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The Creation of a Gene Library of Valuable Plant Species in Armenia

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ABSTRACT

Using DNA banks or gene libraries is the most efficient method of protecting and managing valuable, endangered, and rare gene pools. Genomic libraries are collections of bacteria colonies with restriction sites on their genomic DNA that contain desired genes from a specific organism. The "Scientific Center of Agro-Biotechnology" branch of ANAU has created gene libraries of 34 plant species, which are preserved in the National Genebank of Crops and their Wild Relatives, which forms a basis for genomic selection. This allows genetic identification, barcoding, DNA formula decoding, and, most importantly, is the basis for genome identification. Based on the results, plasmids can incorporate restriction segments of genomic DNA and be efficiently transformed. The high level of correlation between the number of necessary clones and the efficiency of transformation of all studied plant species proves the completeness of the gene pool.

Introduction

In every country, genetic resources are a source of wealth. Due to their natural evolution and human activity, they play an essential role in food production and environmental balance. Breeding cultivated plants and animals contributes to economic growth, national autonomy, and food security (Eastwood, et al., 2022). For preventing genetic resources erosion, utilizing them responsibly, conserving resources, and developing the economy sustainably, the preservation and diversity of the gene pool are essential, being closely related to science, technology, economics, law, ethics, and international politics (Altukhova, 2004; Chandra and Idrisova, 2011).

It is a necessity to create and cultivate varieties of crops that can meet population and production needs within a short period. This is at the current stage of agricultural development. Genomic and classical selection is necessary to improve existing ones. As a result, DNA banks or gene libraries are the most practical way to preserve valuable gene pools, and catalog, locate, and obtain genes.The forced construction of a genomic library can be divided into two types based on the DNA source used: (a) Nuclear Genomic Library: this is the genomic library that contains the entire nucleus' DNA. This type of library is made by extracting nuclear DNA and using to create it. An organelle genome library excludes nuclear DNA and targets the DNA of either mitochondria, chloroplasts, or both (www.biotechnologynotes.com).

An organism's genomic DNA can be inserted into a gene pool by inserting restriction sites into a vector. Colonies like these are considered sources of desired genes. As a result, DNA segments from valuable species are stored in bacterial cells (Goncharenko, 2005). Each bacterial cell contains genomic DNA. Clones are created when a bacterial cell multiplies, receiving hybrid DNA. DNA banks consist of bacterial cells that contain all fragmented DNA fragments from a particular organism (David and Clark, 2019). This study aimed to create gene libraries of 34 plant species preserved in the "Scientific Center of Agro-Biotechnology" branch of the Armenian National Agrarian University (ANAU). By doing this, we established a basis for genomic selection. The mentioned works were performed for the first time in the Republic of Armenia.

Materials and methods

The research was carried out in the Biological Research Laboratory of the "Scientific Center of Agro-Biotechnology" branch of the ANAU, during 2021-2023. Plant material was collected from the ex-situ seed collection of the National Genebank of crops and their wild relatives. The SDS method was used to obtain genomic DNA from crops and wild species (Padutov, et al., 2007). The NanoDrop One spectrophotometer (Thermo Scientific, USA) was used to determine denatured DNA concentration. To obtain colicinogenic plasmids against ampicillin (as a selection marker), ampicillin resistance was identified in *E. coli* according to the following scheme (Gvozdeva, et al., 2012). At 37 ^{0}C for 18 hours, *E. coli* colonies were grown in 100 ml (LB) nutrient medium containing 50 μg/ml ampicillin. Fresh (LB) nutrient medium containing ampicillin was added to a portion of the overnight culture to ensure a 100-fold dilution. After 16-18 hours of growth, the optical density (2-3∗10^8 cells/ml) was reached. The basic degradation method was used to isolate plasmid DNA from bacterial cells (Padutov, et al., 2007; Kutlunina and Ermoshin, 2017).

Genomic DNA and vector were restrictedly digested using *EcoRI* restrictive enzyme (Kayumov, 2016), restriction mixture containing: water – 15 μl, 10x buffer – 2 μl, DNA –2 μl (2 μg/ml), restriction enzyme – 1 μl (5 units/ μl). At 37 ^{0}C, the mixture was incubated for 16 hours. A restriction method was used to determine DNA incorporation into transformed cells (Padutov, et al., 2007). In the case of a restrictive enzyme, to avoid cleaving of the split ends of the vector, dephosphorylation of plasmid DNA was carried out immediately after restriction according to the following scheme: adding 5 μl of 10x buffer (5 μg DNA, 50 μl deionized water, 2 μl alkaline phosphatase) to a 50 μl reaction mixture to avoid all this. At 37 ^{0}C, the mixture was incubated for one hour. A phenol-chloroform method was used to purify DNA, and 96 % ethanol was used to remove phosphatase.

