Research Article

Ekin
Journal of Crop Breeding and Genetics

9(1):19-23, 2023

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Cytoplasmic Genome Prediction in Cucumber (*Cucumis sativus* L.) Hybrid Variety Breeding**

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Citation:
Akar Öztürk L., Gülsen O., Zengin S., Vural GE., 2023. Cytoplasmic Genome Prediction in Cucumber (*Cucumis sativus* L.) Hybrid Variety Breeding. Ekin J. 9(1):19-23.

Received: 15.08.2022 ***Accepted: 30.11.2022*** ***Published Online: 31.01.2023*** ***Printed: 31.01.2023***

ABSTRACT

Breeding studies in Cucurbitaceae species take a long time. It has become necessary to shorten the time and support traditional breeding methods with modern biotechnological methods to get qualified domestic cucumber varieties. Cytoplasmic genome prediction within the scope of molecular-based breeding is a very important application. To increase heterosis in test crosses, reciprocal 'double way' crosses can be made as well as single crosses. Cytoplasmic organelles 'plastid and mitochondria' are considered to be different from each other between individuals and reciprocal crosses are made based on this idea. However it significantly increases the labor. In this study, 4 plastid genome regions (rbcL, psb-trnS, trnHK, trnSt) located within non-conserved regions therefore expected to be variable of 50 donor genotypes were sequenced, analyzed and their cytoplasmic genome prediction was estimated. A total of 6300 bp including four plastid regions indicated no polyfmorphism and all sequences were identical among the 50 donor genotypes analyzed. This may imply no cytoplasmic organelle variation. In conclusion, reciprocal crosses were excluded from our breeding studies. So cytoplasmic genome prediction can provide rapidity and savings in breeding by eliminating unnecessary reciprocal test crosses.

Keywords **:** Breeding, cucumber, *Cucumis sativus* L., cytoplasmic genome prediction, double haploid, hybrid variety breeding

** *This article was oral-presented at fourth International Plant Breeding Congress, November 21-25, 2022, Antalya Türkiye.*

Introduction

Cucumber (*Cucumis sativus* L.) belongs to the family of Cucurbitaceae which is one of the largest vegetable families with 117 genera and 825 species (Gopalakrishnan 2007). It is the top of 10 most cultivated products among fruits and vegetables. Cucumber is a warm-season vegetable crop and grows best at temperatures above 20°C. It consists of 95% water, 4% carbohydrates, 1% protein and negligible fat. It has a production value of 91 million tons in the world according to the latest statistics and Turkey ranks second after China with 1.9 million tons of production. (FAO 2020). It is necessary to carry out effective and rapid breeding studies in order to develop domestic hybrid varieties with high yielding and resistance to both abiotic and biotic stress factors in cucumber cultivation. Cucumber breeding with classical methods take too much time and effort. It has become necessary to shorten the time and support traditional breeding methods with modern biotechnological methods to get qualified domestic cucumber varieties. Cucumber has a small chromosome complement with n=x=7 and a small haploid genome of 367 Mbp/C (Huang et al., 2009) and genetic base of cultivated cucumber is narrow with 3–8% polymorphism within the cultivated genotypes (Behera et al., 2011). This narrow genetic

diversity makes breeding of this species is more difficult (Acquaah 2012). Obtaining haploid plants by double haploid techniuqe and make selection after hybridization with molecular markers is one of the supportive methods for shorten time. Many important horticultural traits such as size/shape of fruits and flowering time are quantitatively inherited and related genes have been recently identified and reported (Pan et al., 2020). Complex genetic variances can be captured with genomic prediction models by using genomic-coverage molecular markers to achieve more accuracy in the selection process (Liu et al., 2021). The genomic prediction has also become more practical in cucumber with the release of the full cucumber genome (Huang et al., 2009; Yang et al., 2012). Chloroplast and mitochondria genomes of plant cell are usually inherited from maternal parent, with rare exceptions (Park et al., 2021). In cucumber, previous studies indicated that chloroplast genome is maternally inherited whereas the large cucumber mitochondrial genome is paternally inherited (Park et al., 2021). To get heterosis in test crosses, reciprocal crosses can be applied as well as single crosses. Reciprocal crosses which are made based on the idea that the cytoplasmic organelles 'plastid and mitochondria' between individuals are different from each other, significantly increases the labor. So cytoplasmic genome prediction within the scope of molecular-based breeding is a very important application. In case where the origins of individuals whose cytoplasm can be estimated by molecular methods are not different, the need for reciprocal crosses will be eliminated, and a significant labor and time savings will be provided. In crop breeding genomic prediction method has been widely used such as rice (Xu et al., 2016), maize (Riedelsheimer et al., 2012) and potato (Sverrisdottir et al., 2018). However, in horticultural crops there are relatively fewer studies such as in pea (Tayeh et al., 2015), strawberry (Gezan et al., 2017) and tomato (Duangjit et al., 2016) while there are no related reports in cucumber. In this study, we tried to figure out our parental lines reciprocal need by cytoplasmic genome prediction for accelerate breeding.

