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Impact of single nucleotide polymorphisms of the B3 gene on growth performance and carcass characteristics in broiler chickens

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Abstract

The experiment was carried out at the poultry field of the Research station, college of Agriculture and Marshes, University of Thi-Qar, during the period from 9/15/2024 to 3/16/2025.The study consisted of two phases: field and laboratory work. The fieldwork phase included raising 300 ROSS 308 broiler chickens starting from the first day post-hatching until 35 days of age. The broilers were tagged with plastic numbers, each having a unique number. To evaluate weight gain and feed conversion efficiency, body weight measurement were conducted at the onset and conclusion of each week during the experimental period. Additionally, other weekly body measurements were taken, including bird length, chest circumference, and chest width. At the end of the production period, blood was drawn from the wing vein of broiler chickens. Thereafter, the birds were slaughtered and weighed in order to assess the primary and secondary carcass yield percentages. The main and secondary cuts, as well as edible viscera (heart, liver, and gizzard), were also weighed. The analytical procedures were carried out at the Marshes Research Center Laboratory affiliated with Thi-Qar University, aiming to extract DNA and determine the phenotypic structures of the TGF-B3 gene. Electrophoretic analysis was conducted on the obtained samples, after which the amplified PCR products were submitted to Macrogen Corporation (South Korea) for sequencing. This was done to determine the nucleotide sequence of the targeted gene fragment and to identify the genotypic variants of the TGF-B3 gene. The relationship between these genotypes and some productive traits of broiler chickens was also studied. The studied fragment was registered in the genetic bank. The results showed that both mutations, 321 C>T and 290 A>G, occurred in intron 2, a non-coding region that does not affect the peptide chain of the TGFB3 protein. The C>T 321 mutation showed a significant effect ($p \leq 0.05$) on the relative weights of the heart and liver, with the CT genotype demonstrating superior performance over the CC genotype. However, No statistically significant differences were detected among the genotypes of the investigated gene at the A>G 290 mutation site with respect to the relative liver weight, heart, and gizzard. Additionally, no significant differences were found in carcass weight, dressing percentages, or carcass cuts in both mutations A>G 290 and C>T 321.

Keywords: Polymorphism, B3 Gene, Broiler Chicken, Carcass.

I. Introduction

The poultry sector represents a key component of animal production industries and contributes notably to enhancing living standards. One of the most prominent challenges facing the world today is the significant gap between the scarcity of food (animal proteins) and the increasing population, leading to an increased demand for animal products, especially poultry. This is due to the rapid growth rate, high feed conversion efficiency, short breeding period, and high-quality meat that is characterized by low fat content (Bahri et al., 2019). Poultry is a primary animal source of protein, playing a crucial role in fulfilling human nutritional requirements. The poultry production field has undergone significant enhancements in recent times, with a substantial increase in productivity and efficiency, thanks to advancements and efforts in applied research in various fields of poultry science (Hameed et al., 2021). Poultry breeds in Iraq are considered

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a primary source of genetic and biological diversity, characterized by genetic traits that enable them to adapt to local environmental conditions, playing an essential economic role. Studying the relationship between genetic and environmental factors carried by individuals in their genetic makeup is also crucial (Ismail, 1997). Transforming growth factor-beta 3 is a cytokine protein involved critically in processes such as cell differentiation, embryonic development, and tissue formation(Sanders and Wride, 1997; Jakowlew, 1991). The TGF-B3 gene, which encodes the above protein in chickens, is linked to chromosome five (Groenen et al., 2000). The aim of this study is to investigate the genetic patterns of the Transforming Growth Factor Beta 3 (TGF-β3) gene and their association with growth performance, carcass traits, the primary and second dressing yield percentage, and the relative weights of the edible cuts.

Materials and Methods The experiment was carried out in the poultry section the Agricultural Research Station, affiliated with College of Agriculture and Marshes, University of Thi-Qar, during the period from (2024/9/15) to (2025/3/16).The study consisted of two phases: field and laboratory.

Fieldwork phase A batch of 300 unsexed broiler chicks was introduced, and the rearing period lasted for five weeks. The broilers were raised on a floor system using wood shavings as bedding material, with continuous lighting for 24 hours. Feed and water were provided ad libitum. All vaccinations and preventive measures were carried out according to the programs recommended by the veterinarian.

