International Journal of Engineering Research & Management Technology

ISSN: 2348-4039

March-April-2021 Volume 8, Issue-2

Email: editor@ijermt.org www.ijermt.org

A CRITICAL STUDY ON GENETIC DIVERSITY BY MITOCHONDRIAL AND NUCLEAR GENES OF MAIZE (Zea mays L.)

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ABSTRACT

Maize is an essential cereal crop in the northwestern Himalayan region of India due to its wide variety of uses, which has led to the release and recommendation of new maize hybrids for the region. The use of progenitor lines with a limited genetic base by private and public seed agencies may pose a threat to genetic diversity. DNA fingerprinting provides knowledge of the genetic relationship between hybrids and parental lines, thereby preventing the danger of increasing uniformity. The method is useful for molecular identification and verifying hybrid purity. Ten maize hybrids and their progenitor lines were subjected to DNA fingerprinting using microsatellite markers for molecular identification and assessment of genetic diversity. Hybrid purity is also an essential criterion for high commercial value and certified hybrid seed production. Molecular characterization of hybrids and their progenitor lines using a set of highly informative molecular markers confers significant advantages over markers for revealing the genetic composition of genotypes derived from various sources. Molecular markers can be used to discriminate even closely related genotypes of a crop. For molecular characterization, microsatellite markers or simple sequence repeat (SSR) markers are the preferred molecular markers.

KEY WORDS: Genetic Diversity, Mitochondrial Gene and Nuclear Genes, Maize.

INTRODUCTION

Maize (Zea mays L.) is the most extensively grown and consumed crop in Africa however, its productivity is affected by numerous biotic and abiotic factors, which can lead to low yields on farmers' fields. Genetic improvement through plant breeding utilizing current biotechnology advancements is a viable and sustainable method for mitigating the negative impacts of these production constraints. The genetic diversity of the available

germplasm is an essential requirement for any breeding program. Through genetic diversity analysis, the level and pattern of genetic diversity can be determined.

In plant breeding, genetic diversity analysis is essential because it allows breeders to classify germplasm based on their genetic relationships. genetic diversity as the likelihood that randomly selected alleles are dissimilar. state that information obtained from genetic diversity analyses can also be utilized for the conservation of plant genetic resources. The genetic distance between various genotypes indicates the level of diversity. Studies of genetic diversity can be conducted at both the phenotypic and genome levels. At the phenotypic level, agro-morphological traits can be used for diversity analysis, whereas molecular markers can be used for diversity analysis at the genome level.

Agro-morphological traits comprise both qualitative and quantitative agronomic and morphological characteristics. Quantitative characteristics are highly influenced by the environment, which reduces the accuracy of the generated results. Molecular diversity analysis, however, is more accurate and informative as it cannot be influenced by the environment.

In the development of hybrid maize, high genetic diversity enables plant breeders to create new, improved hybrids with a high yield potential due to heterosis, which can only be exploited efficiently by crossing unrelated and distant progenitors. Effective exploitation of heterosis increases maize yield because inbred lines from different heterotic groups have a high potential to produce hybrids with increased vigor. A heterotic group is a group of related individuals with comparable combining ability when crossed with individuals from other groups. The maize breeding program at the Agricultural Research Council (ARC) is the premier public research institution in charge of developing maize cultivars for the South African farming community. Using conventional, double haploid, and molecular methods, the ARC creates and maintains numerous inbred lines. The inbred strains are utilized in the creation of single- and three-way hybrids. Determining the extent and pattern of diversity among the ARC maize inbred lines is crucial. This research aids breeders in selecting genetically distinct progenitors for hybrid cultivar development and establishing the preliminary foundation for heterotic grouping.

RESEARCH METHODOLOGY

The current analysis was conducted at the Genomic Resources Laboratory.

COLLECTION OF SAMPLES

During the growing season, samples of S. inferens were taken from four distinct host plants, including maize, rice, sugarcane, and sorghum. Each location yielded at least 20 live larvae. S. inferens' several life phases are displayed.

DIVERSITY ANALYSIS IN INBREDS DERIVED FROMDIFFERENT BASE POPULATIONS

The experiment was conducted at the R&D Farm, in kharif season using a 12 × 12 basic lattice design with two replications. The farm's coordinates are 212 meters above sea level and 11.5990 North 78.5980 East, respectively.

