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# CHROMOSOMES MAP, FUNCTION, CLASSIFICATION, AND GENE DETECTION IN PROSO MILLET (*PANICUM MILIACEUM* L.)

**M.A. KHALAF and M.KH. JABBAR***

Faculty of Agriculture, Al-Qasim Green University, Iraq
*Corresponding author's email: dr.mundher80@agre.uoqasim.edu.iq
Email address of co-author: maha.adel@agre.uoqasim.edu.iq

**SUMMARY**

Molecular information has been available about the genome of proso millet (*Panicum miliaceum* L.), which include about 18 nucleus' chromosomes, one chloroplast chromosome, and more than 100 main genes with a total sequence length of proso millet 854,793,052 nucleotides. All chromosomes also differed in total length, ungapped length, Scaffold N50, and spanned gaps, however, did not differ in scaffold count and unspanned gaps. All genes in the genomic classification resulted into 17 groups according functions. Therefore, some genes encode functional enzymes with links to organelles in the cytoplasm and inside the nucleus to perform a specific function or structural proteins involved in plant biosynthesis. The 17 groups observed had main functions of the protein (ribosomal RNA protein, ATP synthase enzyme, NADH-plastoquinone oxidoreductase enzyme, cytochrome protein, photosystem, ribosomal Protein L, ribosomal Protein S, RNA polymerase, tRNA protein, Hypothetical chloroplast RF, and single proteins). The genes also differed in size, and the smaller gene included 65 nucleotides, while the bigger gene included 2883 nucleotides. The local variety of millet grown in Iraq possesses most genes registered among the international millet varieties of the Gene Bank, according to the National Center for Biotechnology. This study validated such results of gene detection.

**Keywords:** Proso millet (*P. miliaceum* L.), molecular studies, gene map, chromosomes, gene functions, nucleotides, proteins

**Key findings:** The presented molecular study detected 17 functional gene groups in the local cultivar of Proso millet (*P. miliaceum* L.).

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## INTRODUCTION

Proso millet (*Panicum miliaceum* L.) domestication occurred around 10,000 years ago in Northern China. This self-pollinated allotetraploid crop is a climate-resilient and human health-promoting cereal (Khound *et al*., 2022). Molecular genetics refers to a fundamental theory alleging genes direct all life processes through the production of polypeptides. Sometimes, it is a more modest basic theory about the expression and regulation of genes at the molecular level, and occasionally, an investigative approach based on strategies grounded on the basic theory about genes (Waters, 2013; Al-Fatlawi and Jabbar, 2023). In the present era, current studies included chromosomes and genes' information as molecular additions (Orlando, 2000; AL-Hilfi *et al*., 2020).

In crop plants, various studies have been progressive related to plant molecular biology, especially in field crops, such as, wheat, barley, and maize, with these crops used for food, forage, and other purposes. Millet is one of the grass forage crops, and its grains serve as bird feeds, while the green plant parts as cattle forage (Zhang *et al*., 2019). It has the highest and fast growth with high yields, completing the vegetative growth in 30 days. Therefore, it gives several mowing in a crop season in Iraq. Moreover, less breeding research and improvement programs have transpired because of the insufficient information about its genetic material and the knowledge on the genes involve in the plant biology.

The proso millet genome consists of more than 100 genes. The sequence of genetic material is about 854,793,052 base pairs, and the total ungapped length is about 837,869,052 base pairs; however, it does not have a gap between scaffolds. The number of scaffolds consists of 1306, and the scaffold N50 consists of 46,661,915, while scaffold L50 is only eight. The number of contigs is from 5537, which included contig N50, consisting of 368,640, and contig L50 consisted of 423. A total number of chromosomes are about 19, and the number of component sequences (WGS or clone) is about 1306 (SCPS, 2018).