Laboratory work The molecular genetic analyses were performed at the laboratories of the Marshes Research Center/University of Thi-Qar. Blood samples were collected from the wing vein of 50 broilers at 35 days old, with 3 ml of blood collected from each broiler. Samples were placed in tubes containing the anticoagulant EDTA-K3 and transported to the laboratory using a cooled container to maintain sample integrit. The samples were then frozen at temperture (-20°)C until DNA extraction.

DNA Extraction

DNA extraction from bird blood samples was performed according to the manufacturer's instructions using the Geneaid kit provided by the (Korean) company. The extraction was carried out using specialized laboratory equipment for DNA extraction to conduct molecular examination of the studied gene, Transforming Growth Factor B3 (TGF-B3).

Primer Design

Primers specific to the target gene were designed and synthesized by Macrogene (Korean) as lyophilized powder. Each primer came in a separate tube labeled with its nucleotide sequence. The primers were reconstituted by dissolving them in 300 µL of nuclease-free water, resulting in a stock solution with a concentration of 100 pmol/µL. Subsequently, a working solution was prepared by diluting 5 µL of the stock solution in 95 µL of nuclease-free water, resulting in a final concentration of 5 pmol/µL, suitable for PCR amplification.

Table (1): Primer Sequences For *TGF-B3* Gene

Source	Annealing Temperature Ta(C°)	Fragment Size	Primer	Gene
Current study	57	1027bp	F: 5'- CTCTGTGCAGAAGCCACTCA-3' R: 5'- GCAGGTGCTCATCTGGAGTT-3'	*TGF-B3*

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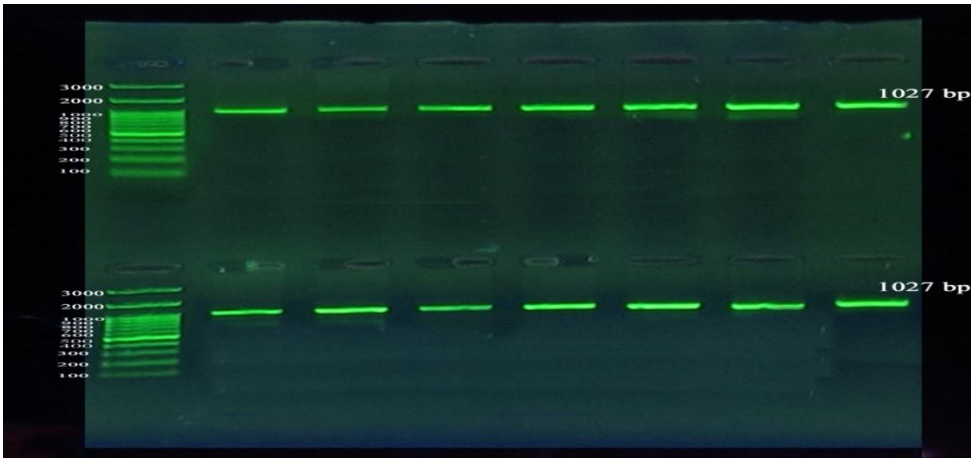
Detection of the Amplified Product

To identify the amplification products, agarose gel electrophoresis was used at a concentration of 1.5%. This involved dissolving 0.3 grams of agarose in 20 mL of 10x TBE buffer using a microwave. Safe View Dye from abm, Canada, was added at 1 µL per gel. A 100 bp DNA ladder was used as a molecular marker, and 4 µL of PCR product was loaded into other wells. The electrophoresis process was performed under conditions of 70 volt and 65 milliamperes for a duration of 45 minutes. Upon completion, DNA fragment size were assessed by visualizing the gel with a gel documentation system.



Figure(1). Electrophoresis of the 1027 bp TGF-B3 gene fragment was performed on a 1.5% agarose gel at 70 V and 85 mA for 35 minutes.

DNA Sequencing

Following verification of the PCR product size corresponding to the target gene through comparison with a DNA ladder, 20 µL from each sample were submitted to Macrogen, a biotechnology company based in south korea. Following purification, the samples were subjected to Sanger sequencing. The resulting sequences were subsequently analyzed using BLAST available on the NCBI platform, in addition to other bioinformatics tools.

