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# Genetic Polymorphism Under Therapy Genotypes From Cucumber (*Cucumis sativus*) And Sunflower (*Helianthus annuus*)

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**Abstract:** Seven F1 hybrids of Sunflower and Cucumber from two families were studied, both lines and hybrids of the first generation.The denaturations processes of kinetics of different genotypes isolated from DNA of Sunflower and Cucumber was investigated. The thermal denaturation of DNA macromolecules have proved high melting temperatures at sunflower hybrids, when compared with cucumber genotypes. This fact denotes the increased stability of DNA macromolecules. The level of heterogeneity has varied in dependence of genotype and it has correlated with the highest values of morphological and physiological parameters which determines the effect of hybrid vigor in the studies of Sunflower and Cucumber genotypes. The kinetics of denaturation processes, realized from two cucumber families, has proved that the first generation hybrids DNA macromolecules have higher melting temperature , with an increased content of G-C content (%) and also a higher level of heterogeneity (2σ%) from Sunflower, in comparison with parental lines. Molecular studies on Sunflower and Cucumber would be a stepping stone and of great advantage to agriculture, where farmers deliberately crossbreed different strains or varieties of plants to take advantage of heterosis, thereby producing hybrids with improved traits such as; high crop yields, a better disease resistance or enhanced agricultural productivity.

**Keywords:** *DNA; Denaturation; Genetic Polymorphism; Genotype; Hybrid; Heterogeneity.*

## 1. Introduction

Genetic polymorphism is a huge reservoir of hereditary variations staple, high selective value, which allows adaptation to new environmental conditions or rapid changes, moreover, it likely existence of the species [23]. Polymorphism may be identified at various levels of organization of animal and plant organisms by analyzing the various characters such as; morphological, physiological, cytogenetic, biochemical, and molecular [1]. Analysis of existing variations in the macromolecules of DNA from different genotypes is of particular importance in assessing the genetic polymorphism of various organisms. The amount of genetic material (DNA) and its structure in living organisms varies largely. In general, the organisms evolved and the amount of DNA, specifically genetic information is much higher compared to simpler organisms [1,2]. Study of DNA from different species of prokaryotes and eukaryotes indicates that it undergoes significant changes from one species to another with regard to their bases containing quantity different and, especially, the relationship between cytosine and guanine, adenine and Thymine [ 2.3].

The denaturations processes of kinetics from Sunflower and Cucumber of different genotypes isolated from their DNA was investigated. The thermal denaturation of DNA macromolecules have proved high melting temperatures at sunflower hybrids, in comparison with cucumber genotypes, this fact denotes the increased stability of DNA macromolecules. The level of heterogeneity has varied in dependence of genotype and it has correlated with the highest values of morphological and physiological parameters which determines the effect of hybrid vigor in studies of genotypes [20]. The kinetics of

denaturation processes, realized on two cucumber families, has proved that the first generation hybrids DNA macromolecules had the melting temperature higher, an increased content of G-C and a higher level of heterogeneity, in comparison with parental lines.

Most works devoted to analysis of variability of the species in the polynucleotide sequences of DNA to a specific plant [23]. However, this makes it likely that their genome is far more than variable animals [4.8]. Numerous researches have shown that genetic variability is very different from related species [3]. Following investigations were determined by a number of correlations between genetic variability and genome size [9]. Normally, macromolecules as DNA is double-helical strand, maintained for 2 types of forces; the hydrogen bonds between base pairs arranged perpendicular to the axis of the spiral and staking interaction between successive bases [22]. The fact that DNA macromolecule is composed of two complementary chain increases its stability and assures a regular structure [10]. AT exists between two hydrogen bonds and the GC-three hydrogen bonds, hence the DNA macromolecule regions rich in G-C has a higher stability when compared with A-T rich.

DNA molecule may undergo changes, that can be distorted by the action of physical and chemical factors as temperature, exposure to a high pH, decrease the dielectric constant of aqueous environment under the influence of alcohol and ketone [11]. Distortion of the DNA molecule is called DNA melting and melting temperature corresponds to 50% when the distortion of the molecule (corresponding to the midpoint of the melting curve) and is specific for each type of DNA [12-14]. The melting temperature of nucleic acids can serve as an indicator of the stability of DNA macromolecules and research processes of DNA distortion at different plant genotypes can provide information on the composition of different types of DNA bases and the degree of heterogeneity of hereditary material. The current study determine the complexity of the genome melting temperature of DNA and the overall kinetic process of distortion of DNA molecules in different genotypes of Sunflower and Cucumber.