The millet genomics included 136 genes distributed among the various chromosomes found in the nucleus and the cytoplasm. All the genes have their own biological functions and divided into two types, according to mRNA molecules, to translate to a functional protein called enzymes (Kalinova and Moudry, 2006; Zimmermann *et al*., 2013). Several studies on nucleotides of the millet genome have proceeded, with the first study conducted in 1992 (Taniguchi *et al.,* 1992). However, as per the literature, the last study on millet genome was unpublished; although, its introduction continued in the College of Bioengineering, Shanxi University, China (Shen *et al*., 2018). The pop set about 30 and the bio projects have studied 22 projects, and the bio-samples have registered 450 (NCBI, 2018).

Genetic studies about the genes of proso millet are limited, and therefore, little information relates to the genome of the crop. Therefore, the promising study ensued to conduct and get an aide-mémoire booklet, including information about the proso millet genome.

## MATERIAL AND METHODS

### Genetic material and procedure

The presented study began in the Laboratory of the Department of Field Crops, Al-Qasim Green University, Iraq. The planting of proso millet seeds in special soil reached harvest after 15 days, with the plant leaves taken for RNA extraction.

### RNA extraction

Ribonucleic acid (RNA) extraction was the first step in studying the gene's expression. Different types of RNA molecules include the messenger RNA (mRNA), ribosomal RNA (rRNA), and small interfering RNA (siRNA). The mixture of total RNA was generally evident in the proso millet leaves and shoots samples. The TRIzol® protocol used for RNA extraction from the leaf had the general RNA extraction from three sources: tissues, adherent cells, and suspension cells. The protocol proceeded

according to the TRIzol® RT FDmix Kit (Wizbiosolution, Seongnam, South Korea)**.**

**Reverse transcriptase**

Conversion of the total single-stranded RNA into complementary DNA (cDNA) progressed according to the protocol of WizScript RT FDmix Kit (Wizbiosolution, Seongnam, South Korea).

**Reaction, thermal cycling program, and primers**

Using the standard Kit application for a single reaction started by adding only water and placing the RNA on a template to the RT FDmix (Hexamer) tube. The reaction components appear in Table 1. Carrying out the thermocycler program followed the procedure of the FDmix Kit (Table 2). Immediately, using the synthesized cDNA as a product, continued with the product prepared for PCR electrophoresis material (Xiang *et al.*, 2007). The used primers are available in Table 3. The genes' amplification happened in Real-time PCR, and then, transferred a product for the detection of genes through electrophoresis.

**Chromosomes and other genes**

The study sent samples of the local proso millet grown in Iraq to the Biotechnology Center in China for evaluation. Detection and tests ensued there, providing the data on chromosomes and other genes that had been undetected in Iraq.

**Table 1.** Reaction components for converting single-strand RNA to complementary DNA (cDNA).

| No. | Reagent | Volume |
|---|---|---|
| 1. | RT_FDmix (Hexamer) | 1 tube containing all components for the reaction |
| 2. | Primers | 1 µL in all primer |
| 3. | Template RNA | < 5 µL |
| 4. | RNase free water | Up to 20 µL |

**Table 2.** Thermal cycling program for conversion of RNA into cDNA.

| Stage | Temperature (°C) | Time | Cycle No. |
|---|---|---|---|
| cDNA synthesis | 50 | 20 min. | Hold |
| Denaturation Initial | 95 | 10 min. | Hold |
| Denaturation | 95 | 30 s | |
| Annealing | 62 | 30 s | |
| Extension | 72 | 1 min. | 35 |
| Extension | 72 | 5 min. | Hold |

**Table 3.** Primers and gene size were studied in the Proso millet. (Nie *et al.,* 2018; Zou *et al.,* 2019).

| Gene name | Forward Primer (5′–3′) | Reverse Primer (5′–3′) |
|---|---|---|
| atpE | AGCACHCCHSARATGTGGC | CGDGGHAYRTGRTACCATC |
| ndhC | TAACTCACCTCAACTCGCTC | AACAGCACCTGAAGTTACCA |
| petA | ATGCTCGAGAAGATTGCTGA | ACAGGTCCAAATGGCATGTA |
| psaC | GGATGTCCACTCTTCCAGAC | GCTCAATGGTAACCAATAGG |
| rpl14 | GGTTTAAGCGGTGAAGTTG | TTGTCCAATTCCTTGAGGA |
| psbH | AAGCTGCTCTTGCCTATGAT | CTGCTAGTTGGTGAGGCATA |
| rpoA | GATGCACGATTTCGCCATAA | GGTGGAAGCAGAAACAATCC |
| rps8 | CATACCGTGAGCAAAACAGG | GGCAATGAAACTGATGCACT |
| trnA-UGC | TCAAACCCACCCATGTCTTC | CGCGTGAGTGTGGTAATCTG |
| ysf 4 | CTCCGGTGAAATCCATCATC | TTGCAGCGGAAGAACAAATA |