Statistical Analysis

Data obtained from the study were statistically analyzed using the Statistical Analysis System (SAS) software package (SAS, 2018). The analysis included studying the effect of the genetic polymorphism of the TGF-B3 gene on the studied traits during the five weeks of the breeding period (growth and production performance traits) according to the mathematical model below. To identify differences between means, Duncan's Multiple Range Test(Duncan, 1955) was applied within the framework of a Completely Randomized Design, as follows:

$$Y_{ijk}=\mu+G_I+S_J+ E_{ijkL}$$

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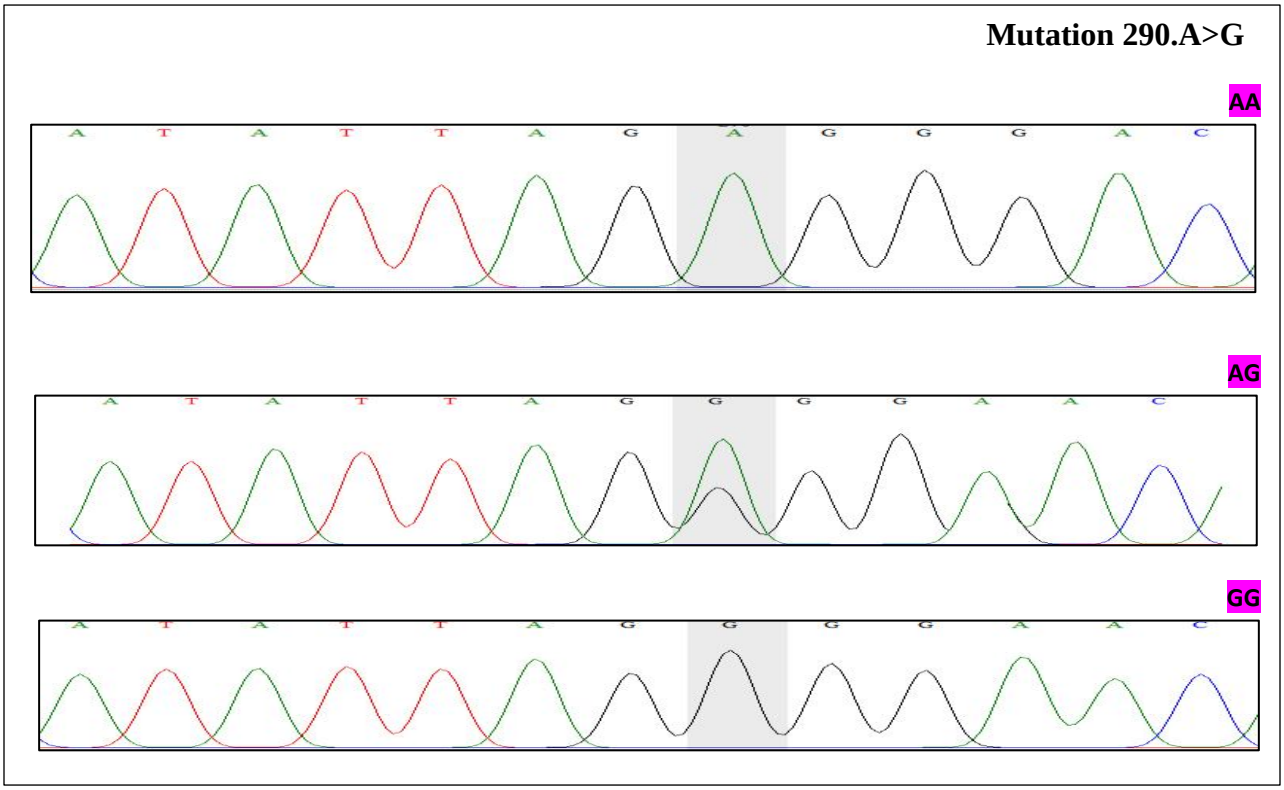
II. Results and Discussion

3-1-Identification of SNP in the *TGF-B3* Gene Following the molecular examination of the targeted region the TGF-B3 gene, which included part of intron 2 and the entire exon 3, both mutations 321 C>T and 290 A>G were found to occur in intron 2. Since this is a non-coding region, these mutations did not affect the peptide sequence of the amino acids specific to the TGFB3 protein.