## 2 Methodology

### 2.1 Sample Identification

The World Flora online (WFO) at http://www.worldfloraonline.org/ was used to confirm these plants' scientific names.

### 2.2 Sample Collection

Seveven F1 hybrids of sunflower and cucumber from two families were studied, both lines and hybrids of the first generation.

### 2.3 Isolation of DNA

Extraction of nucleic acids in the experimental material was made using extra bags Tris-OH 133mM, NaEDTA 6.7mM, 0.95M NaCl, 1.33% Na sarcosil, β-mercaptoetanol 1.33%, pH = 7.8 in relation to the ratio of 1:3. Samples were incubated at 65ºC for about 60 minutes, reversing their regular in every 10 minutes [20].

After the samples were brought to room temperature, a solution volume of chloroform isoamyl alcohol was added at a ratio of 24:1 and reversed relative slow until a single-phase was achieved [20].

To collect the nucleic acids, which are in the aqueous phase, centrifugation is performed at 4 thousand r/min., for 20 minutes., at 4ºC. DNA precipitation was accomplished with NaCl up to its final concentration of 0.2M. Then, one volume of isopropanol was added, stirred slowly until the advent precipitate reversal characteristic form of jellyfish was appeared. Samples were centrifuged for 5 minutes to 5 thousand r/min., at 4ºC. The sediment obtained was added 2 ml of alcohol 76% 0.2M Na

acetate samples were centrifuged for 5 minutes, 5 thousand r/min., at 4ºC. The dried sediment was added with 100 ml distilled water. The DNA obtained can be stored at temperature-20°C [15].

**2.4 Distortion of DNA**

This took place in a small temperature range and is reflected in dynamic variations of several physical properties of DNA such as, for example, optical density variation. The degree of distortion of DNA can be analyzed proceeding from the change of absorption intensity of rays with λ=260nm of the solution containing the denatured DNA-based effect that occurs as a result of dark unwind DNA molecule. The DNA distortion of an increase in light absorption at 26nm of about 40%, depending on the DNA type [15, 20].

The total amount of light absorbed by denatured DNA was about equal to that absorbed by an equivalent number of free mononucleotide [12.13], and increased absorption at 260nm, produced by heating native DNA solution, is directly dependent on the contents of pairs of A-T bases, the proportion of A-T base pairs is greater, the more intense UV rays of light are absorbed [16,17]. Proceeding from this principle, [25] and his associates have outlined a method for determining the content of DNA nucleotides as Tt (melting temperature) [18].

**2.5 DNA preparation was dissolved in SCC buffer solution with the following components:**

0.15M NaCl+0.015M CH3COONa, pH 7.0. Tanks were maintained in a water bath for 10 minutes, at a temperature of 2ºC, then to-end solution optical density at λ=260nm and λ=320 nm. Kinetics research denaturing process, the temperature rises to 50ºC, where tanks were maintained for 10 minutes. And the optical density of the solution was determined. Temperature rises gradually, each time with 2ºC, and the solution was allowed for about 10 minutes to achieve equilibrium, to determined the optical density at given temperature [21].

Based on the results obtained is logically chart dark effect. For this, the temperature falls at x-axis and y-axis, the ratio of optical density of DNA solution, that was determined at a certain temperature, to the optical density of the solution at t= 25ºC (At/A25) [22].

The melting temperature corresponds to 50% when distorting the DNA and corresponding means of melting curve.

Melting temperature (Tt) increases linearly with the content of G-C base pairs, which are more stable due to the presence of three hydrogen bonds [12,16,17]. The curves of distortion was determined by the nucleotide composition, experience was carried out under standard conditions for determining empirical dependence between G-C content and T-T. The standard solution of SCC (0.15M NaCl+ 0.015M CH3COONa) this relationship was as follows [18]:

$$G + C = (Tt\text{-}69, 3) \cdot 2{,}44,$$

Where 2.44- tg angle of the curve determined the actual value of the G-C.

Using the relationship given, the composition of the nucleotides of DNA solution was determined containing from 30 to 70% of G-C.

Under this method, the level of heterogeneity of DNA was determined [19] [23]:

$$2\sigma\ \text{-}(Tt\text{-}30)\ 2{,}44$$

Where, 2σ – is the level of heterogeneity of DNA;

Tt: the melting temperature, which was determined by curve distortion.