## RESULTS AND DISCUSSION

### Chromosome map and description

The results showed the proso millet (*P. miliaceum*) genome consisted of 19 chromosomes, including 18 chromosomes in the nucleus and one chromosome in the chloroplast, where all chromosomes differed in total length (Table 4). The first chromosome was the largest, comprising 69,183,459 nucleotides, while the 18th chromosome was of smaller length (32,237,550 nucleotides). The one chromosome found in chloroplast showed the length of about 9,477,950 nucleotides. The scaffold count was similar in all the chromosomes by giving the unity (1) value. However, the ungapped length of chromosomes was different; the first chromosome provided the highest length of 67,843,739 compared with the 18th chromosome, which gave an ungapped length of 32,044,379, while the chloroplast chromosome length gave 9,477,950.

The scaffold N50 was the highest in the first chromosome (69,183,459) and the lowest in the 18th chromosome (32,237,550). The chromosome found in the chloroplast provided 28,187 scaffold N50s. The spanned gaps were 85 in the first chromosome and the least was 29 in the eighth chromosome. The unspanned gaps were unidentifiable. Plant species vary in the number of chromosomes due to their genetic material content. A noticeable constant chromosome number of 2n = 18 among the genotypes of proso millet existed, regardless of their sources. However, despite differences in chromosome number, the basic chromosome number x = 9 has remained for this species. Previous reports indicated the basic chromosome number in *Pennisetum* varies, from x = 5, x = 7, x = 8, and x = 9 (Harlan and de Wet, 1971; Morakinyo and Adebola, 1991; Techio *et al.,* 2006). Proso millet belongs to the x = 9 group, and thus, study findings are consistent with previous reports. Polyploidy is an important phenomenon that can play a role in genome rearrangement, adaptation, reproductive isolation, and ultimately, species. It is crucial in plant breeding because it can affect hybridization, fertility, and gene expression (Adams and Wendel, 2005).

**Table 4.** The description of the chromosomes included their length, scaffold, and gaps.

| Molecules | Total Length | Scaffold Count | Ungapped Length | Scaffold N50 | Spanned Gaps | Unspanned Gaps |
|---|---|---|---|---|---|---|
| All | 848,351,880 | 466 | 838,886,533 | 48,259,421 | 829 | 0 |
| Chromosome 1 | 69,183,459 | 1 | 67,843,739 | 69,183,459 | 85 | 0 |
| Chromosome 2 | 61,153,219 | 1 | 60,497,902 | 61,153,219 | 59 | 0 |
| Chromosome 3 | 57,970,102 | 1 | 57,341,442 | 57,970,102 | 50 | 0 |
| Chromosome 4 | 56,286,655 | 1 | 55,895,376 | 56,286,655 | 34 | 0 |
| Chromosome 5 | 54,126,031 | 1 | 53,410,514 | 54,126,031 | 52 | 0 |
| Chromosome 6 | 52,839,179 | 1 | 52,069,300 | 52,839,179 | 46 | 0 |
| Chromosome 7 | 51,234,605 | 1 | 50,270,538 | 51,234,605 | 67 | 0 |
| Chromosome 8 | 48,259,421 | 1 | 48,045,998 | 48,259,421 | 29 | 0 |
| Chromosome 9 | 45,112,342 | 1 | 44,590,768 | 45,112,342 | 70 | 0 |
| Chromosome 10 | 44,648,547 | 1 | 44,079,361 | 44,648,547 | 53 | 0 |
| Chromosome 11 | 43,177,482 | 1 | 42,827,818 | 43,177,482 | 30 | 0 |
| Chromosome 12 | 42,466,157 | 1 | 42,020,767 | 42,466,157 | 30 | 0 |
| Chromosome 13 | 40,720,392 | 1 | 40,083,665 | 40,720,392 | 50 | 0 |
| Chromosome 14 | 38,490,750 | 1 | 38,190,433 | 38,490,750 | 32 | 0 |
| Chromosome 15 | 34,360,906 | 1 | 34,225,472 | 34,360,906 | 34 | 0 |
| Chromosome 16 | 33,613,985 | 1 | 33,444,542 | 33,613,985 | 45 | 0 |
| Chromosome 17 | 32,993,148 | 1 | 32,526,569 | 32,993,148 | 25 | 0 |
| Chromosome 18 | 32,237,550 | 1 | 32,044,379 | 32,237,550 | 38 | 0 |
| Unplaced | 9,477,950 | 448 | 9,477,950 | 28,187 | 0 | 0 |