Ligation was used to seal the insert into the vector, which is done by using T4 DNA ligase, according to the following scheme: A 10x buffer was used, a plasmid DNA sample (0.1 μg/ml) of 1.5 μl, restriction fragments of genomic DNA sample of 3 μl (0.1 mg/ml), a T4 DNA ligase sample of 1 μl, and water were used and incubated at 16 ^{0}C, for 2 hours (Kayumov, 2016; Zhuravleva, 2022).

According to (Yprintsev, et al., 2008), complementary cells were obtained by treating them with 0.1 M calcium chloride:

1. *E. coli* colonies from the Petri dish were transferred to a tube containing 2 ml of LB nutrient medium at 37 ^{0}C and were incubated overnight for 16-18 hours.
2. 200 μl of the overnight culture was transferred to a tube containing 10 ml of LB and was grown for 2.5 hours at 37 ^{0}C.
3. 1.5 mL of cell culture was centrifuged at 13.000 rpm for 1 minute.
4. Cells were suspended in 200 μl cold and sterile solution of 0.1 M $CaCl_2$, then transferred to ice under 4 ^{0}C conditions for 2 hours.

In this case, heat shock was used to carry out the genetic transformation (Koltovaya, 2010).

Clark's formula was used to determine the amount of recombinant DNA (clone) needed (Yeprintsev, et al., 2008):

$$N = Ln(1-p)/Ln(1-f),$$

where N = number of recombinants, p = probability, and f = fraction of the genome contained in a single average insert. According to (Zhuravleva, 2022), transformation efficiency was calculated by the formula:

$$T=M/A,$$

where T = transformation efficiency, M = the total number of transformed colonies per cluster, and A = the amount of incorporated DNA (μg).

Results and discussions

In creating gene libraries, inserting as many restriction segments of genomic DNA of a specific organism into the vector as possible and undergoing genetic transformation is extremely significant. Vectors are DNA molecules that self-replicate within cells of different organisms, transferring genetic information to the recipient. Genomic DNA restriction insert size guides vector selection. As a result of digestion with restriction enzymes, these inserts are generated.

The current study used the EcoRI restriction enzyme, which has the following nucleotide sequence at its site:

5′-G-A-A-T-T-C-3′
3′-C-T-T-A-A-G-5′

EcoRI recognizes six bases, so they cut every 46 kb or 4096 bases. According to this fact, plasmids were chosen as vectors. It is possible to clone DNA inserts up to 15 kb on most plasmids (Goncharenko, 2005).

DNA bank formation is directly influenced by the number and efficiency of clones carrying restriction inserts. Our study found that breadfruit had the largest genome size among the plant species we studied (table). The range was from $1.68*10^6$ kb to $17*10^6$ kb. Spinach (*Spinacia tetrandra Steven*.) has a genome size of $989*10^3$ kb, while Physalis (*Physalis alkekengi L.*) has a genome size of $157*10^3$ kb.

Depending on the number of clones, restriction inserts of genomic DNA are more likely to be inserted into the genome (90 %).

The number of clones needed to capture the genomic DNA of the studied plant species, the number of clones for DNA extraction is presented in the same table as well. Based on the numerical values, it becomes evident that the number of clones needed depends on the genomic DNA size. Hence, clones are needed more when the genome is large. The largest number of clones was 1920-19131 kb, and in other cases, it ranged from 174.4 kb to 1292 kb (table).