The experimental field was well-equipped to produce a quality crop. Application rates for inorganic fertilizers were 135 kg N, 62.5 kg P2O5, and 50 kg K2O/ha. Just before sowing, a full dose of P2O5 and K2O were administered as a basal dose, whereas N was applied in three splits.

GENOMIC DNA EXTRACTION

All 10 inbred plants' young leaves were used to extract the entire genomic DNA, which was done largely in accordance with the CTAB extraction methodology (Doyle and Doyle, 1987). The steps in the process were as follows:

- Young leaves from seedlings that were 2 weeks old were selected. After wiping off the leaf lamina with 70% alcohol, leaf samples were collected.
- 400 l of CTAB buffer (2% Cetyl Trimethyl Ammonium Bromide, 1.4M NaCl, 20mM EDTA at pH 8.0, 100mM Tris-HCL at pH 7.5, 0.2% 2-mercapto ethanol, and 2% Polyvenyl pyrolidone) were used to envelop the leaf tissue.
- The leaf tissue was crushed and heated to 650°C for an hour in a water bath.
- The supernatant was then removed after centrifuging the incubated sample at 12000 rpm for 5 minutes.
- 400 l of choloroform:isoamyl alcohol (24:1) were added to the supernatant, and the mixture was gently inverted.
- After two minutes, it was spun for ten minutes at 12000 rpm, and the supernatant was collected.
- Following that, 400 l of extremely cold isopropanol was added, and it was then incubated at -20 oC for two hours.
- The solution was spun at 12000 rpm for 10 minutes after two hours.

- The pellet that had formed at the bottom of the tube was washed with 100 l of 70% alcohol after the supernatant was discarded.
- After being overnight air dried, the pellet was dissolved in 100 l of T10E1 buffer.

Gel electrophoresis was used to confirm the DNA extraction's purity. To ensure their quality, the samples were run on a 0.8% agarose gel in 0.5X TBA buffer, stained with ethidium bromide, and compared to a standard undigested DNA sample. An sufficient amount of TE buffer (pH 8.0) was used to dilute a portion of the DNA sample, resulting in a working concentration of 5ng/l of DNA, which was then stored at 4oC.

Genetic diversity of *S. inferens* by microsatellite markers

For each location in relation to host, about 20 larvae in live or dead condition were collected (Table 1). Each population of collected larvae from a specific site and host was kept separately in 95% ethanol at -80°C and utilized for DNA extraction. To prevent the contamination of any other DNA, all larvae were completely cleaned in formaldehyde and alcohol, and the gut contents were removed. A kit for animals (QIAGEN cat# 69504) was used for the genomic DNA isolation in accordance with industry standards.

RESULTS AND DISCUSSION

Genetic diversity by mitochondrial and nuclear genes

Using nuclear gene Elongation Factor Alpha-1 (EF1) and mitochondrial markers like Cytocrhome oxidase subunit I (CO-I) and CO-II, the genetic diversity of S. inferens populations was investigated.

Mitochondrial marker: *Cytocrhome oxidase subunit I* (CO-I) gene

From the DNA of 20 S. inferens populations, the mitochondrial COI gene was amplified and sequenced. The sequences were evaluated after being aligned. The accession numbers for each sequence were received when they were all submitted to GenBank at the NCBI.

Twenty S. inferens populations from India collected on four host crops, along with out groups (one each from China and Korea), were used for the phylogenetic analysis of the COI gene. The phylogenetic analysis revealed two major clusters that distinguished the S. inferens populations from those in India, China, and Korea. However, the COI gene sub-clusters revealed no genetic diversity among the populations of S. inferens gathered on several host crops, including sorghum, maize, sugarcane, and rice. The pink stem borer populations from India are

genetically distinct from those from Korea and China, according to comparative research, with genetic variation ranging from 0.000% to 0.061%.

Mitochondrial marker: *Cytochrome oxidase subunit II* (COII) gene

Twenty COII sequences of S. inferens populations were sequenced. These sequences were then aligned and examined after being found using BLAST at the NCBI database. The twenty sequences were all used for analysis and entered into NCBI's GenBank. The accession numbers are listed in Table 15 of the publication. Twenty populations from India were employed in the phylogenetic analysis of the COII gene, among the four host crops, and one population each from China, Spain, and Greece. The phylogenetic analysis's findings revealed two primary clusters among the S. inferens populations from India, China, Korea, and Greece. However, a closer analysis at the sub clusters of the CO-II gene reveals that even among the many hosts, the Indian population is not that diverse. In the end, it was shown that the S. inferens populations from India that were taken from the sorghum, maize, sugarcane, and rice crops did not differ significantly in terms of mitochondrial molecular diversity. The pink stem borer populations from India are genetically distinct from those from China, Spain, and Greece, with genetic variation ranging from 0.000% to 0.146%, according to a comparison of the gene sequences of the populations in this study with those retrieved for S. inferens from those three countries.