**Gene map, classification, and functions**

In proso millet, the genes' division into two types depended upon the sites, genes in the nucleus, and genes in cytoplasm, such as, in chloroplast, ribosomes, and mitochondria. All the gene transcripts can build the proteins (enzymes). The gene map of proso millet appears in Table 5 and discussed herein.

- Four ribosomal RNA genes transcript ribosomal RNA gr proteins identified and named as 5S, 4.5S, 23S, and 16S, which is one of the ribosomal groups;
- Six ATP synthase enzymes found in the proso millet genome (CF1 alpha subunit, CF1 beta subunit, CF1 epsilon subunit, CF0 subunit I, CF0 subunit III, and CF0 subunit IV) transcripted from *ATP (adenosine triphosphate)* genes. These ATP enzymes showed as responsible for the energy synthesis (Nie *et al*. 2018);
- The *cem-A* gene is responsible for the chloroplast envelope membrane from a protein named chloroplast envelope membrane protein;
- One protease enzyme named Clp protease proteolytic subunit transcript from the *ClpP* gene. This protein appeared in the ribosome, and peptide chain connection was its function;
- One gene (EHI_096750) transcripted Alanine aminotransferase, a putative protein. It was a recognized transporter enzyme to alanine (Yoshimura *et al*., 2005);
- The *infA* gene is an important gene as a co-factor. It transcripts translational initiation factor-1 protein enter in genomic transcription;
- The *mat*K gene transcripts two enzymes responsible for plant maturity, named maturase. This enzyme enters in the maturity process in crop plants (Jungcurt *et al.*, 2012);
- Eleven NADH-plastoquinone oxidoreductase subunit proteins incurred transcription from *ndh* genes in subunits (2, 7, 1, 6, 4L, 4, 5, 3, K, J, and I). These proteins function as co-enzymes and participate in the food synthesis from oxidation and reduction reactions and play an important role in respiration chain to product cell battery (ATP) (Nie *et al*., 2018);
- The ORF-42 gene is responsible for transcription hypothetical protein and also has multi-functions;
- One gene gives protein enters in genome sequencing and analysis management. This protein named as Pc12g09330 also has multi-functions (Van *et al.,* 2008);
- There are five cytochrome proteins transcript from different genes (petA, petG, petL, petN, and ccsA). These proteins received the names Cytochrome f, Cytochrome b6/f complex subunit V, Cytochrome b6/f complex subunit VI, and Cytochrome c heme attachment protein. They function as transporters in the cell;
- There are 20 genes transcript photosystem proteins. These proteins enter in the photosynthesis process (Kiel *et al*., 2006);
- The *rbc*L gene transcript enzyme named Ribulose 1,5-bisphosphate carboxylase/oxygenase. These genes are responsible for oxidation and carboxylation processes (Zou *et al*., 2019);
- Twenty-two gene types (rpl and rps) emerged responsible for transcription mRNA, which translates ribosomal protein types (L and s). These proteins have many functions and connected with the translation of mRNA in ribosomes (Nie *et al*., 2018);
- Four rpo gene transcript RNA polymerases were notably highly crucial for cell division and DNA transcription (Lie *et al.*, 2016);
- There were 21 *trnA* genes, which transcript the tRNA proteins, and also responsible for the translation of mRNA molecules in the ribosomes (Haas *et al*., 1997);
- The *vcf* genes transcript four hypothetical chloroplast proteins and one non-chloroplast protein named hypothetical protein found in ribosome s12. These proteins also enter the photosynthesis process (Van *et al*., 2008).

