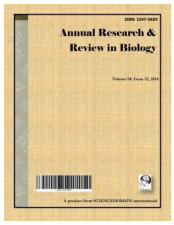

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Molecular Genetic Markers for Assessing the Genetic Variation and Relationships in *Lactuca* Germplasm

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The genus *Lactuca* L. belonging to the *Asteraceae* family, is widely distributed in different geographical and ecological areas. Lettuce and most of the other species of the genus *Lactuca* L. have been cultivated for their economic and medicinal importance. This review summarizes recent knowledge of the application of biochemical (isozymes) and molecular technologies (restriction fragment length polymorphism; random amplified polymorphic DNA; amplified fragment length polymorphism; microsatellites or simple sequence repeats; single nucleotide polymorphism) in *Lactuca* germplasm in order to better understand the genetic variation, interspecific relationships, taxonomy and breeding as a basis for further research studies. Undoubtedly, this would in turn provide a better platform for germplasm improvement, utilization and conservation.

Keywords: Biochemical markers; conservation; genetic variation; Lactuca; molecular marker technologies.

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1. INTRODUCTION

Plant genetic resources comprise all agricultural crops and their wild relatives of valuable traits. The genus *Lactuca L*. is one of the economically and medicinally important genera of the *Asteraceae* family, subfamily *Cichorioideae*, tribe Lactuceae [1-3]. The genus *Lactuca* L. is divided into seven sections (*Lactuca* /subsection *Lactuca* and *Cyanicae*/, *Phoenixopus*, *Mulgedium*, *Lactucopsis*, *Tuberosae*, *Micranthae* and *Sororiae*), and include two geographic clusters – African and North American [3-5]. The subsection *Lactuca* includes cultivated *Lactuca* sativa (lettuce) *L. aculeata*, *L. altaica*, *L. azerbaijanica*, *L. dregeana*, *L. georgica*, *L. livida*, *L. saligna*, *L. serriola f. serriola and f. integrifolia* and *L. virosa*. The other species of the genus *Lactuca L*. are widely distributed within the other sections in different geographical and ecological areas [3-5]. Genetic variation allows species to adjust to a changing environment, whether these changes are due to natural or human factors. Genetic variation studies are vital for providing information for propagation, taxonomy, disease resistance, and breeding programs as well as conservation and utilization of *Lactuca* genetic resources. Genetic diversity can be evaluated based on morphological, cytogenetic, biochemical and molecular markers [6-10]. However, the evaluation of genetic variation based on morphological and cytological traits has the disadvantages of being affected by both genetic and environmental factors and may not provide an accurate measure [11]. Hence, advanced molecular genetic technologies including biochemical and molecular markers, have been developed to overcome those limitations of morphological and cytological traits. The aim of this study is to summarize recent knowledge of the application of biochemical (isozymes) and molecular technologies (RFLP, Restriction Fragment Length Polymorphism; RAPD, Random Amplified Polymorphic DNA; AFLP, Amplified Fragment Length Polymorphism; microsatellites or SSR, Simple Sequence Repeats; SNP, Single Nucleotide Polymorphism) in *Lactuca* germplasm in order to better understand the genetic variation, interspecific relationships, taxonomy and breeding as a basis for further research studies towards a better platform for germplasm improvement, utilization and conservation.

2. BIOCHEMICAL MARKERS

Isozymes belong to the biochemical protein markers and could be defined as structurally different molecular forms of an enzyme with the same catalytic function [3,12]. Isozymes originate through amino acid changes, which in turn result in changes in the net charge, or the spatial structure of the molecules of enzyme and their electrophoretic mobility [3]. Allele variation and frequency data are used to obtain a number of measures which include average level of heterozygosity, average level of polymorphism and mean number of alleles per locus [3,12].

