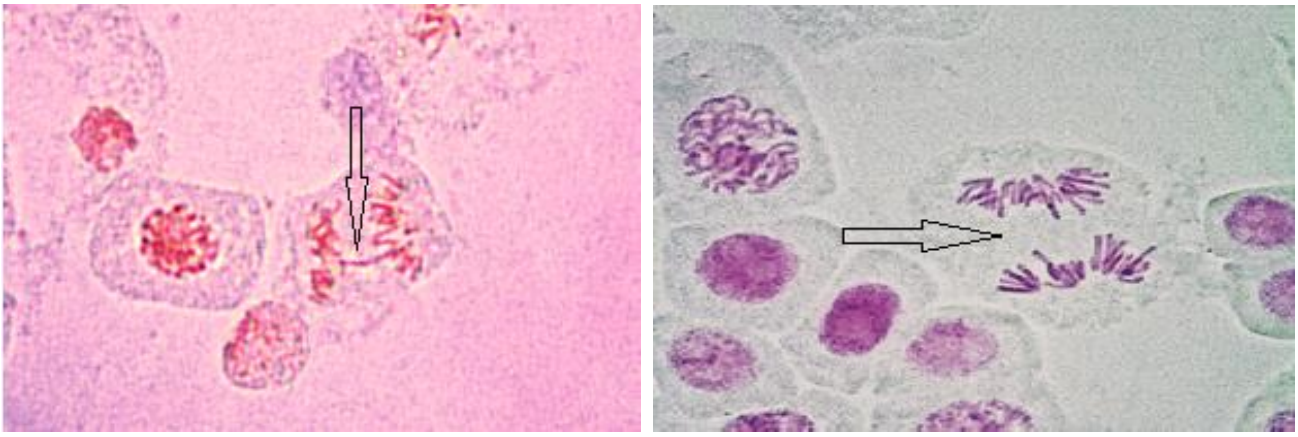
3. Results and Discussion

As a result of this study, we determined the harmful effects of acetic acid on onion chromosomes, varying according to the amount of % and application times. Acetic acid is a chemical substance that enters our daily lives, especially our kitchens, in different forms. In addition to its attractive positive effects such as ease of use and taste, this substance, which has some harmful effects in terms of human health, is also widely used in industry [2]. In order to observe the possible effect of this substance on chromosomes, we chose the common onion (Allium cepa) which we use frequently in our kitchen and whose chromosomes can be observed easily, as research material. Although similar studies in the literature were mostly done with bean (*Vicia faba* L.) chromosomes, there are studies with onions [8].
In the study, we observed that with the effect of acetic acid, which we used in different % and amounts for different periods, abnormalities in the form of abnormalities outside the normal appearance of the chromosomes occurred during mitosis in the root tips of the onion. These deformities in the chromosomes, which are caused by the effect of acetic acid, are determined as abnormalities and named in the light of the literature with different definitions. In this study, we named some chromosomal abnormalities that we observed differently from the literature as fan chromosomal abnormalities. In the literature, many chromosome studies have been carried out using different plants and different chemicals. These investigators also found chromosomal abnormalities similar to our findings [6-[10]. There are very few studies in the literature similar to our study with acetic acid. Sugiyama et al. observed the effect of using acetic acid during the examination of barley chromosomes (*Hordeum vulgare* L.). They stated that the acetic acid used for the removal of pollutants during the examination, on the other hand, damaged the chromosomes [12]. In another study on the effect of acetic acid on plants, the effect of acetic acid in coping with drought of the plant called Mayok (*Manihot esculenta* Crantz) was investigated [13].

In another study, different concentrations of xylene and different times were applied to the pod chromosomes in the study; unlike our study, they observed abnormalities such as C chromosome and shrinkage [14]. In our study, unlike the literature, spectrum chromosomal abnormalities were observed. These results show that the effects on chromosomal abnormalities vary in application types such as different chemicals, different times, different materials.

The numerical values we obtained as a result of microscopic studies were used for statistical analysis and the results were shown in the tables. In Table 1 regarding the mitotic index and chromosomal abnormality diversity due to acetic acid application in root tip cells, the application with the highest mitotic index belongs to the period coded with 6 and the highest value in percentage (70/12 hours). The lowest mitotic index was seen in the application with the lowest time and percentage coded with 1. Accordingly, it can be said that mitosis increases with the increase of time and amount of acetic acid.