**Table 5.** The genes, protein size, and product in Panicum miliaceum L.

| No. | Name of Gene | Gene size (the unit = nt: nucleotide) | Protein size (aa: amino acid) | Product | No. | Name of Gene | Gene size (the unit = nt: nucleotide) | Protein size (aa: amino acid) | Product |
|---|---|---|---|---|---|---|---|---|---|
| 1. | A3271_gr004 | 121nt | 39 aa | 5S ribosomal RNA | 54. | rbcL | 1431 nt | 476 aa | Ribulose 1,5 -bisphosphate carboxylase / oxygenase |
| 2. | A3271_gr003 | 95 nt | 30 aa | 4.5S ribosomal RNA | 55. | rpl 2 | 1482 nt | 273 aa | ribosomal Protein L 2 |
| 3. | A3271_gr002 | 2883 nt | 960 aa | 23S ribosomal RNA | 56. | rpl14 | 412 nt | 123 aa | ribosomal protein L14 |
| 4. | A3271_gr001 | 1492 nt | 496 aa | 16S ribosomal RNA | 57. | rpl16 | 1411 nt | 469 aa | ribosomal protein L16 |
| 5. | AtpA | 1524 nt | 507 aa | ATP synthase CF1 alpha subunit | 58. | rpl 20 | 139826 nt | 119 aa | Ribosomal Protein L20 |
| 6. | AtpB | 1497 nt | 498 aa | ATP synthase CF1 beta subunit | 59. | rpl22 | 450 nt | 149 aa | ribosomal protein L22 |
| 7. | AtpE | 414 nt | 137 aa | ATP synthase CF1 epsilon subunit | 60. | rpl 23 | 282 nt | 93 aa | Ribosomal Protein L 23 |
| 8. | AtpF | 1382 nt | 188 aa | ATP synthase CF0 subunit I | 61. | rpl 32 | 192 nt | 63 aa | Ribosomal Protein L32 |
| 9. | atpH | 246 nt | 81 aa | ATP synthase CF0 subunit III | 62. | rpl 33 | 139826 nt | 66 aa | Ribosomal Protein L33 |
| 10. | atpI | 744 nt | 247 aa | ATP synthase CF0 subunit IV | 63. | rpl36 | 114 nt | 37 aa | ribosomal protein L36 |
| 11. | ccsA | 969 nt | 322 aa | Cytochrome c heme attachment protein | 64. | rpoA | 1020 nt | 339 aa | RNA polymerase alpha subunit |
| 12. | cemA | 693 nt | 230 aa | chloroplast envelope membrane protein | 65. | rpoB | 3228 nt | 1075 aa | RNA polymerase beta subunit |
| 13. | ClpP | 651 nt | 216 aa | Clp protease proteolytic subunit | 66. | rpoC1 | 2052 bp | 683 aa | RNA polymerase beta |
| 14. | EHI_096750 | 1452 nt | 483 aa | Alanine aminotransferase, putative | 67. | rpoC2 | 4575 nt | 1524 aa | RNA polymerase beta' subunit |
| 15. | infA | 324 nt | 107 aa | translational initiation factor 1 | 68. | rps2 | 711 nt | 236 aa | ribosomal protein S2 |
| 16. | matK | 1902 nt | 513 aa | Maturase | 69. | rps3 | 675 nt | 224 aa | ribosomal protein S3 |
| 17. | ndhA | 2095 nt | 357 aa | NADH-plastoquinone oxidoreductase subunit 1 | 70. | rps4 | 606 nt | 201 aa | ribosomal protein S4 |
| 18. | ndhB | 2243 nt | 510 aa | NADH-plastoquinone oxidoreductase subunit 2 | 71. | rps8 | 411 nt | 136 aa | ribosomal protein S8 |
| 19. | ndhC | 401 nt | 120 aa | NADH-plastoquinone oxidoreductase subunit 3 | 72. | rps11 | 432 nt | 143 aa | ribosomal protein S11 |
| 20. | ndhD | 1503 nt | 500 aa | NADH-plastoquinone oxidoreductase subunit 4 | 73. | rps14 | 312 nt | 103 aa | ribosomal protein S14 |
| 21. | ndhE | 306 nt | 101 aa | NADH-plastoquinone oxidoreductase subunit 4L | 74. | rps 15 | 237 nt | 78 aa | Ribosomal Protein s 15 |
| 22. | ndhF | 2301 nt | 766 aa | NADH-plastoquinone oxidoreductase subunit 5 | 75. | rps 15 | 273 nt | 78 aa | Ribosomal Protein S15 |
| 23. | ndhG | 531 nt | 176 aa | NADH-plastoquinone oxidoreductase subunit 6 | 76. | rps16 | 1096 nt | 364 aa | ribosomal protein S16 |
| 24. | ndhH | 1182 nt | 393 aa | NADH-plastoquinone oxidoreductase subunit 7 | 77. | rps 18 | 492 nt | 163 aa | Ribosomal Protein S18 |
| 25. | ndhJ | 480 nt | 159 aa | NADH-plastoquinone oxidoreductase subunit J | 78. | rps 19 | 282 nt | 93 aa | Ribosomal Protein s 19 |

