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Equation Design of Primer As An Effective Factor For Molecular Test of Iraqi Cultivars of Bread Wheat

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Abstract: To design equation of primer effective factor, the experiment was needed get morphological traits by planting six Iraqi cultivars of bread wheat were used as organism to get morphological traits and molecular technique such as RAPD (5-mer oligonucleotide primers of RAPD indicator were used) . In this study, the measurements were polymorphic molecular markers and three morphological traits. To find an equation that determines the primer effective factor for appearing DNA fragments with different genotypes that belong to the same species or geneses. After equation design and applied , the results were appeared: primers OP-M06 and OP-I02 showed a significant effect on the phenotypic traits studied, they gave an effect factor greater than 4% for all phenotypic traits, while the OP-R14 primer came in second which is given an effect between 3.25-3.5%, the primers OP-M20 and OP-V02 were the least influential in phenotypic traits and in a manner Specific to the OP-V02 primer which gave a low effect was less than 2.25%.

Keywords: equation, molecular markers, morphological traits, primer.

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Introduction

Molecular markers are one of the modern important indicators in biotechnology programs because of their importance in the determining type of active genes and their effectiveness on morphological traits in a specific genotype. Also, these indicators do not change by environmental impacts comparison the morphology and productivity indicators. During the previous decades, Molecular Biology witnessed rapid development, and genetic engineering achieved continuous successes, which led to the emergence of a new type of genetic marker called DNA markers (1). These indicators (DNA indicators) that depend on the DNA material present in all the cells of the organism were characterized equally so that analyzing any part of that organism at any age stage will give us identical and comprehensive results and no change or difference occurs, the case of the proteomic analysis of the same the organism give differences in quantity and depend on the content of the genetic material (2). These indicators include the RFLP, AFLP, SSR, ISSR, and RAPD which have been fixed (3, 4), and are accompanied by other physiological and productive indicators (5, 6).

RFLP, AFLP, SSR, ISSR, and RAPD are the indicators depend on the

interaction of DNA chain replication (PCR), it has been used to study genetic affinity and detect genetic diversity, the basis of its role depends on the presence of random primers linked to compatible sites and it does not need to specify the nucleotide sequence (7). The studies of morphological traits are basic standard in organism studies, they include growth, yield, and quality studies (8), in recent years, many studies on the organisms include morphological studies with molecular indicators (9), but it did not study the relationship between them. Primers are the means to detect DNA and RNA fragments, these fragments may be genes, mRNA molecules, or random fragments of DNA. These fragments may be known sequences such as genes, or unknown sequences as in fragments that are cloned from primers of RABD, REFLP, ISSR, etc. (10). Most studies did not give data that included the connection between these fragments and morphological traits. Most studies include the transcript fragments of a specific genetic material have studied the phenotypic expression of the organism in many tables and detection of genes or DNA fragments without knowing the primer efficiency effect in each genotype, so it was necessary to find a factor or indicator that determines the primer efficiency in the morphological traits in different genotypes.

Materials and methods

Plant material

Six Iraqi cultivars of bread wheat were used in the present investigation to determine polymorphic molecular markers and some morphological traits were measured to a limitation of the primer effective factor. Samples of six varieties were provided by different Institutes that were affiliated with the ministry of higher education and ministry of agriculture.

Oligonucleotide primers

There were 5-mer oligonucleotide primers of RAPD indicator were designed (Table. 1) by primer design online program in the centre of the biotechnology web, the primers were lyophilized, were dissolved in the free ddH2O to give a final concentration of 100 pmol.µL-1 as stock solution and keep stock at -20 to prepare 10 pmol.µL-1 concentration as work primer suspended، 10µL of the stock solution in 90 µL of the free ddH2O water to reach a final volume of 100 µL was investigated by IDT (Integrated DNA Technologies company، Canada.(DNA extraction: Total DNA was extracted from green leaves, and Total genomic DNA was extracted according to the standard procedure (11) with some modifications by intron biotechnology /Korea, the PCR program was remembered from (12).