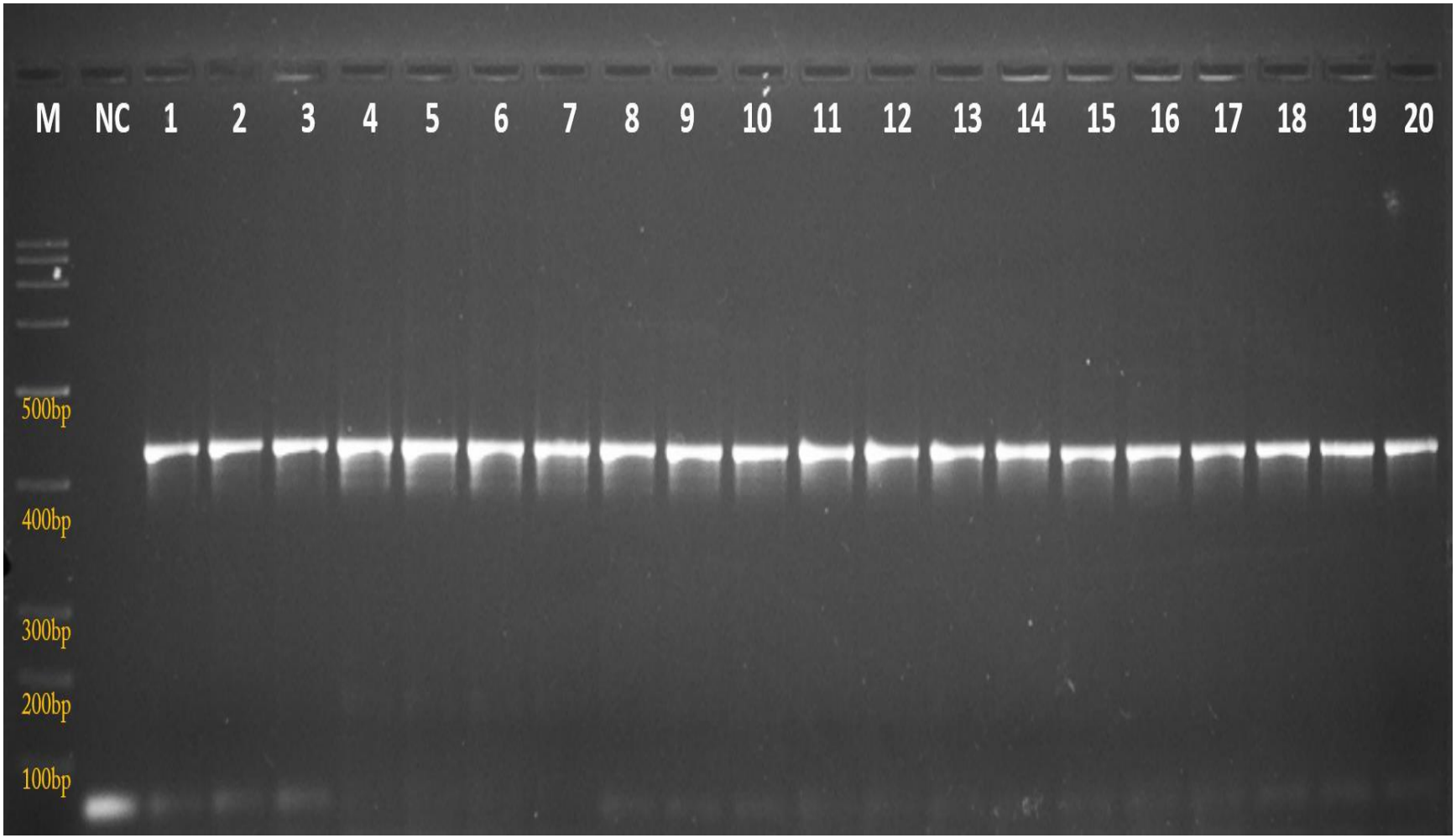


FIGURE-1: USING 20 INDIAN POPULATIONS FROM FOUR DISTINCT HOST PLANTS AND THE PRIMERS LC01490 FORWARD AND HC02198 REVERSE, THE CYTOCHROME OXIDASE I SUBUNIT WAS AMPLIFIED.