Materials and Methods

Plant materials

The plant material used in this study was consisted of 44 cucumber parental donor lines and 6 selected promising lines from Antalya Tarım Seed Company's cucumber gene pool (Table 1). These total 50 donor lines were germinated in controlled conditions for DNA isolation.

Molecular Marker analysis

Single fresh leaf was used for DNA extraction. Total DNA was extracted from 100 mg fresh leaf tissue according to a modified CTAB DNA extraction procedure (Doyle and Doyle 1990). DNA pellets were diluted with 100 uL of TE (10 mM Tris, 0.1 mM EDTA, pH 7.4) and their concentrations were measured with Qubit fluoremeter. 10 ng ul^{-1} DNA templates for PCR amplifications were made using with distilled water. Four plastid genome regions (rbcL, psb-trnS, trnHK, trnST) of 50 donor genotypes were sequenced. Region spesific polymorphic universal primers were used for PCR assays (Gulsen and Ceylan 2011) (Table 1). Regions were amplified by using specific primers designed for this purpose at 60°C annealing temperature in a 50-uL reaction and 15 ul of PCR-amplified products were separated by agarose gel electrophoresis for control. These marker analyses were done by Agromar Seed Company. Remaining 35 ul of PCR products, which has seen as a single band, were analysed with MEGA Genetic Analysis Program by MASGEN Ar-Ge Ltd.Sti for statistical analyses.

Results and Discussion

Molecular Marker Analysis

Four plastid genome regions which are assumed to be non-conserved due to their intergenic location were amplified with polymorphic universal primers targeting rbcL, psb-trns, trnHK and trnST regions. Primer pairs produced non-polymorphic, size expected fragments and those fragments are 100% reproducible. (Fig.1). The resulting band sizes are approximately about 900, 1500, 3000 and 1400 base pairs. Obtained results are consistent with previous studies (Gulsen and Ceylan 2011).

Statistical Analysis

Illumina DNA libraries of the PCR products were prepared and barcoded ('indexed') for sequencing by MASGEN Ar-Ge Ltd.Ş., Antalya. The bioinformatics processes (quality filtering processes, demultiplex processes and assembly processes) of each of the PCR products to be obtained as a result of sequencing, were carried out by the company. According to alignment results which achieved by using MEGA Genetic Analysis Program, there were no cytoplasmic genome difference among 50 donor genotypes although these regions are assumed to be non-conserved due to their intergenic locations.

In a previous study the chloroplast genomes of some cucumber lines were fully assembled however the mitochondria genomes could not which also same in our study (Park et al., 2021). In this study, the plastid genomes of the 50 cucumber lines were checked, however, the mitochondria genomes were not because generally chloroplast copy number is much higher than that of the mitochondria (Alverson et al., 2011).

Conclusions

The plastid genome length of cucumber is 150,501 base pairs, and its length of about 6300 base pairs was analyzed and estimation done for the rest according to these results in this study. It is found that four intergenic plastid regions, which were known to be polymorphic in previous studies, were exactly the same for 50 genotypes so all came from same maternal origin. Only plastid specific primers were used in this study, therefore our results indicate only maternal origins not paternal origins. Therefore combined use of plastid and mitochondria specific primers may produce more comprehensive results about need of reciprocal crosses. Since these results were assumed that there was no cytoplasmic genome difference between the hundreds of inbred lines obtained from 50 genotypes, reciprocal crosses were excluded. This analysis can provide rapidity and savings in breeding by eliminating unnecessary reciprocal test crosses.