PCR amplification and electrophoresis PCR amplification was performed in 1x PCR buffer 10)mM Tris-HCl pH 8.3, 50 mM KCl, 0.1% Triton 1.5(mM MgCl2, 0.2 mM of each dNTP, 25 pM of each primer, 10-50 ng of genomic DNA per 25, l of reaction volume and 2 units of Taq polymerase. The amplifications were carried out on a crocodile III (Appligene) programmed for one cycle of denaturalizing at 94°C for 5 min. and 35 cycles of 30 s at 94°C, annealing of 1 min. at 60°C and extension of 1 min. at 72°C, a final extension of 7 min. at 72°C3)). The wheat cultivars were used in this study: Babil 113: C1, Euphrates: C2, Baghdad

3: C3, Boohuth 10: C4, Tamooz 2: C5, Rasheed: C6.

Table (1): The primers of RAPD indicators and their sequences were used in this study

NO.	Primer	Sequence 5'…….3'
1	OP-M06	CTGGGCAACT
2	OP-R14	CAGGATTCCC
3	OP-M20	AGGTCTTGGG
4	OP-I02	GGAGGAGAGG
5	OP-V02	AGTCACTCCC

Morphological traits

Six genotypes were entered into the study, three phenotypic traits were studied and the measurements were taken, the statistical analysis was calculated for including it in the study. The phenotypic traits included: Plant height, Grain weight and Grain density.

Data analysis

The data were analyzed according to the factorial experiments design, and the statistical averages were tested using the least significant difference test at the 5% level.

Results

Molecular measurements were limited by the appearance and number of bands, if RAPD or ISSR and other molecular tests markers were used, it was necessary to know many primers, all genotypes or treatments gave different bands according to the effectiveness so that the number of bands was necessary to the comparison. In morphological measurements, the value of the trait might be having a positive or negative relationship with molecular indicators. The study included RAPD markers, 5 primers used in this study gave different bands according to the fragments or genes which appear after amplifying, the sum of bands took in all genotypes (Figure 1) (Table. 2), and three morphological traits were used for comparison (Table 3), morphological traits measured in table 3 included mean and total values in all traits. The results of 6 genotypes appeared to be a difference in the number of bands; there were also variations in morphological traits.