Table. Analysis of the transformation efficiency of the studied plant species*

№	Plant species/varieties	Genome size (kb)	The number of clones (N)	Transformation efficiency (T)
1	*Triticum urartu Tumanian ex Gandilyan*	$5*10^6$	5625	$2.4*10^8$
2	*Triticum araraticum Jakubz*	$5*10^6$	5625	$2.2*10^8$
3	*Triticum boeoticum Boiss.*	$5*10^6$	5625	$2*10^8$
4	*Triticum aestivum L.. variety "Alti Aghaj"*	$17*10^6$	19131	$3*10^8$
5	*Triticum aestivum L.. variety "Voskehask"*	$17*10^6$	19131	$3.8*10^8$
6	*Triticum aestivum L.. variety "Gyulgiani"*	$17*10^6$	19131	$2.8*10^8$
7	*Triticum aestivum L.. variety "Garaseferyani"*	$17*10^6$	19131	$3.6*10^8$
8	*Triticum aestivum L.. variety "Qrik"*	$17*10^6$	19131	$2.4*10^8$
9	*Triticum aestivum L.. variety "Galgalos"*	$17*10^6$	19131	$2*10^8$
10	*Hordeum bulbosum L.*	$5.3*10^6$	5987	$3.2*10^8$
11	*Hordeum vulgare L.. variety "Hayk 1"*	$5.3*10^6$	5987	$3.7*10^8$
12	*Hordeum vulgare L.. variety "Hayk 2"*	$5.3*10^6$	5987	$3.1*10^8$
13	*Hordeum vulgare L.. variety "Marina"*	$5.3*10^6$	5987	$3*10^8$
14	*Aegilops tauschii Cosson.*	$4.2*10^6$	4704	$2.8*10^8$
15	*Aegilops umbellulata Zhuk.*	$4.2*10^6$	4704	$2.5*10^8$
16	*Lactuca serriola L.*	$2.5*10^6$	2881	$3.6*10^8$
17	*Spinacia tetrandra Steven.*	$989*10^3$	1098	$3.8*10^8$
18	*Daucus carotaL.*	$473*10^3$	530.1	$4.2*10^8$
19	*Beta macrorhiza Steven.*	$758*10^3$	850.8	$3.8*10^8$
20	*Beta lomatagona F. et M.*	$758*10^3$	850.8	$2.1*10^8$
21	*Beta corolliflora Zoss.et Butler*	$758*10^3$	850.8	$2.8*10^8$
22	*Beta vulgaris L. variety "Bordeaux 237"*	$758*10^3$	850.8	$2.7*10^8$
23	*Physalis alkekengi L.*	$157*10^3$	174.4	$3.7*10^8$
24	*Coriandrum sativum L.*	$213*10^4$	2395	$2.4*10^8$
25	*Phaseolus vulgaris L.* "Buzhakan local" variety-population	$587*10^3$	658.4	$3.2*10^8$
26	*Phaseolus vulgaris L.* "Goris local" variety-population	$587*10^3$	658.4	2.2*108
27	*Phaseolus vulgaris L.* "Armenian red" variety-population	$587*10^3$	658.4	2.8*108
28	*Phaseolus vulgaris L..* "Kotayk local" variety-population	$587*10^3$	658.4	$4.2*10^8$
29	*Cicer arietinum L.* variety" Leninakan 313"	$740*10^3$	830.6	$3.6*10^8$
30	*Cicer arietinum L.* variety" Karin"	$740*10^3$	830.6	$2.4*10^8$
31	*Glycine max Merr.* variety "Milena"	$115*10^3$	1292	$2.8*10^8$
32	*Vitis vinifera ssp. sylvestris*	$500*10^3$	560.4	$4.4*10^8$
33	*Rubus L.*	$240*10^3$	267.8	$3.3*10^8$
34	*Pistacia mutica Fisch et C.A.Mey.*	$600*10^3$	673	$3.2*10^8$

*Composed by the author.

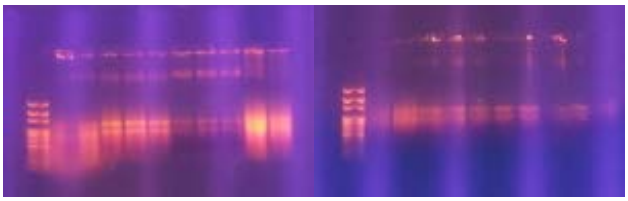

Figure. The results of DNA recombination in transformed cells.

Transformations in the gene pool indicate outcomes. The highest transformation results were recorded in wild grape ($4.4*10^8$), local Kotayk bean variety ($4.2*10^8$), and wild carrot ($4.2*10^8$) species. While the lowest was observed in the Galgalos wheat variety, wild Triticum boeoticum, Triticum araraticum species, the wild Beta corolliflora species of the beet, and the local variety-population of bean from Goris, which equaled $2*10^8$, $2*10^8$; $2.2*10^8$, $2.8*10^8$ and $2.2*10^8$ respectively. There were also cases where it ranged between $2.4*10^8$ – $3.8*10^8$. According to the study, genomic DNA is fully incorporated into the genome of the studied plant species. Agarose gel electrophoresis was used to determine DNA incorporation into transformed cells (Figure).

As can be seen from the agarose gel, recombinant cells of the tested plant species contain a vector carrying recombinant DNA. As a result, the genome is complete. After growing transformed colonies in LB medium for 8 hours and preserving them in 50 % glycerol solution, a 3:1 ratio formed the DNA bank. To preserve transformed cells for a long period, they were stored at -70 ^{0}C.

Conclusion

Species from the Poaceae family had the largest genome size among the studied plant species, whereas *Physalis alkekengi L.* had the smallest. Due to their 15 kb size, plasmids can efficiently transform genomic DNA while containing restriction segments. Gene pool completeness is demonstrated by the high correlation between the number of necessary clones and transformation efficiency for all species studied.

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