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The Application of Quantification Genomics in the Development of Autogamous Plants with Chloroplast DNA Variability in Wild Brassicas and their Biology Research

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Abstract

In order to select inbred lines more effectively in light of the rising demand for food, it is necessary to employ techniques and alternatives. Particularly when the pedigree technique is applied to autogamous plants, quantitative genetics play a significant role in this regard. This study suggests using the best linear unbiased predictor (BLUP) in conjunction with relationship information between progenies to provide breeding values that are more accurate and, as a outcome boost genetic benefits via selection. A proposal is put forth to speed up the process of obtaining perennial plant inbreds and use as much data as possible during selection to ensure optimal accuracy. Inbreds that are superior to the ones already available might be made accessible more frequently in this way, helping the agricultural sector meet the demand for perennial plants.

In order to characterise the cytoplasm and conduct population genetics and phylogeographic analysis, it is crucial to assess the diversity of the chloroplast DNA (cpDNA) in wild relatives of crop brassicas. The former is helpful for breeding programmes that involve extensive hybridization and the synthesis of alloplasmic lines, whereas the latter is crucial for developing conservation methods. Consequently, the PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) technique was used to examine the cpDNA diversity in 14 wild brassicas, including 31 accessions, and the outcomes showed the presence of 219 polymorphic fragments in total. The combination of polymorphisms obtained by using only two primer pair-restriction enzyme combinations was sufficient to distinguish all 14 wild brassicas. Moreover, 11 primer pairs-restriction enzyme combinations revealed intraspecific polymorphisms in eight wild brassicas (including endemic and endangered species, *B. cretica* and *B. insularis*, resp.). Thus, even within a small number of accessions that were screened, intraspecific polymorphisms were observed, which is important for population genetics analyses in wild brassicas and consequently for conservation studies.

Keywords: Autogamous; Polymerase Chain Reaction; Polymorphism; Phylogeographic

Introduction

Autogamous plants are those in which self-fertilization predominates; in other words, where the cross-fertilization rate is under 5%. These plants are bred using methods specific to their form of reproduction. Information at hand shows that this variety of plant has been successfully bred in various parts of the world.

The bulk technique and pedigree method, the two most common approaches for dealing with the segregating offspring of autogamous plants, were put forth in Europe around the tail end of the 19th and the start of the 20th centuries. The shortcomings of the first two selection techniques were then addressed by the proposal of new techniques. Among them, the single seed descendent (SSD) and the bulk approach within progenies F2 or F3 have both been heavily utilised [1]. These approaches have been compared over time, and while discrepancies between them have occasionally been found, it has been found that all of them are effective when used properly.

It can be concluded that, despite the fact that it has occasionally happened, breeders of autogamous plants have used quantitative genetics far less frequently than they have for alogamous plants. The need for food is anticipated to increase significantly over the next few decades, along with population expansion. The major way to meet the demand for grains, fruit, and fibres is by raising yields because uncultivated land is no longer as readily available [2].

By making improvements to crop management, you have a choice of ways to raise production. This is the setting for the increased use of irrigation water, herbicides, and fertilisers. However, there are some limitations on the expanding usage of these inputs, some through limitations in availability and price and others through concerns connected to potential environmental repercussions.

So, it's anticipated that genetic advancement will be a key to achieving the boost in output. To help identify inbreds that are becoming more and more effective, various novel alternatives are being put forth in this context. Utilizing information from biometrics and quantitative genetics more heavily is one of these options [3]. In this study, several applications of quantitative genetics will be explored, with a focus on breeding autogamous plants, particularly when the pedigree technique is applied.

Crop brassicas' wild relatives serve as both a gene bank for genes granting tolerance to various biotic and abiotic challenges as well as a source of cytoplasm that causes male sterility in cultivars. The maternal lineage of related species can be shown by evaluating the

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diversity of the chloroplast genome in wild brassicas because maternal inheritance of the mitochondrial and chloroplast genomes has been shown in Brassica species. This is crucial for breeding programmes since brassicas' cytoplasm/maternal lineage can affect the direction of crosses and the degree of success in extensive hybridization [4]. Additionally, examination of the differences in chloroplast DNA (cpDNA) can show genetic relationships between and within wild and domesticated species. Research on cpDNA diversity is crucial for assessments of population genetics and phylogeography in rare, endemic, and endangered species. Considering that many of the wild relatives (such as Brassica insularis and B. cretica) are endemic and/or threatened species, population genetics studies of these species are crucial for developing conservation strategies. In order to conduct such genetic and conservation research, it is necessary to first analyse the wild brassicas' chloroplast genome for interspecific and intraspecific polymorphisms [5].

PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) is an easy, quick, and repeatable method that amplifies portions of the chloroplast genome using universal primers, then employs restriction enzyme digestion to identify fragment length polymorphisms. Very few studies have examined the chloroplast genome of brassicas using PCR-RFLP of cpDNA to distinguish between three diploid varieties.cultivated radish's maternal lineage was easier to grasp thanks to the application of the PCR-RFLP technology to identify interspecific polymorphisms in Raphanus sp. Brassicas have been subjected to phylogenetic and genetic diversity investigations using simple sequence repeat markers of cpDNA, short noncoding portions of cpDNA, and dCAPS markers to identify polymorphisms [6]. These methods require either a sequencing facility or Polyacrylamide Gel Electrophoresis (PAGE) with silver staining. However, PCR-RFLP, also known as CAPS, is a faster, more dependable, and easier method that just requires agarose gels. It can also cover a vast area of the chloroplast genome for analysis because there are numerous universal primers available.