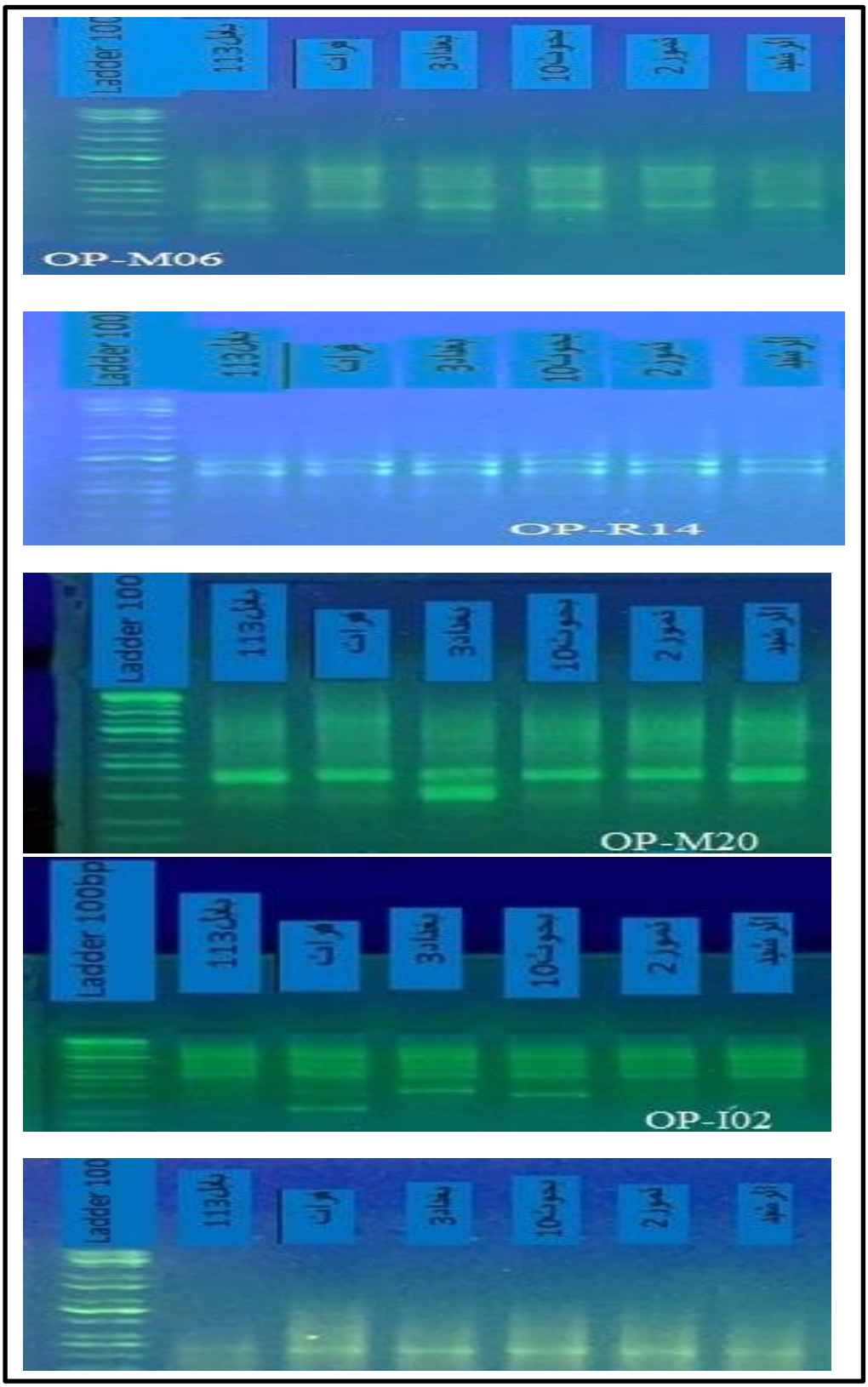

Figure (1): Primer effective factor included the effect of five primers (OP-M06, OP-R14, OP-M20, OP-I02 and OP-V02)

Genotypes	OP-M06	OP-R14	OP-M20	OP-I02	OP-V02	Total
C1	5	4	3	4	3	19
C2	6	6	4	6	4	26
C3	7	6	4	7	3	27
C4	7	6	5	8	4	30
C5	7	5	5	7	4	28
C6	5	4	4	5	2	20
Total	37	31	25	37	20	150

Table (3): The study traits in the genotypes

Genotypes	Plant height (cm)	Grain weight (mg)	Grain density (mg/ mm)
C1	102.2	43	1.90
C2	105.1	32	1.96
C3	101.6	37	1.85
C4	95.0	36	1.73
C5	70.8	28	1.3
C6	109.4	36	1.78
Total	584.1	212	10.52

The results in (Table. 2) showed that all the genotypes gave bands of DNA fragments with variation among genotypes, all primers included gave DNA fragments for each genotype, the primers differed in the number of bands which gave in all the genotypes, the primers OP -I02 and OP-M06 had given the highest number of bands 37 compared to the primer OP-V02 which gave the lowest number of bands 20. As for morphological traits, the results in (Table. 3) showed a difference among the genotypes in the phenotypic traits, the C6 genotype outperformed in plant height and the C1 genotype exceeded in grain weight, while the C2 genotype was superior in grain density.

However, no relationship determines the effect of the primers on the average number of DNA segments that appeared and the morphological characteristics that were calculated, so the following equation was calculated to find the type of effect for each primer to the average of the primers with all treatment in genotypes included in the study:

$$\textbf{PEF} = \frac{\Sigma PgTg}{Ptotal * Ttotal} \textbf{*100\%}$$

PEF: Primer effect factor

Pg: number of fragments (primer × genotype) such as (OP-M06: C1) = 5, (OP-V02: C6) =2 (Table. 2).

Tg: Values of morphological trait in genotypes such as (plant height: C1 = 102.2), (plant height: C6 = 109.4) in (Table. 3).

P_{total} **:** sum of fragments in all primer is (150) in (Table. 2).

T_{total} **:** sum of morphological trait values such as (plant height =584.1) , (grain density = 10.52) in (Table. 3) .

The application

For example, to apply the above equation, we take the data of the first primer (OP-M06) from (Table. 2) and the data of plant height from (Table. 3), and then apply the equation to get the result below:

$$\text{PEF (OP-M06)} = \frac{5*102.2+6*105.1+7*101.6+7*95+7*70.8+5*109.4}{150*584.1} \text{ *100\%……of plant height}$$

By applying the equation to the other primers with studied traits, a new Table is obtained that determines the effect of each fragment of primer in the phenotypic shape of the studied traits (Table. 4).