**Table 5.** (cont'd.)

| No. | Name of Gene | Gene size (the unit = nt: nucleotide) | Protein size (aa: amino acid) | Product | No. | Name of Gene | Gene size (the unit = nt: nucleotide) | Protein size (aa: amino acid) | Product |
|---|---|---|---|---|---|---|---|---|---|
| 26. | ndhK | 750 nt | 249 aa | NADH-plastoquinone oxidoreductase subunit K | 79. | rps 7 | 471 nt | 156 aa | Ribosomal Protein S7 |
| 27. | Ndhl | 543 nt | 180 aa | NADH-plastoquinone oxidoreductase subunit I | 80. | rps12 | 375 nt | 124 aa | Ribosomal Protein s 7 |
| 28. | orf 42 | 174 nt | 57 aa | Hypothetical protein | 81. | trnA-UGC | 883 nt | 293 aa | tRNA protein A for UGC codon |
| 29. | Pc12g09330 | 1056 nt | 397 aa | Pc12g09330 | 82. | trnC-GCA | 71 nt | 22 aa | tRNA protein C for GCA codon |
| 30. | petA | 1003 nt | 320 aa | Cyochrome f | 83. | trnD-GUC | 74 nt | 23 aa | tRNA protein D for GUC codon |
| 31. | petG | 114 nt | 37 aa | Cytochrome b6/f complex subunit V | 84. | trnE-UUC | 73 nt | 23 aa | tRNA protein E for UUC codon |
| 32. | petL | 96 nt | 31 aa | Cytochrome b6/f complex subunit VI | 85. | trnF-GAA | 73 nt | 23 aa | tRNA protein F for GAA codon |
| 33. | petN | 90 nt | 29 aa | cytochrome b6/f complex subunit VIII | 86. | trnfM-CAU | 54 nt | 17 aa | tRNA protein fM for CAU codon |
| 34. | psaA | 2253 nt | 750 aa | photosystem I P700 apoprotein A1 | 87. | trnG-UCC | 71 nt | 22 aa | tRNA protein G for UCC codon |
| 35. | psaB | 2205 nt | 734 aa | photosystem I P700 apoprotein A2 | 88. | trnH-GUG | 75 nt | 24 aa | tRNA protein H for GUG codon |
| 36. | psaC | 246 nt | 81 aa | Photosystem I subunit VII | 89. | trnI-CAU | 74 nt | 23 aa | tRNA protein I for CAU codon |
| 37. | psaJ | 135 nt | 44 aa | Photosystem I subunit IX | 90. | trnK-UUU | 2542 nt | 846 aa | tRNA protein K for UUU codon |
| 38. | Psal | 111 nt | 36 aa | Photosystem I subunit VIII | 91. | trnL-CAA | 81 nt | 26 aa | tRNA protein L for CAA codon |
| 39. | psbA | 1062 nt | 353 aa | Photosystem II protein D1 | 92. | trnM-CAU | 73 nt | 23 aa | tRNA protein M for CAU codon |
| 40. | psbB | 1527 nt | 508 aa | photosystem II CP47 chlorophyll apoprotein | 93. | trnN-GUU | 72 nt | 23 aa | tRNA protein N for GUU codon |
| 41. | psbC | 1422 nt | 473 aa | photosystem II CP43 chlorophyll apoprotein | 94. | trnP-UGG | 75 nt | 24 aa | tRNA protein P for UGG codon |
| 42. | psbD | 1062 nt | 353 aa | photosystem II protein D2 | 95. | trnQ-UUG | 73 nt | 23 aa | tRNA protein Q for UUG codon |
| 43. | psbE | 252 nt | 83 aa | Photosystem II cytochrome b559 alpha subunit | 96. | trnR-ACG | 74 nt | 23 aa | tRNA protein R for ACG codon |
| 44. | psbF | 120 nt | 39 aa | Photosystem II cytochrome b559 beta subunit | 97. | trnS-GCU | 88 nt | 28 aa | tRNA protein S for GCU codon |