Figure(2): The *TGF-B3* gene region under investigation exhibited a single nucleotide polymorphism (A>G) at position 290

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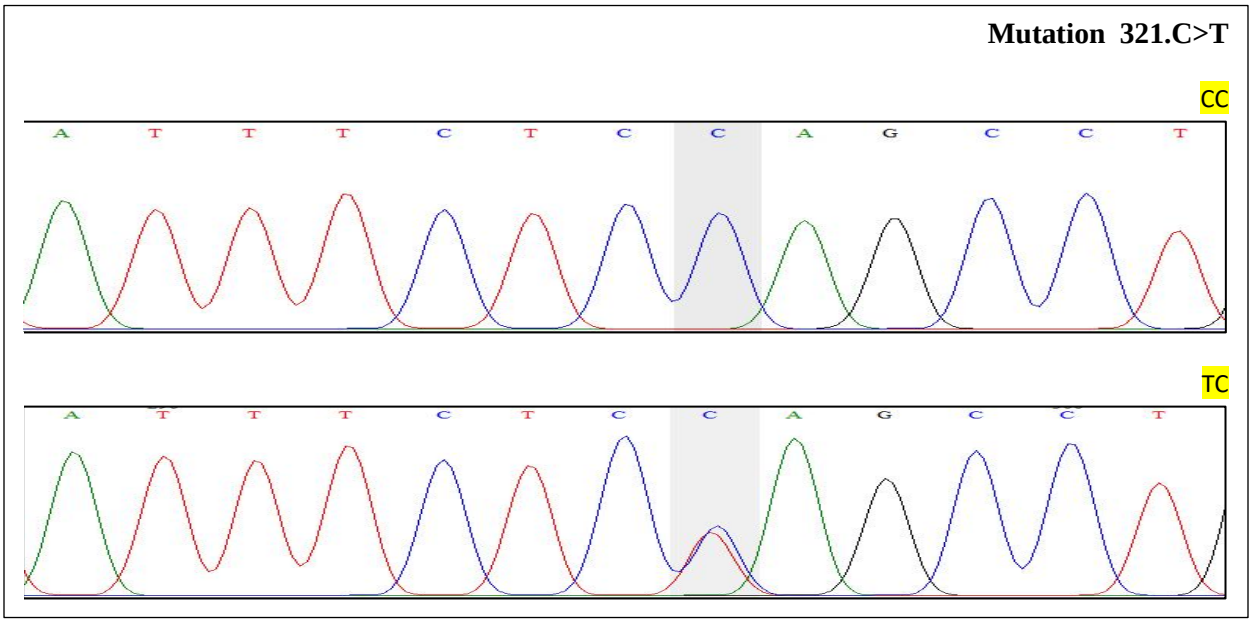

Figure(3): The mutation that occurred at position C>T 321 within the analyzed region of the TGF-B3 gene

The polymorphism of the TGF-B3 gene and allelic frequencies in Ross 308 broiler chickens for the .290 G>A mutation.

Table (2) shows the number and percentages of polymorphism for the TGF-B3 gene in the studied sample. The genotypes were determined based on the AG nucleotide variation site, and three genotypes were identified based on sequence analysis results. Highly significant differences (P≤0.01) were observed among the AA, AG, and GG genotypes. The AG genotype had the highest frequency (52%), the AA genotype ranked second with a frequency of 40%, whereas the GG genotype exhibited the lowest occurrence at 8%. Regarding allelic frequencies, the frequency of allele A was 0.66, and allele G was 0.34. Given the high frequency of allele A in the studied gene, it can be used in selection programs. These results differ from those found by Sahib et al., (2021) in their study of the TGF-B3 gene, where the genotype AA had a frequency of(57.33%), followed by CA (24%), and CC (18.67%). The differences in genotypic and allelic frequencies may be attributed to variations between breeds and genetic structures.