Over the past decades, isozyme markers have been used for various purposes in plant biology, e.g., to study phylogenetic relationships, assess genetic variation and taxonomy, study population genetics and breeding practices, and to manage and conserve plant genetic resources. Only a few studies have been carried out (Table 1), focusing on the study of genetic diversity and phylogenetic relationships of cultivated lettuce and wild *Lactuca* species using isozymes analysis [3,6,13-23]. These studies summarized the applications of isozyme markers for the delineation of phylogenetic relationships and estimation of genetic variation among cultivars and wild populations of *Lactuca* species (*Lactuca serriola*, *Lactuca aculeata*, *Lactuca virosa*, *Lactuca saligna*). Isozyme markers would be useful for the study of inter- and intra-species diversity. The results revealed a lower level of intra-species than inter-species diversity. Roux et al. [18] used isozyme markers to demonstrate that *Lactuca aculeata* is a part of the *Lactuca serriola* complex, confirming their genetic relatedness with *Lactuca sativa*, and also revealed that *Lactuca saligna* and *Lactuca virosa* are very distinct from the other species.

Kesseli and Michelmore [13] and El-Esawi [6] characterized the levels of genetic variation and phylogenetic relationships of wild and cultivated *Lactuca* populations using isozyme markers. Isozyme data suggested a polyphyletic origin of *Lactuca sativa* [14]. *L. sativa* is generally assumed to have a progenitor similar to *Lactuca serriola* [3]. Dziechciarková et al. [16] studied genetic variation of *Lactuca serriola* based on isozymes. Out of 66 isoforms observed, 42 displayed polymorphism. A significant relationship was found between isozyme polymorphism and taxonomic status of *Lactuca serriola* varieties. The above summary of studies demonstrates that isozyme markers exhibit a high level of polymorphism and has proven to be useful for assessing the genetic variability and taxonomic relationships in *Lactuca* species. However, the polymorphism of closely related

species was relatively low, resulting in limiting the delineation of these relationships [3]. Therefore, molecular markers have been used to overcome such disadvantages of isozyme markers.

3. MOLECULAR MARKERS

Molecular markers are powerful tools for assessing the genetic variation within and among *Lactuca* populations (Table 2). These markers include Restricted Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatellites (SSRs), Internal Transcribed Spacers (ITS-1), and Single Nucleotide Polymorphisms (SNPs).

3.1 Restriction Fragment Length Polymorphism (RFLP)

RFLP technique is based on the cleavage of DNA by restriction endonucleases at specific nucleotide sequences [3]. Size fractionation is carried out by gel electrophoresis. After transfer to a membrane using Southern blotting, fragments of interest can be identified by hybridization with radioactive labelled probes. Different sizes or lengths of restriction fragments are typically produced when different individuals are analyzed. This polymorphism could be used to distinguish plant species, genotypes and individual plants [24]. RFLP markers proved to be powerful tools in constructing genetic map and assessing genetic variation and phylogenetic relationships in plants including *Lactuca* species [25-27] (Table 2).

Kesseli et al. [26] used RFLP markers to assess the genetic variation in *Lactuca* species and the origin of cultivated lettuce (*Lactuca sativa*). Sixty-five accessions representing the different morphotypes of cultivated *Lactuca sativa* (looseleaf, butterhead, cos, crisphead and latin types) and five wild *Lactuca* species (*L. saligna, L. serriola*, *L. virosa*, *L. indica, L. perennis*) were analyzed. The genetic variation among populations was higher than that resided within populations. RFLP data revealed that *Lactuca sativa* is closely related to *Lactuca serriola*, but not to any of the other species included in this study (*L. virosa*, *L. saligna, L. indica* and *L. perennis*). Cultivars of *Lactuca sativa* formed distinct clusters, confirming that RFLP markers could be suitable for studying the intraspecific variation and among closely related taxa. This study also suggested a polyphyletic origin of *Lactuca sativa*.

RFLP genetic markers provided the opportunity to develop a detailed genetic map of lettuce, for use in selection and breeding studies. The cross, Calmar × Kordaat, was developed as the source of the segregating population to develop a genetic linkage map of lettuce using RFLP markers [25]. The above summary of studies demonstrates that RFLP markers are powerful tools in the identification of individuals, segregation analysis of progenitors and the assessment of genetic diversity and relationships in lettuce germplasm collections. However, this technique is labour intensive and expensive, being the main reasons to look for new molecular genetic methods to overcome those technical limitations of RFLPs.