In light of this, our goal was to determine the applicability of the PCR-RFLP technique to examine cpDNA variations in a few wild brassicas belonging to the same cytodeme, which can facilitate I population genetics and phylogeographic studies for conservation purposes and (ii) analyses of maternal lineage and genetic relatedness, which are crucial for breeding brassicas [7].

Materials and Methods

Improving the Efficiency of the Pedigree Method

In breeding autogamous plants, the pedigree approach is the most popular technique, and numerous publications describe its process. The ability to determine the degree of coancestry between generations—in other words, to have the genealogy of the chosen inbreds—is one of the many benefits of the pedigree approach that is frequently emphasised. Although it is usually always possible to collect this knowledge, when it does so, very few people are actually able to use it. With a view to enhancing the method's effectiveness, it should be used more expressively, especially given the effort required to attain it.

Utilizing the data from the genealogical records at the time of selection through the use of the mixed model statistical approach is advised for the aim of maximising genetic benefits when employing the pedigree method. It is important to emphasise that effective experimentation is inextricably linked to the success of selecting superior offspring since statistical analysis cannot produce accurate predictions of BLUP without accurate estimates of genetic and environmental variations [8]. It is also common knowledge that, at the time of analysis, the phenotypic data are always given more weight than the additional information included, such as coancestry.

Strategies for Perennial Plant Breeding in the Pedigree Method

Despite focusing on the coffee plant (Coffea arabica), the remarks can be applied to any perennial autogamous plant. Given its significant social and economic impact on Brazil, coffee was chosen as the subject. The public sector alone is responsible for running breeding initiatives. Prior to it, the pedigree method of population segregation was the breeding methodology in use.

It is well known that the main challenge in genetic breeding for any species is minimising the impact of the environment and genotype by environment interaction on phenotypic expression, or having a good representative of the genotype in the phenotype. A perennial plant, like the coffee crop, has a protracted juvenile phase and pronounced annual production oscillation, necessitating multiple years of assessment, making this element much more expressive in the plant [9].

Any approach that this species' breeders take can be used to determine the base population. Even Nevertheless, the ideal scenario is to encourage good crossings by involving parents who have a track record of good performance whenever possible. The 10 best available inbreds will be combined to create the segregating population, as a suggestion. Due to their superior performance in yield, insect resistance, and grain quality—performances that have already been mentioned—these inbreds would be chosen. The best case scenario would be for them to come from existing breeding operations.

This tactic offers certain benefits. I It promotes greater dynamism in breeding initiatives for perennial plants like the coffee crop. The proposal is appropriate for a system of ongoing selection. The most successful offspring may be recombined as F3,4 of the generation, together with inbreds from other projects and sources of pest and disease resistance. (ii) Because of the vast number of replications required by the procedure, selecting the best progenies at each stage—especially F3, 4 is more accurately done. (iii) A larger chance of spotting descendants with more flexibility and stability [10]. This truth is evident from the previous information. It must be emphasised that stability can be evaluated in terms of both years and places as well as the alternating nature of yield every two years.

Plant Material

From the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, seeds were collected for 31 accessions that belonged to 14 wild cousins of the brassicas in the tribe Brassiceae. Each accession's seeds were planted twice in pots, the plants were kept in the university of Delhi's Botanical Garden at Zakir Husain College. Although the NBPGR maintains proper identification of germplasm, morphology-based classification using Flora Europaea was used to further confirm the plant species. For DNA extraction, fresh leaves from individuals of each accession/species were collected, frozen, and kept at 80°C. In addition, leaf material from a naturally expanding population of Cardamine flexuosa (tribe Cardamineae) was gathered.

DNA Extraction, Amplification, and Digestion

Six pairs of universal cpDNA primers (CD, DT, HK, K1K2, TF, and VL) were utilised for PCR amplification. For each primer pair, PCR was performed three times. The producer of the enzyme, Merck, gave 30 L of reaction mixture including 0.2 M of each primer, 200 M of each

of the four dNTPs, 2 mM MgCl2, 1 U of Taq DNA Polymerase in 1x buffer, and 15 ng of genomic DNA for the amplification. The PCR was started with a 4 minute cycle at 94 degrees, then 30 cycles of 45 seconds each at 94, 50, 54, 72, and 10 minutes of extension at 72 degrees. PCR products were run on an agarose gel (1.2%) in 1X TBE buffer with a 1 kilobase (kb) ladder as a molecular size marker. To break down the amplified products, HinfI and TaqI restriction enzymes were utilised. Following digestion, the fragments were run at 3 V/cm for 3 hours to separate them on 2.4 percent agarose gels using 50- and 100-base pair (bp) ladders as molecular size markers. The electrophoresis and all restriction digestions were carried out three times. Along with the samples, negative controls for PCR amplifications and restriction digestions were also set and run on gels. Gel Doc XR+ (BioRad) with Image Lab TM software was used to take pictures of and record the gels after they had been stained with ethidium bromide.