| 45. | psbH | 222 nt | 73 aa | photosystem II phosphoprotein | 98. | trnT-GGU | 65 nt | 20 aa | tRNA protein T for GGU codon |
| 46. | psbI | 156 nt | 51 aa | photosystem II protein I | 99. | trnV-GAC | 72 nt | 23 aa | tRNA protein V for GAC codon |
| 47. | psbJ | 123 nt | 40 aa | Photosystem II protein J | 100. | trnW-CCA | 74 nt | 23 aa | tRNA protein W for CCA codon |
| 48. | psbK | 186 nt | 61 aa | photosystem II protein K | 101. | trnY-GUA | 84 nt | 27 aa | tRNA protein Y for GUA codon |
| 49. | psbL | 117 nt | 38 aa | Photosystem II protein L | 102. | ycf 3 | 1988 nt | 661 aa | Hypothetical chloroplast RF 34 |
| 50. | psbM | 105 nt | 34 aa | photosystem II protein M | 103. | ycf 15 | 300 nt | 99 aa | Hypothetical chloroplast RF 15 |
| 51. | psbN | 132 nt | 43 aa | photosystem II protein N | 104. | ycf 68 | 405 nt | 134 aa | Hypothetical chloroplast RF68 |
| 52. | psbT | 111 nt | 36 aa | photosystem II protein T | 105. | ysf 4 | 558 nt | 185 aa | Photosystem I assembly protein y c f 4 |
| 53. | psbZ | 189 nt | 62 aa | photosystem II protein Z | | | | | |

Plant species vary in their content of genetic groups. Proso millet is characteristic of lacking genetic groups, with only 17 groups being identified. Hence, it classifies as a genetically poor plant species. This is a direct impact on the morphological composition of the plant, as an herbaceous plant with a short life cycle of less than two months with limited economic yield (Khound *et al.,* 2022; Francis *et al.*, 2023). Thus, the study indicates the plant can contribute in the genetic improvement programs by introducing other genes unavailable in the plant genome. Energy genes play an influential role in increasing the progress and speed of vegetative growth of the plant. The millet crop has a short period of vegetative growth due to its content of genes, helping it do so. Therefore, this plant can be a source of genes in gene transfer experiments (Nie *et al*., 2018). Additionally, about 20 genes are responsible for photosynthesis, giving a great impetus to the number of enzymes and proteins responsible for this process, and is, therefore, a physiologically considered resource for providing large quantities of sugar (Kiel *et al*., 2006).

**Gene detection**

The results further revealed genes 1-6 appeared in proso millet plants (Figure 1). The size of *atpE*, *ndhC*, *rpl14*, and *rps8* genes were 138, 121, 124, and 137 bp, respectively, while *psaC* and *psbH* genes showed the sizes of 82 and 74 bp, respectively. The two other genes (*petA* and *rpoA*) detected gave the sizes of 321 and 340 bp, respectively (Figure 2). However, the *trnA-UGC* and *ysf4* genes did not appear in local proso millet plants.