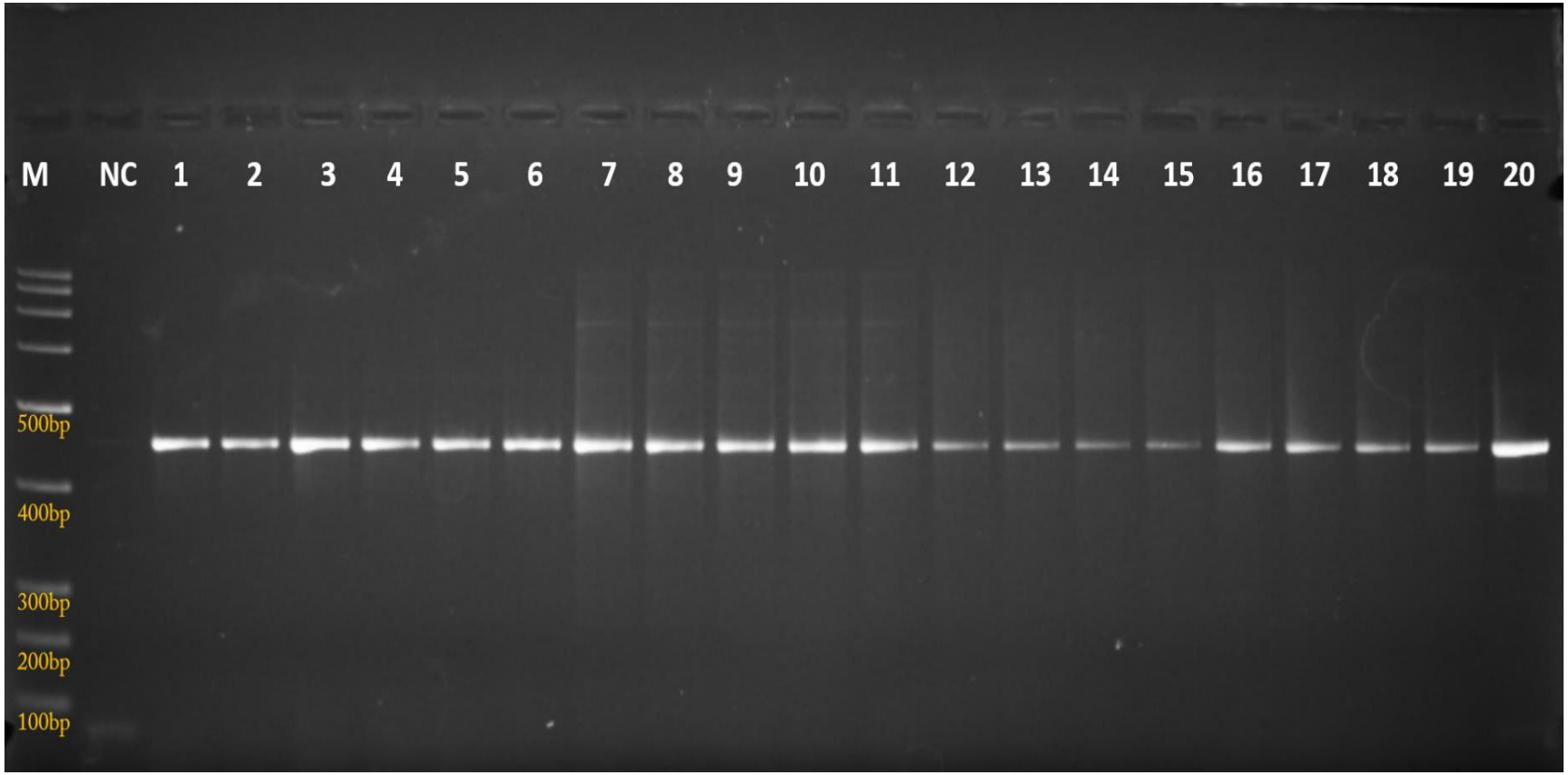