Table (4): Primer effective factor in all morphological traits

Trait \ Primer	OP-M06	OP-R14	OP-M20	OP-I02	OP-V02	Morphological Traits mean
Plant height	4.063	3.436	2.74	4.0554	2.184	3.296
Grain weight	4.069	3.415	2.733	4.047	2.189	3.291
Grain density	4.076	3.452	2.738	4.0657	2.203	3.307
Primers mean	4.069	3.434	2.737	4.056	2.192	
L.S.D$_{0.0S}$	**Morphological Traits = NS Primer= 0.323 Interaction= 0.811**					

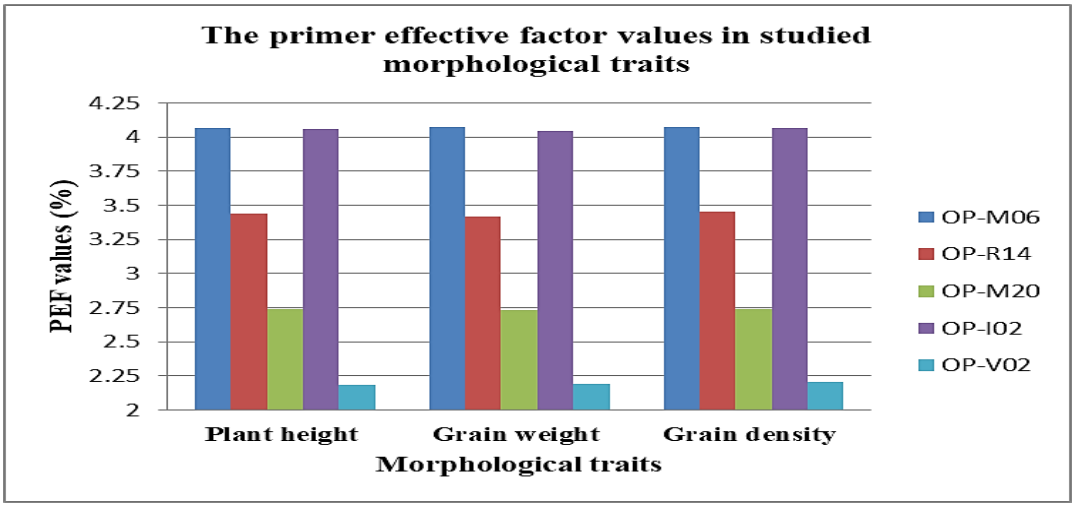

Figure (1): Primer effective factor included the effect of five primers (OP-M06, OP-R14, OP-M20, OP-I02 and OP-V02) on three morphological traits (Plant height, Grain weight and Grain density).

From Table (4) and Shape (1), Primers OP-M06 and OP-I02 showed a significant effect on the phenotypic traits studied, they gave an effect factor greater than 4% for all phenotypic traits, while the OP-R14 primer came in second which is given an effect between 3.25-3.5%, the primers OP-M20 and OP-V02 were the least influential in phenotypic traits and in a manner Specific to the OP-V02 primer, which gave a low effect was less than 2.25%.

Discussion

Primers differ in the number of DNA fragments given in each genotype, this difference is due to the presence of regions where a primer can be linked (13), these regions when increased, it shows a good relationship between the primer and the genetic material studied, so if the primer gives a large number of DNA fragments in the most genotypes, it is evidence of the high efficiency of the primer in binding and detecting different genes or DNA fragments (14). The DNA fragments have shown by the primer participate directly or indirectly in the gene expression that affects the phenotypic trait, the genotype which is rich in genetic material has the most influence in the presence of phenotypic trait with an increase in the quantity of the trait is linked to gene expression (15).

Primers differ in the number of DNA fragments that are detected, some primers show a large number of DNA fragments, while others show a few. Primers that show a large number of DNA fragments are required because they are more important in showing genetic variations among different genotypes in the same species or genus, while primers show a few numbers of DNA fragments, they may not give genetic variations clear, so it is preferable to exclude these primers in the subsequent study (16).