## 3. Findings and Discussion

The acceptance of genetically modified and molecular breeding crops remain limited despite advancements in agricultural research [25]. To comprehend the molecular and genomic breeding patterns studies like this one is crucial. The single-step genomic relationship from individual genotypes to pedigree information across various phenotyped species, amalgamating the genomic relationship of distance plant species to achieve desired traits in breeding programs [20].

Proceeding from the fact that the melting temperature of DNA molecules is specific for each type of DNA to highlight heterogeneity of DNA molecules on hybrids of sunflower and cucumber was studied based on the kinetic processes of distortion (Figure 1). Data analysis regarding the distortion of the molecule of DNA from different sunflower hybrids has revealed different melting temperatures for the genotypes studied (Table 1). Values melting temperature of DNA molecules varied according to genotype within 67, 7-89,0°C (Figure 1). The highest melting temperature was found to hybrid 11 (89.0ºC) and the lowest - the hybrid 12 (67.7°C). High melting temperatures have been confirmed also in hybrids 11, 17 and 18, showing a higher stand-lished a molecule of DNA and a larger number of G-C base pairs in the genome given.

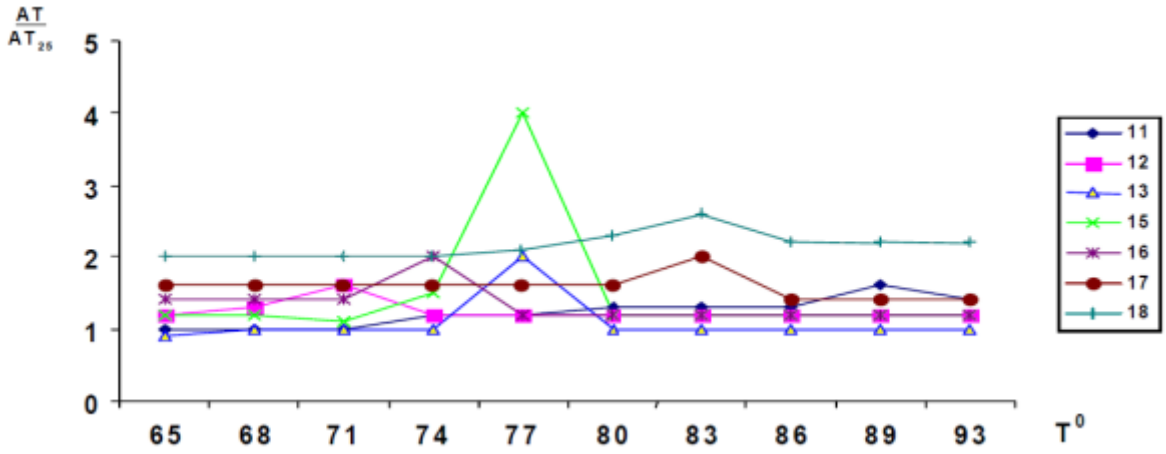


Figure1: Denaturing kinetics of DNA extracted from different sunflower genotypes.

The data in Table 1 shows that the highest G-C content is characteristic of the hybrid 11 (44.408) and the lowest for hybrids 12 and 15 (14.884).

Table 1: G-C content (%) and level of heterogeneity (2σ%) from different genotypes Sunflower

| Hybrid | 11 | 12 | 13 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|
| Contents G-C (%) | 44,408 | 14,884 | 15,128 | 14,884 | 15,128 | 30,256 | 22,448 |
| 2σ % | 140,30 | 110,776 | 111,02 | 110,776 | 111,02 | 126,148 | 118,34 |

The maximum level of heterogeneity of DNA is observed in hybrids 11 and 17, and the minimum level of hetero-genital DNA - from hybrids 12 and 15.

The comparative study of sunflower hybrids (data not presented in the article) revealed the hybrids 11 and 17 maximum Morpho-physiologic parameters, which shows pronounced heterosis for these genotypes. Thus, higher degree of heterogeneity was established in genotypes with pronounced heterosis, which also had higher melting temperature of DNA molecules. The study is in conformity

with [24] in the study of genetic characterization of wild sunflower species and interspecific hybrids based on brromrape resistance.