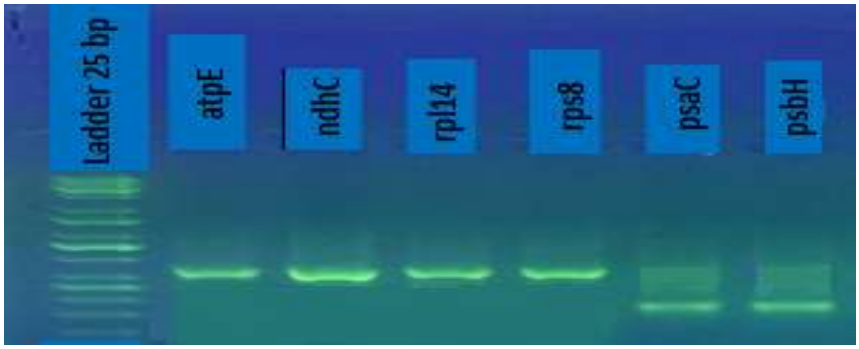


**Figure 1.** Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of all genes detected in Proso millet, first line: 50 bp DNA ladder, lines (2, 3, 4, 5, and 6) positive results in *atpE*, *ndhC*, *rpl14*, *rps8*, *psaC*, and *psbH* genes.

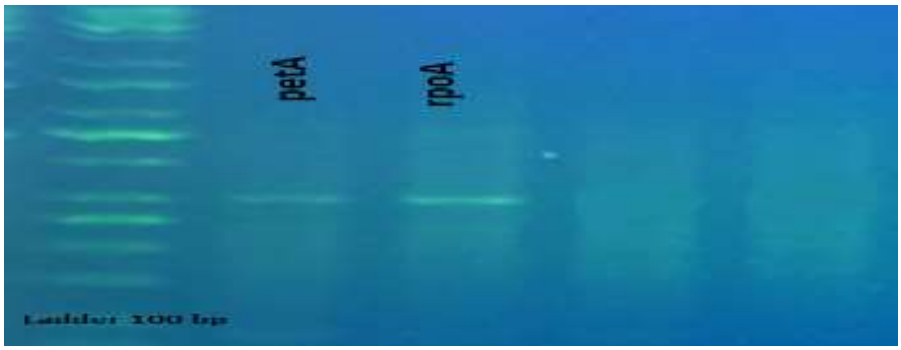


**Figure 2.** Gel electrophoresis (1% agarose, 7 V/cm for 90 min) of all genes detected in Proso millet, first line: 50 bp DNA ladder, lines (2, 3) positive results in (*petA*, *rpoA*) genes, lines (4, 5) negative results in (*trnA-UGC, ysf 4*) genes.

These results distinguish the proso millet cultivar grown in Iraq from other international cultivars. Therefore, it is evident from the above figures that eight genes were highly essential in the proso millet cultivars because they appeared in the local cultivar and became registered in the international cultivars. The two genes did not appear in the local cultivar of proso millet, but occurred in other cultivars and registered globally in the NCBI. Therefore, these genes may not necessarily be essential in the proso millet genome (Nethra *et al*., 2014; Makhmudov *et al*., 2024). The genes contained in the same plant species vary between genotypes grown from one region to another. The loss or presence of certain genes in a genetic combination does not mean their presence in other combinations of the same species (Van *et al*., 2008 ; Lie *et al.*, 2016). These are the results the study has revealed to us.

## CONCLUSIONS

In proso millet, 17 types of genes emerged, which can serve as a source for this gene type. As the local variety grown in Iraq is an important raw material, it is beneficial in genetic improvement programs according to the results of genetic detection. The main genes will be crucial in molecular plant breeding programs, genetic variations in cultivars of proso millet, and a resource of some genes in transgenic to other plants which lose these genes. The pertinent study provided sufficient information on the proso millet genome for plant breeders to take advantage in molecular plant breeding programs.

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