Recommendations

It is necessary to conduct studies related to the quality of the primers used in molecular measurements (ISSR, RAPD, RFLP..etc.) Before they are generalized to researchers for including them in their studies and adopt the primers that show the largest DNA fragments for obtaining large genetic variations, or the process of examining the primers can be conducted in studies conducted by researchers in the field of molecular measurements.

References

1. Stansfield, W. D. (1986). Theory and Problems of Genetics. Mcgraw-Hill Book Compan, New York.
2. Nantawan K., S. Jirawat, S. Pranee and T. Piyada. (2011). Genetic diversity analysis of rice cultivars (*Oryza sativa L.*) differing in salinity tolerance based on RAPD and SSR markers. Electronic Journal of Biotechnology, 14(6) 1-17.
3. Mueen, A. S. and M. Kh. Jabbar. (2019). Creation of molecular databases to Iraqi bread wheat cultivars, Plant Archives, 19(2): 3607-3612.
4. Sofalian, O., N. Chaparzadeh , A. Javanmard and M. S. Hejazi. (2008). Study of the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. Int. Journal of Agriculture and Biology 10(3): 466-468.
5. Jabbar, M. Kh. (2019a). Effect exposure of non-specialist tissue to ultraviolet treatments on gene expression and growth under cold stress in some breeds of beans, 2nd International Science Conference: IOP Conf. Series: Journal of Physics: Conf. Series, 1294.
6. Srisaikhan, S. (2021). A Comparison of Nutritional Values, Bioactive Compounds, Amino Acids, and Antioxidant Activities of Alfalfa (Medicago sativa L.) Plant and Pellet for Use as Beneficial Material Ruminant Feed Walailak Journal Science & Technology 18(5): 1-16.

7. Khavarinejad, M. S. (2014). Comparison of obtained wheat genetic divergence by molecular and morphological analysis using a cluster, Scientia Agriculturae 6(3): 107-113.
8. Jabbar, M.K.h. (2018). Response of Some Genotypes of Bread Wheat (*Triticum aestivum L.*) and Gene Expression under Salt Stress (In vitro and In vivo), Ph. D. Dissertation. Institute of genetic engineering and biotechnology for higher studies/ University of Baghdad-Iraq.
9. Jabbar, M.K.h. (2019b). Study of the Adaptation and Gene expression in Coffee Beans after Exposure to Mutation, Indian Journal of Ecology, 46(2): 399-401.
10. Haider Turky Mousa Al-Mousawi1, Al-Bderee Nadhim Mushtaq and Alyaa Hasan Bohan. (2022). Molecular and immunological activity of *Terminalia chebula* extracts. Journal of Advanced Biotechnology and Experimental Therapeutics, Journal Advance Biotechnological Experimental Therapy.; 5(1): 176-188,
11. Vierling, R.A. and Nguyen, H.T. (1992). Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. Theory Applied Genetics Journal 84(7-8): 835- 8.
12. Noah, L.H. (2018). Selection Some of Drought Tolerance Genotypes in bread wheat (*Triticum aestivum L.*) Under In Vitro and In Vivo Conditions Based on Conventional and Molecular Approaches. M.S.A A thesis, institute of genetic engineering and biotechnology for higher studies – university of Baghdad, Iraq.
13. Attia, H.J. and M.K.H. Jabbar. (2021). Gene expression of hkt and seedling growth parameters of three plant crops under salinity stress, Plant Cell Biotechnology and Molecular Biology, 22(5-6):1-7.
14. AL-Salihy, A.A., I. Hassan, M. K. Jabbar. (2018). Study of tasos4 gene and yield, chemical traits in some bread wheat genotypes under salinity conditions, Iraqi Journal of Agricultural Sciences, 49(4):586-591.
15. Qabas Neamah AL-Hajjar and Haider Turky Mousa Al-Mousawi, (2021). Immunological and Molecular Diagnosis of Cytomegalovirus Infection between Aborted & Pregnant Women in Babylon City. Baghdad Science Journal, 18:1-8.
16. Haider Turky Mousa Al-Mousawi. (2022). Remarkable association of the highly frequent rs1801133 snp of mthfr gene with growth hormone deficiency in children. Web of scientist: International Scientific Research Journal 3:1-5.