Data Analysis

All polymorphic restriction pieces that could be resolved clearly received a score of 1 (present) or 0. (absent). SJ = nxy/(nxy + nx + ny), where nx and ny are the total number of fragments studied in people x and y, respectively, and nxy is the total number of fragments shared by the two individuals, was used to generate a matrix of similarities between each pair of samples. The SAHN-clustering and TREE algorithms were used in conjunction with the similarity matrix to create a UPGMA dendrogram. By comparing the two matrices using the Mantel matrix correspondence test in the MXCOMP programme of the NTSYS-pc package, a cophenetic matrix was created from the tree matrix to test the goodness of fit of the cluster analysis to the similarity matrix on which it was based.

Results

In order to amplify a 13.5 kb area of the chloroplast genome from wild relatives of brassicas, six pairs of universal cpDNA primers were utilised. In every species, the size of the amplified fragments with each primer pair was the same. Fragments produced with HK-TaqI were not included in the studies since they could not be clearly resolved in any of the 12 combinations (i.e., 6 primer pairs + 2 restriction enzymes).
For the examination of polymorphisms, a total of 219 restriction fragments (between 1 kb and 100 bp) were scored. The presence or lack of polymorphic fragments that were acquired from the PCR-RFLP patterns of each of the 14 wild brassica species was combined with other factors to determine the interspecific/intergeneric variations between the species. Out of 11 primer pair-restriction enzyme combinations, two (K1K2-TaqI and DT-TaqI) were sufficient to differentiate all wild brassicas.

Discussion

All of the wild Brassica species were grown in the field for the current experiment until flowering and/or fruit set. Field-grown plants' morphological traits were investigated, and Flora Europaea was used to confirm the species identification of every plant. All 31 accessions from 14 wild brassicas were subjected to PCR-RFLP of six cpDNA regions (using 11 primer pair-restriction enzyme combinations), revealing intergeneric/interspecific and intraspecific polymorphisms. However, members of the oleracea group, such as wild B. oleracea, B. alboglabra, B. bourgeaui, and B. montana, did not exhibit interspecific and intraspecific polymorphis Here, it should be highlighted that, despite belonging to the same diploid cytodeme, the species used in the two investigations are distinct. discovered 38 of 110 combinations (10 primer pairs 11 restriction enzymes) efficient in separating only four diploid farmed crucifers (B. nigra, B. oleracea, B. rapa, and Raphanus sativus). Two combinations—DT-TaqI and K1K2-TaqI—out of the 11 primer pair-restriction enzyme combinations used in this investigation produced the most distinct PCR-RFLP patterns With various primer pair-restriction enzyme combinations, intraspecific polymorphisms were also discovered in eight wild species. Population genetics and phylogeographic research, which are essential for developing conservation strategies, can be facilitated by this information. Five and nine primer pair-restriction enzyme combinations, respectively, may show intraspecific differences in B. cretica and B. insularis. Although before, B. cretica's population genetic structure was studied using cpDNA SSR markers. An additional set of cpDNA markers for similar investigations is provided by the current set of polymorphisms discovered using the PCR-RFLP approach. There were one to three accessions per species that were examined. It is important to highlight that intraspecific polymorphisms were found even among the few accessions that were screened. This outcome promotes the use of the PCR-RFLP technology for a wider range of wild species and their accessions for chloroplast genome investigations. Except for B. elongata and B. gravinae, which belong to the oleracea lineage, the dendrogram illustrating the genetic relatedness of the wild brassicas largely agreed with other findings. Here, it might be suggested that PCR-RFLP of a larger number of noncoding regions of cpDNA (which can detect a greater number of pertinent interspecific polymorphisms) can aid in understanding genetic links even further.

In previous studies, the psbD-trnT sequence, which corresponds to the amplicon of the DT primer pair (used in the current investigation), and the trnT-trnF sequence, which corresponds to the amplicon of the primer pair TF, were used along with other noncoding sequences and revealed trustworthy genetic relationships among members of the Brassicaceae.

Conclusion

Wild brassicas can exhibit intra- and interspecific polymorphisms, which can be discovered using PCR-RFLP of cpDNA. The primer-restriction enzyme combinations that have discovered intraspecific polymorphisms in the wild brassicas, including B. cretica and B. insularis (endemic and endangered species), can be helpful for phylogeographic studies and population genetic structure assessments, which are crucial for developing conservation strategies. It is possible to characterise or confirm the maternal ancestry of natural hybrids and alloplasmic lines produced by crossing wild and crop brassicas by using the appropriate combinations of PCR-RFLP that can reveal a variety of interspecific polymorphisms (e.g., DT-TaqI and K1K2-TaqI). The use of PCR-RFLP of cpDNA in marker-assisted Brassica breeding programmes is thus possible.

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Conflict of Interest

The author has no known conflicts of interested associated with this paper.

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