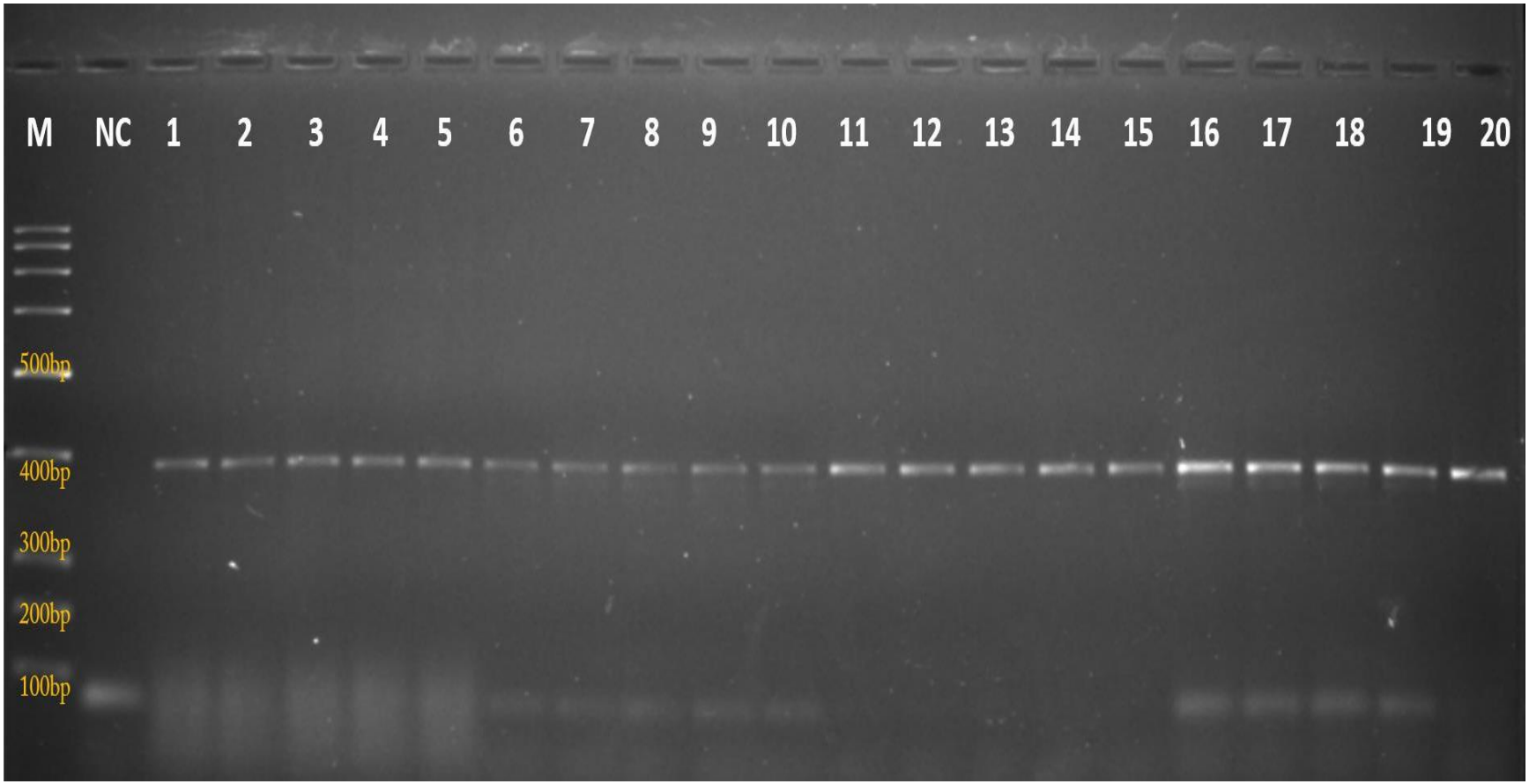