In the tables showing statistical evaluations, the results were evaluated at the significance levels of $P < 0.05$ and $P < 0.01$. According to Table 3, chromosomal aberration coded with B has statistically similarity between 0.01 and 0.05 significance levels compared to other abnormalities. In the light of this result, we can say that adhesion chromosomal abnormalities are the most triggered abnormality by the effect. On the other hand, although the bridge chromosomal abnormality coded with A is the highest percentage, it does not have statistically significant values, they act independently and do not interact with other abnormalities. According to Table 4, apart from the acetic acid application, which was coded as 4 (25% / 4 hours), as a result of the Pearson correlation test, there was a similarity between 0.05 and 0.01 significance levels. With this result, we can say that this type of acetic acid application shows independent results compared to other applications.



a b

Figure1. a: Biridge b: Fan chromosomal abnormalities caused by the action of acetic acid

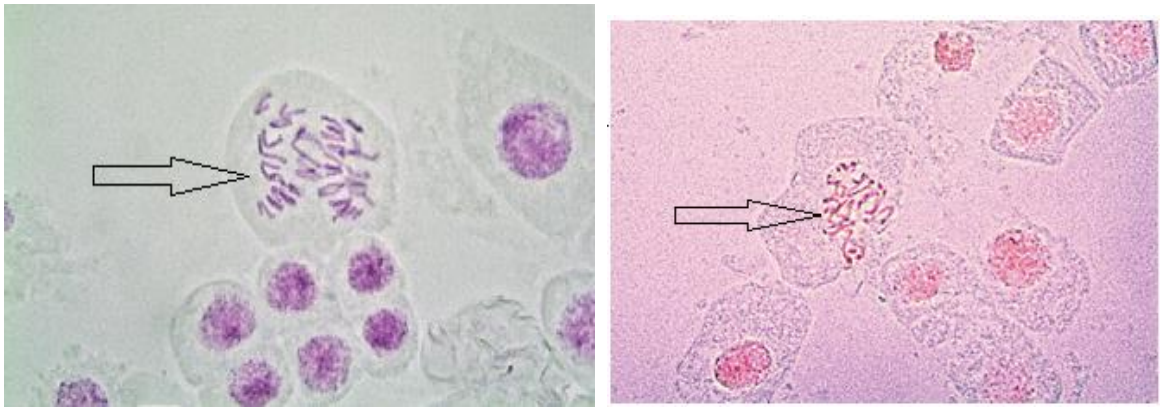
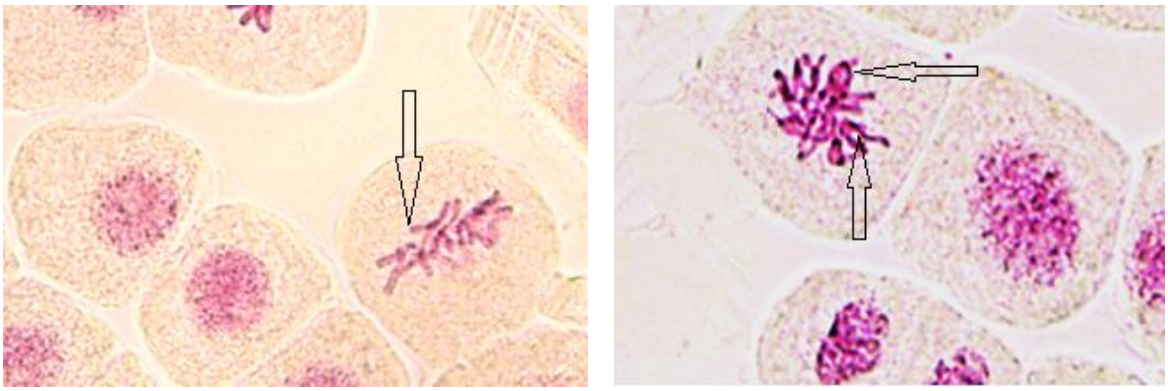


Figure 2. Irregular distribution chromosomal abnormalities caused by the action of acetic acid .



a b

Figure 3. a: Adhered b: Ring chromosomal abnormalities caused by the action of acetic acid