Table 2: Genotypic and Allelic Frequencies of the TGF-B3 Gene for the .290 A>G Mutation

Chi-Square Value	Percentage %	Number	Genotype
10.320**	40	20	AA
	52	26	AG
	8	4	GG
	%100	50	Total
	Alleles		
		0.66	A
		0.34	G
** P≤0.01			Probability Level

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Polymorphism of the TGF-B3 Gene and Allelic Frequencies in Ross 308 Broiler Chickens for the 321.C>T Mutation.

Table (3) shows the number and percentages of TGF-B3 gene polymorphisms in the studied sample. The genotypes were determined based on the CT base polymorphism site. Two genotypes were identified based on sequence analysis results, which showed highly significant differences (P≤0.01) between the CC and CT genotypes. The CC genotype was the most prevalent, accounting for 54%, while the CT genotype was observed in 46% of the individuals. Regarding allele frequencies, the C allele frequency was 0.77, and the T allele frequency was 0.23. Accordingly, the C allele may serve as a potential marker for selection, given its elevated frequency within the gene. These percentages differ from those found by Sahib and Alkhalisy (2021). The difference in genotypes and allele frequencies may be attributed to the variation between breeds and genetic structures.

Table (3): Frequencies of alleles and genotypes related to the C>T polymorphism at position 321 in the TGF-β3 gene.

Chi-Square Value	Percentage %	Number	Genotype
25.320**	54	27	CC
	46	23	CT
	0	0	TT
	%100	50	Total
	Alleles		
		0.77	C
		0.23	T
** P≤0.01			Probability Level

3-4-Association of TGF-B3 gene polymorphism with relative weights of carcass parts in ROSS 308 broiler chickens for A>G 290 mutation.

Table (4) shows no significant differences in the relative weight of heart, liver, and gizzard. The relative weight of gizzard for the AA, AG, and GG genotypes were 1.871, 1.951, and 1.964, respectively.

Table (4): Shows the relative weights of internal organs % ± SE among different genotypes of TGF-B3 gene in A>G 290 mutation.

Mean ± Standard Error (Mean ± SE)			Number	Genotype
Gizzard(g)	Liver(g)	Heart(g)		
1.871± 0.047	1.645± 0.050	0.572± 0.024	20	AA
1.951± 1692 0.035	1.683± 0.040	0.552± 0.018	26	AG
1.964± 0.094	1.696± 0.113	0.603± 0.059	4	GG
NS	NS	NS	50	Significance Level

N.S :Not Significant

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3-5 Association of TGF-B3 gene genotypes with relative weights of carcass parts in ROSS 308 broiler chickens for C>T 321 mutation.

The results in Table (5) Demonstrate notable differences in the proportional weights of the heart and liver, where the CT genotype outperformed the CC genotype. The mean relative weights of heart were (CT 0.595, CC 0.537) and liver were (CT 1.736, CC 1.611). However, no significant differences were observed in the relative weight of gizzard between the CC and CT genotypes (1.884 and 1.962).

Table (5): Shows the relative weights of internal organs % ± SE among different genotypes of TGF-B3 gene in C>T 321 mutation.

Mean ± Standard Error (Mean ± SE)			Number	Genotype
Gizzard(g)	Liver(g)	Heart(g)		
1.884± 0.034	1.611± 0.039 B	0.537± 0.018 B	27	CC
1.962± 0.043	1.736± 0.042 A	0.595± 0.020 A	23	CT
0	0	0	0	TT
NS	*	*	50	Significance Level

*: Significant (P ≤ 0.05) N.S. :Not Significant

3-6 Association of TGF-B3 gene genotypes with carcass weight and first and second net percentages in broiler chickens for A>G 290 mutation.

The results in Table (6) Indicate that no statistically significant differences in carcass weight were observed among the AA, AG, and GG genotypes (1718.75, 1740.69, and 1896.000 g, respectively). These findings align with those reported by Amirinia et al. (2011) on Iranian commercial broiler lines, which found similar results for the AA, AB, and BB genotypes. Analysis of the first and second dressing percentages revealed no statistically significant differences among the AA, AG, and GG genotypes. Furthermore, no prior research was identified regarding the influence of the investigated gene on dressing percentage.