FIGURE-2: USING 20 INDIAN POPULATIONS FROM FOUR DISTINCT HOST PLANTS AND THE 2993+ FORWARD AND A3772 REVERSE PRIMERS, THE CYTOCHROME OXIDASE II SUBUNIT WAS AMPLIFIED.


M
NC
1
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500bp
400bp
300bp
200bp
100bp

FIGURE-3: ELONGATION FACTOR ALPHA-1 (EF-1) AMPLIFICATION USING 20 INDIAN POPULATIONS FROM FOUR DISTINCT HOST PLANTS USING F2-557F FORWARD AND F21118R REVERSE PRIMERS

Mitochondrial marker: Elongation Factor Alpha-1(EF1α)

Following sequence amplification and BLAST searches at the NCBI database, the 20 S. inferens populations that were obtained for the EF1 gene were sequenced for each population before being aligned and evaluated. The twenty sequences were all used for analysis, entered into GenBank at NCBI, and given accession numbers.

Three sequences from France were employed in the phylogenetic study of the EF1 gene among the twenty populations from India, the four host crops, and the outgroup. The analysis's findings revealed two major clusters among the populations of S. inferens in India, China, Korea, and Greece. However, a closer analysis at the subclusters of the EF1 gene revealed that the Indian population lacked significant variability among hosts. In the end, it was found that there was little to no mitochondrial molecular diversity among the Indian populations of S. inferens that were collected from the host crops of sorghum, maize, sugarcane, and rice.The COI, II, and nuclear (EF1) genes show little evidence of considerable genetic variability across S. inferens populations across geographical areas in India.

The pink stem borer populations from India are genetically diverse from those of France, with genetic variation ranging from 0.000% to 0.061%, according to a comparison of the gene sequences of the study's populations with the three sequences recovered for S. inferens from France.

Bacterial community from *S. inferens*

In the current investigation, it was discovered that several different insect species were home to the identified bacterial isolates. Using 16S rRNA gene sequencing, a total of 32 culturable commensal bacteria were recovered from the mid-gut of S. inferens live larvae. The three phyla of Proteobacteria, Firmicutes, and Bacteroidetes were used to categorize the different bacterial species. In the Proteobacteria, the species were divided into eight genera and four families, including the Enterobacteriaceae (Citrobacter, Enterobacter, Serratia, Klebsiella, and Xenorhabdus), Pseudomonadaceae (Pseudomonas sp.), Moraxellaceae (Acinetobactor), and Comamonadaceae (Comamonas). Staphylococcaceae (Staphylococcus), Bacillaceae (Lysinibacillus), Streptococcaceae (Lactococcus), and Enterococcaceae (Enterococcus) are the four families and four genera under which the species of Firmicutes were categorized. Only one bacterial species, Chryseobacterium, was classified as belonging to the Flavobacteriaceae family in the phylum Bacteroidetes. The Enterobacteriaceae family demonstrated dominance in the mid-gut of the S. inferens with five commensal bacterial taxa, including Citrobacter, Enterobacter, Serratia, Klebesilla, and Xenorhabdus. Four gram positive genera and nine gram negative genera were found to exist.

Species level diversity in gut bacteria of S. inferens

S. inferens populations obtained from maize crops at various locations showed substantial endosymbiont bacterial diversity. Using the 16S rRNA gene, 32 culturable commensal bacteria were identified from S. inferens collected from 12 locations. Acinetobacter sp., A. bereziniae, and Klebsiella oxytoca were found in the S. inferens specimen taken from Koppala. Additionally, strains of Enterobacter asburiae, Chryseobacteruim sp., and C. gleum from Shimoga, Lysinibacillus fusiformis and Staphylococcus sp. from Bangalore, Serratia nematodiphila and Serratia sp. from Kolar, Acinetobacter sp., Enterococcus disper, Enterococcus sp. S. inferens collected on rice had the following gut bacterial species: Xenorhabdus poinarii from Hyderabad (Enterococcus gallinarum, Lactococous sp., L. lactis and Comamonas sp. from Killikulam (Klebsiella sp., Lactococcus lactis, Lactococcus sp. and Pseudomonas sp. from Pattambi.

Serratia ureilytica, Serratia sp., and Enterococcus sp. were the gut bacteria found in S. inferens obtained from sugarcane, while Citrobacter sp., C. freundii, and C. werkmanii were found in S. inferens collected from sorghum in the Gulbarga region. Insects from different host plants had significantly more diverse bacterial populations than insects from the same host plant in different locations. According to the findings, the host plant has a greater impact on S. inferens' midgut bacterial diversity than the host plant's actual location.

Phylogeny analysis

At the phylum level, all the isolated commensal bacteria from the mid-gut of S. inferens were categorized. Using Bacteroides acidifaciens as the outgroup, phylogenetic analysis revealed that all bacteria species clustered into three main clades that represented the phyla Proteobacteria, Firmicutes, and Bacteroidetes. Proteobacteria, which are divided into four families and eight genera, predominated among the gut bacterial communities of S. inferens.