Comparative analysis of melting temperatures of DNA molecules in two families of cucumbers, which were studied both parental lines and hybrids of the first generation has revealed melting temperatures were within the limits of about 61-70º C (Figure 2). In the genotypes of cucumbers were found minimum melting temperatures (61.5°C) in maternal and paternal lines of hybrid 1 and the paternal line of hybrid 3 and maximum melting temperatures were recorded in both hybrids (67.5 and 70.5ºC). Thus, first generation hybrids have higher melting temperatures of DNA molecules compared with parental lines. Determining the degree of heterogeneity of DNA (Table 2) shows that the highest level of heterogeneity is characteristic of hybrid 3, in which maternal line had a melting temperature greater than paternal line.

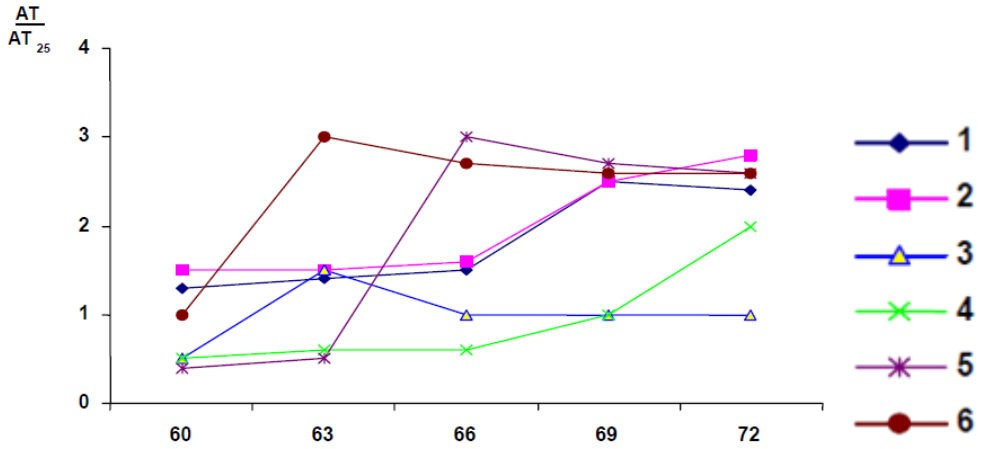


Figure 2: Denaturing kinetics of DNA extracted from different genotypes of cucumbers. (1-hybrid 1,2-maternal line,3-paternal line,4-hybrid 3,5 maternal line,6-paternal line).

Table 2: The level of heterogeneity (2σ%) in different genotypes of cucumber

| Genotype | *Hybrid 1* | ♀ 1 | ♂ 1 | *Hybrid 3* | ♀ 3 | ♂ 3 |
|---|---|---|---|---|---|---|
| 2σ % | 91,5 | 76,86 | 76,86 | 98,82 | 84,18 | 76,86 |

Thus, consistent analysis of parameters investigated in various genotypes of sunflower and cucumber has called for greater values of melting temperature for molecules of DNA in sunflower genotypes compared with the cucumbers. It shows a higher stability of the molecules of DNA in sunflower, caused by the presence of a high content of GC. Also, the degree of heterogeneity of DNA sunflower hybrids is greater than the hybrids of cucumber, while lines had lower values compared with hybrids.

## 4 Conclusion

Thermal distortion of DNA macromolecules showed maximum melting temperatures in sunflower hybrids compared with genotypes of cucumbers, which shows increased stability of macro-molecules of DNA. The degree of heterogeneity varied depending on genotype and correlated with mean values of parameters of morpho-physiology determining the effect of heterosis of the given genotypes studied. Kinetic processes of distortion, conducted in two families of cucumbers and sunflower, showed that first-generation hybrids have higher melting temperature of DNA molecules and the degree of hetero-sexual higher when compared with parental lines. The current study has shown that, genetic polymorphism plays a crucial role in therapy genotypes of both Cucumber and Sunflower. This also involves the inherent genetic variability within these plant species that could influence their response

to various treatments, environmental conditions, or breeding programs. Considering the genetic polymorphism in both Cucumber and Sunflower helps in selecting the most suitable varieties or genotypes that exhibits desirable traits, such as disease resistance, higher yield, adaptability to specific environments, and improved overall performance under particular therapeutic interventions or breeding strategies. Overall, future research on genetic polymorphism of different genotypes of Sunflower and Cucumber is recommended, this will enable a deeper understanding of diversity of these plant species.

## 5. Author's Contribution

Dogan Ozdemir carried out the experimental and Abdulrahman Mahmoud Dogara analysed and drafted the Manuscript all authors read and approved the final version.

## 6. Conflict of interest

No Conflict of interest

## 7. Acknowledgement

Not applicable

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