Table (6): Shows carcass weight (g) and dressing percentages ± SE among different genotypes of TGF-B3 gene in A>G 290 mutation.

Mean ± Standard Error (Mean ± SE)			Number	Genotype
Net Percentage 2	Net Percentage 1	Carcass Weight(g)		
65.401± 1.786	69.164± 1.717	1718.75± 52.873	20	AA
64.246± 1.621	68.553± 1.653	1740.69± 64.655	26	AG
70.157± 1.292	73.884± 1.985	1896.00± 39.549	4	GG
NS	NS	NS	50	Significance Level

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3-7 Association of TGF-B3 gene genotypes with carcass weight and first and second Net percentages in ROSS 308 broiler chickens for C>T 321 mutation.

The results in Table (7) No significant differences were observed in carcass weight among the CC, CT, and TT genotypes, with mean values of (1716.56 g, 1776.96 g, and 0 g), respectively. These findings corroborate the results reported by Amirinia *et al*. (2011) on Iranian commercial broiler chicken lines, where they obtained AA, AB, and BB genotypes. However, no significant variation was observed in the first and second net percentages across the CC, CT, and TT genotypes. Additionally, literature lacks studies examining the influence of this gene on net percentage.

Table (7): Shows carcass weight (g) and dressing percentages ± SE among different genotypes of TGF-B3 gene in C>T 321 mutation.

Mean ± Standard Error (Mean ± SE)			Number	Genotype
Net Percentage 2	Net Percentage 1	Carcass Weight(g)		
63.563± 1.510	67.626± 1.556	1716.56± 53.520	27	CC
67.079± 1.609	71.099± 1.535	1776.96± 60.425	23	CT
0	0	0	0	TT
NS	NS	NS	50	Significance Level

3-8 Association of TGF-B3 gene genotypes with relative weights of carcass cuts in ROSS 308 broiler chickens for A>G 290 mutation.

Table (8) no significant differences were observed in the relative weights of the breast, thigh, back, and wing among the AA, AG, and GG genotypes. These findings contrast with those reported by Amirinia *et al.* (2011) in Iranian broiler lines. Furthermore, the relative weights of the neck were not significantly different among the AA, AG, and GG genotypes, recorded at (2.223%, 2.072%, and 2.137%), respectively.

Table (8): Shows relative weights of carcass cuts ± SE among different genotypes of TGF-B3 gene in A>G 290 mutation.

Mean ± Standard Error (Mean ± SE)					Number	Genotype
Wing(g)	Neck(g)	Back(g)	Thigh(g)	Breast(g)		
4.876± 0.117	2.223± 0.106	14.396± 1.011	24.466± 1.311	34.044± 1.549	20	AA
4.867± 0.144	2.072± 0.07	14.808± 0.695	26.368± 0.773	39.058± 1.139	26	AG
4.713± 5000 0.147	2.137± 0.087	13.370± 1.120	25.501± 1.959	36.417± 1.975	4	GG
NS	NS	NS	NS	NS	50	Significance Level

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3-9 Association of TGF-B3 gene genotypes with relative weights of carcass cuts in ROSS 308 broiler chickens for C>T 321 mutation.

Table (9) no significant differences were detected in the relative weights of the breast, thigh, back, and wing among the (CC, CT, and TT) genotypes. These findings contrast with those reported by Amirinia *et al.* (2011) in Iranian broiler lines. Moreover, the relative weights of the neck showed no significant variation among the CC, CT, and TT genotypes, with values of (2.094%, 2.189%, and 0%), respectively.

Table (9): Shows relative weights of carcass cuts ± SE among different genotypes of TGF-B3 gene in C>T 321 mutation.

Mean ± Standard Error (Mean ± SE)					Number	Genotype
Wing(g)	Neck(g)	Back(g)	Thigh(g)	Breast(g)		
4.827± 0.117	2.094± 0.082	14.293± 0.699	25.670± 0.924	37.034± 1.395	27	CC
4.896± 0.135	2.189± 0.081	14.803± 0.865	25.382± 1.021	36.615± 1.192	23	CT
0	0	0	0	0	0	TT
NS	NS	NS	NS	NS	50	Significance Level

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