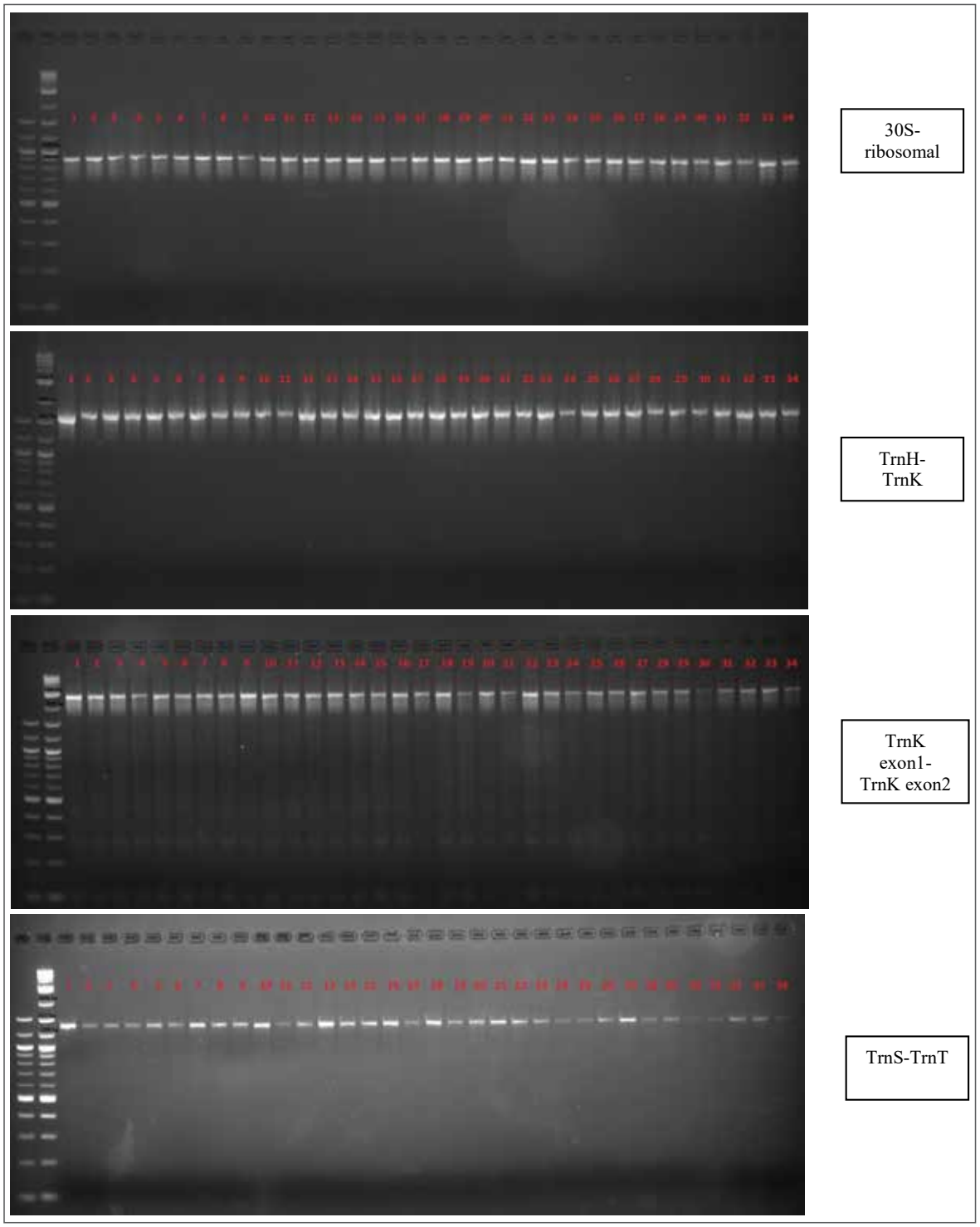

Figure 1. Gel images of uncut PCR products with primers which targets four plastid genome regions.

Table 1. Genotypes and sources used in this study.

Genotype	Source	Genotype	Source	Genotype	Source
Baby F_1	Multi Tohum	Petek Çıtır	Petek Tarım	Kıtır F_1	Yüksel Tohum
Çaka F_1	Antalya Tarım	Emek F_1	Antema Tarım	Yazel 801	Remitto
Umur F_1	Antalya Tarım	Tofida F_1	Teksin	Seçkin F_1	Multi Tohum
Ayda F_1	Takii Seed	Zincir F_1	Mars	Egemen F_1	Seminis
Multistar	Rıjk Zwaan	Uçar F_1	Yüksel Tohum	SV2709 CB	Seminis
PTK40	Petek Tarım	Captainstar	Rıjk Zwaan	Faris F_1	Gavrish
Senyal F_1	Rıjk Zwaan	Kıvılcım F_1	Tasaco	Gözde F_1	Multi Tohum
Süvari F_1	Seminis	Assos F_1	Seminis	Solo F_1	Yüksel Tohum
Çakıl F_1	Axia	Zirve F_1	Yüksel Tohum	Y*225 F_1	Yüksel Tohum
Efsane F_1	AG Tohum	Amisos F_1	Altın Tohumculuk	Tenedos F_1	Seminis
Şampiyon	Altın Tohumculuk	Malazgirt	Altın Tohumculuk	Gordion F_1	Seminis
Nur F_1	Eastern Seed	Özde F_1	Yüksel Tohum	PS 64 F_1	Seminis
Sardes F_1	Seminis	Falconstar	Rıjk Zwaan	Bellastar F_1	Rıjk Zwaan
Titanik F_1	Yüksel Tohum	Çılgın F_1	Vilmorin	Tribün F_1	AG Tohum
Haylaz F_1	Vilmorin	Carrera F_1	Fito		

Table 2. Region specific universal primer list.