CONCLUSION

In terms of global output and area, maize (Zea mays L., 2n = 2x = 20), the crop with the highest average yield per hectare, comes in third place behind rice and wheat. As a low-cost source of nourishment, it is used in underdeveloped nations as a staple diet, pasture for livestock, and a raw material for industry. Its increased use as poultry feed and prospective applications as forage and a source of biofuel make it a crucial cereal crop for the future. Maize is cultivated in the majority of the world's regions up to 3000 m above sea level (masl) and exhibits the C4 physiological route, a wide range of genetic variety, and greater adaptability. For maize improvement

programs to be successful, populations must have sufficient genetic diversity. Although significant challenges to maize variety have been predicted for more than 50 years in the vicinity of its origins, maize breeders have not only protected but also increased the genetic diversity of their crops at numerous research facilities throughout the globe. The construction of heterotic pools for breeding superior hybrids, composites, and line development benefits from a greater understanding of the genetic diversity that already exists. When parents are genetically varied and belong to opposing pools rather than closely related groups, the high level of heterosis in emerging hybrids can be utilized. Due to environmental interactions, the assessment of genetic diversity among genotypes based on morphological data may not accurately depict the precise genetic relationships. In order to evaluate the genetic diversity, molecular markers and phenotypic features have both been used. The links between inbred lines from distinct heterotic groups have been examined in maize using a variety of molecular markers.

REFERENCES

1. Adu GB, Badu-Apraku B, Akromah R, Garcia-Oliveira AL, Awuku FJ, Gedil M. Genetic diversity and population structure of early-maturing tropical maize inbred lines using SNP markers. PloS ONE. 2019;14:e0214810.
2. Ertiro BT, Semagn K, Das B, Olsen M, Labuschagne M, Worku M, Wegary D, Azmach G, Ogugo V, Keno T, Abebe B, Chibsa T, Menkir A. Genetic variation and population structure of maize inbred lines adapted to the mid-altitude sub-humid maize agro-ecology of Ethiopia using nucleotide polymorphic (SNP) markers. BMC Genomics. 2017;18:777.
3. Wu Y, Vicente FS, Huang K, Dhliwayo T, Costich DE, Semagn K, Sudha N, Olsen M, Prasanna BM, Zhang X, Babu R. Molecular characterization of CIMMYT maize inbred lines with genotyping–by–sequencing SNPs. Theor Appl Genet. 2016;129:753–65.
4. Viana JMS, Valente MSF, Silva FF, Mundim GB, Paes GP. Efficacy of population structure analysis with breeding populations and inbred lines. Genetica. 2013;141:389–99.
5. Van Inghelandt DV, Melchinger AE, Lebreton C, Stich B. Population structure and genetic diversity in a commercial maize breeding program assessed with SSR and SNP markers. Theor Appl Genet. 2010;120:1289–99.
6. Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK. Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. Crop Sci. 2003;43:1275–82.

7. Reif JC, Melchinger AE, Xia XC, Warburton ML, Hoisington DA, Vasal SK. Use of SSRs for establishing heterotic groups in subtropical maize. Theor Appl Genet. 2003;107:947–57.
8. Badu-Apraku B, Garcia-Oliveira A, Petroli CD, Hearne S, Adewale SD, Gedil M. Genetic diversity and population structure of early and extra-early maturing maize germoplasm adapted to sub-Saharan Africa. BMC Plant Biol. 2021;21:96
9. Dias KOG, Piepho HP, Guimarães LJM, Guimarães PEO, Parentoni SN, Pinto MO, Noda RW, Magalhães JV, Guimarães CT, Garcia AAF, Patina MM. Novel strategies for genomic prediction of untested single-cross maize hybrids using unbalanced historical data. Theor Appl Genet. 2020;133:443–55.
10. Silva KJ, Guimarães CT, Guilhen JHS, Guimarães PEO, Parentoni SN, Trindade RS, Oliveira AA, Bernadino KC, Pinto MO, Dias KOG, Bernardes CO, Dias LAS, Guimarães LJM, Pastina MM. High-density SNP-based genetic diversity and heterotic patterns of tropical maize breeding lines. Crop Sci. 2020;60:779–87.
11. Cantelmo NF, Von Pinho RG, Balestre M. Genome-wide prediction for maize single-cross hybrids using the GBLUP model and validation in different crop seasons. Mol Breed. 2017;37:51.