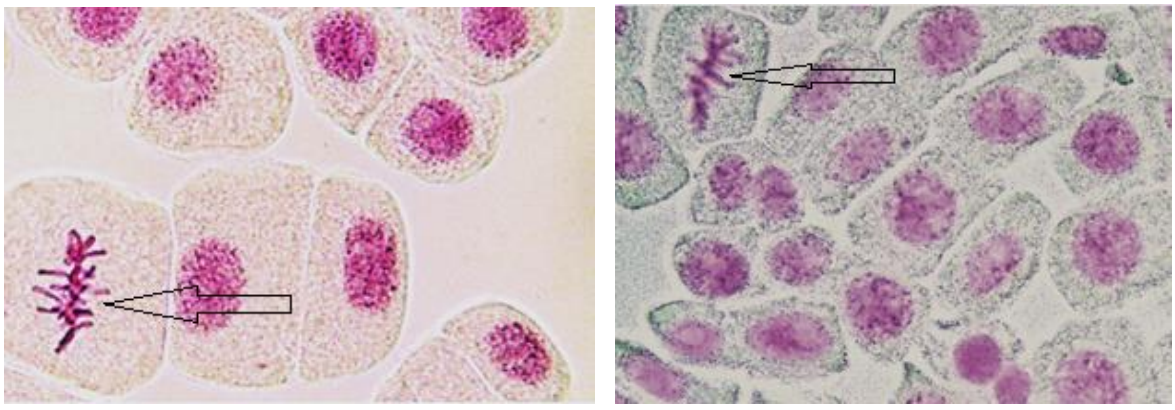


Figure 4. Fishbone chromosomal abnormalities caused by the action of acetic acid.

Table 1. Diversity of mitotic index and abnormality in root tip cells due to acetic acid application.

	Control	**% 5**		**% 25**		**%70**	
		2hours (1)	**12hours (2)**	**2hours (3)**	**12hours (4)**	**2hours (5)**	**12hours (6)**
Mitotic index (%) ± SD	22 ± .2	7.3 ± 1.3	9 ± 9.2	12± 8.1	15 ± 1.2	21 ± 5.8	29 ± 1.3
Total abnormality (%)	00.00	01.35	02.60	04.13	6.19	13.67	23.22
Number of abnormality kinds	00.00	2	4	5	4	5	6

S.D: Standart Deviation

Table 2. A Ratio of chromosomal abnormalities in % and administration times

Chromosome Abnormalities (%)		**% 5**		**% 25**		**%70**	
		2hours (1)	**12hours (2)**	**2hours (3)**	**12hours (4)**	**2hours (5)**	**12hours (6)**
Bridge chromosome	**A**	4.10	4.30	2.70	4.10	9.40	6.10
Adhesion	**B**	3.50	5.70	1.00	3.10	3.70	3.90
Irregular distribution	**C**	3.80	4.70	0.00	2.80	2.40	4.70
Fan chromosome	**D**	4.60	2.60	1.00	0.00	4.90	2.10
Ring chromosome	**E**	1.00	4.40	0.00	1.00	1.00	5.70
Fishbone chromosome	**F**	1.50	3.10	2.60	1.60	0.00	2.70

Table 3. Chromosomal abnormalities due to the effect of acetic acid (Pearson's correlation)

	A	B	C	D	E
B	0,216				
	0,681				
C	0,153	0,784			
	0,772	0,035*			
D	0,606	0,010**	0,278		
	0,203	0,912	0,593		
E	0,312	0,244	0,050*	0,438	
	0,547	0,641	0,901	0,384	
F	0,636	0,033*	0,256	0,557	0,864

* P< 0.05 and ** P< 0.01 Significance level
A-F: Chromosomes abnormalities codes

Table 4. Comparison of acetic acid applications (Pearson's correlation)

	1	2	3	4	5
2	0,002**				
	0,997				
3	0,016*	0,316			
	0,975	0,541			
4	0,340	0,784	0,226		
	0,509	0,065	0,667		
5	0,723	0,050*	0,407	0,478	
	0,104	0,921	0,423	0,338	
6	0,150	0,589	0,021*	0,410	0,322
	0,777	0,219	0,968	0,419	0,534

* P< 0.05 and ** P< 0.01 Significance level
1-6: % Application and application time codes

(%5/2 hours): **1**, (%5/12 hours): **2**, (%25/2 hours): **3,**
(%25/12 hours): **4** (%70/2 hours): **5**, (%70/12 hours): **6**

4. Conclusions

In our research, we tried to determine the negative effects of acetic acid on plant chromosomes. We know that acetic acid, which we frequently use in our daily life, makes our life easier, and it has visible damages and negative effects in terms of human health with the use of inappropriate amounts. In this study, we observed that besides these visible damages, if not used in appropriate amounts, it can cause damage to the chromosomes that will carry our codes to future generations. With this result, we have observed once again that we should be careful not to use such chemicals as much as possible or to use the appropriate doses that are harmless.

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