Primer Name	Forward Primer	Reverse Primer
rbcl	TAGTTTCTGTTTGTGGTGACAT	AAGTAGTAGGATTGGTTCTCAT
psb-trns	GGTCGTGACCAAGAAACCAC	GGTTCGAATCCCTCTCTCTC
trnHK	ACGGGAATTGAACCCGCGCA	CCGACTAGTTCCGGGTTCGA
trnST	CGAGGGTTCGAATCCCTCTC	AGAGCATCGCATTTGTAATG

References

Acquaah G, (2012). Principles of plant genetics and breeding: Breeding Cucumber. 2. New York: Wiley; pp. 676–681

Alverson AJ, Rice DW, Dickinson S, Barry K. and Palmer JD, (2011). Origins and recombination of the bacterial-sized multichromosomal mitochondrial genome of cucumber. Plant Cell 23, 2499–2513.

Behera TK, Staub JE, Behera S, Delannay IY, Chen JF, (2011). Marker-assisted backcross selection in an interspecific *Cucumis* population broadens the genetic base of cucumber (*Cucumis sativus* L.). Euphytica 178, 261-272.

Doyle JJ and Doyle JL, (1990). Isolation of plant DNA from fresh tissue. Focus 12, 13–15.

Duangjit J, Causse M, and Sauvage C, (2016). Efficiency of genomic selection for tomato fruit quality. Mol. Breed. 36:29.

FAO, (2020). FAO of the United Nations. FAOSTAT, http://www.fao.org/faostat/en/#data/QC Accessed 05 February 2020.

Gezan SA, Osorio LF, Verma S, and Whitaker VM, (2017). An experimental validation of genomic selection in octoploid strawberry. Horticult. Res. 4:16070.

Gopalakrishnan TR, (2007). Vegetable crops. In: Peter KV, Swaminathan MS, Editors. Horticulture Science Series – 4. India: New India Publishing Agency; p. 103

Gulsen O, Ceylan A, (2011). Elucidating polyploidization of bermudagrasses as assessed by organelle and nuclear DNA markers. OMICS A Journal of Integrative Biology 15 (12): 903-912.

Huang, S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B and Ni P, (2009). The genome of the cucumber, *Cucumis sativus* L. Nat. Genet. 41, 1275–1281.

Liu C, Liu X, Han Y, Wang X, Ding Y, Meng H, and Cheng Z, (2021). Genomic prediction and the practical breeding of 12 quantitative-inherited traits in cucumber (*Cucumis sativus* L.). Front. Plant Sci. 12:729328

Pan YP, Wang YH, McGregor C, Liu S, Luan FS, Gao ML, et al., (2020). Genetic architecture of fruit size and shape variation in cucurbits: A comparative perspective. Theoretic. Appl. Genetics 133, 1–21.

Park HS, Lee WK, Lee SC, Lee HO, Joh HJ, Park JY, Kim S, Song K. and Yang TJ, (2021). Inheritance of chloroplast and mitochondrial genomes in cucumber revealed by four reciprocal F_1 hybrid combinations. Scientifc Reports 11:2506.

Riedelsheimer C, Czedik-Eysenberg A, Grieder C, Lisec J, Technow F, Sulpice R, et al., (2012). Genomic and metabolic prediction of complex heterotic traits in hybrid maize. Nat. Genet. 44, 217–220.

Sverrisdottir E, Sundmark EHR, Johnsen HO, Kirk HG, Asp T, Janss L, et al., (2018). The Value of expanding the training population to improve genomic selection models in tetraploid potato. Front. Plant Sci. 9:1118.

Tayeh N, Klein A, Le Paslier MC, Jacquin F, Houtin H, Rond C, et al., (2015). Genomic prediction in pea: Effect of marker density and training population size and composition on prediction accuracy. Front. Plant Sci. 6:941.

Xu SZ, Xu Y, Gong L, and Zhang QF, (2016). Metabolomic prediction of yield in hybrid rice. Plant J. 88, 219–227.

Yang LM, Koo DH, Li YH, Zhang XJ, Luan FS, Havey MJ, et al., (2012). Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. Plant J. 71, 895–906.