3.2 Random Amplified Polymorphic DNA (RAPD)

RAPD is a PCR-based technique, based on enzymatic amplification of target or random DNA fragments using arbitrary primers [3]. The amplification products are separated on agarose gels in the presence of ethidium bromide and are viewed under ultraviolet light [28]. RAPD technique is fast, less technical, cheap and involves no radioactivity and hybridization. For repositories with large collections, this technology represents an important advance towards a detailed characterization of individual accessions at the molecular level [29]. RAPD markers have been successfully used in linkage map construction [30], characterization of different resistance genes [31], hybrid origin identification [32] and breeding utilization [33]. RAPD technique has also been successfully used for evaluating the genetic variation within and among *Lactuca* populations, and to establish differences between varieties of apparently closely related populations in germplasm collections [6,29,30,34,35] (Table 2). Waycott and Fort [29] analyzed 10 populations of butterhead and one of crisphead lettuce using 13 RAPD primers. Nine out of the 10-butterhead lines were homogeneous, but the tenth line was highly heterogeneous. Seven out of 10 lines revealed a within-line genetic purity of 96% or more. Thus, most of the lines could be identified using only 8-10 primers. The dendrogram of the RAPD data confirmed the close relationship of the 9 butterhead lines *vs* the heterogeneous tenth line.

Table 1. Survey of protein and isozyme markers used for characterization of *Lactuca* germplasm

Technique	*Lactuca* species studied	Aim of the work	Reference of study
Electrofocusing	*L. sativa*, *L. serriola*, *L. saligna*, *L. aculeata*, *L. virosa*	characterization of *L. sativa* and related species by electrofocusing of esterases.	Roux et al. [18]
Isozymes	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*	genetic variation and phylogenies study.	Kesseli and Michelmore [13]
Isozymes	*L. sativa*	electrophoretic characterisation of lettuce cultivars.	Mejia and Mc Daniel [19]
Isozymes	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*	characterization of wild populations of 4 *Lactuca* species using 10 enzyme systems.	Cole et al. [20]
SDS-electrophoresis	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*	characterization and identification of *Lactuca sativa* cultivars and wild relatives.	Vries de [14]
Isozymes	*L. aculeata*, *L. altaica*, *L. canadensis*, *L. dregeana*, *L. indica*, *L. perennis*, *L. saligna*, *L. sativa*, *L. serriola*, *L. taraxacifolia*, *L. tatarica*, *L. tenerrima*, *L. virosa*, *L. viminea*	characterization of germplasm collection of *Lactuca* species using 4 enzyme systems, confirmation of taxonomy determination.	Lebeda et al. [15,21] Doležalová et al. [23]
Isozymes	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*,*L. indica*	characterization of polymorphism, linkage analysis and genetic markers for lettuce breeding.	Mizutani and Tanaka [22]
Isozymes	*L. serriola*	genetic variation in European *Lactuca serriola* germplasm.	Dziechciarková et al. [16]
Isozymes	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*, *L. indica, L. altaica*, *L. dregeana*, *L. indica*, *L. perennis*, *L. tatarica.*	genomic characterization and genetic improvement of some *Lactuca* spp.	El-Esawi [6]
Isozymes	*L. aculeate, L. serriola*	genetic polymorphism in *Lactuca aculeate* populations and occurrence of natural putative hybrids between *L. aculeata* and *L. serriola*.	Lebeda et al. [17]

Table 2. Survey of molecular markers used for characterization of *Lactuca* germplasm

Technique	*Lactuca* species studied	Aim of the work	Reference of study
RFLP	*L. sativa* (Calmar × Kordaat cross)	a construction of lettuce genetic map.	Landry et al. [25]
	L. sativa, *L. saligna*, *L. serriola*, *L. virosa*, *L. perennis*, *L. indica*	study of relationships between cultivated lettuce and five related wild *Lactuca* species; study on the origin of cultivated lettuce (*L. sativa*).	Kesseli et al. [26]
	L. sativa, *L. serriola*, *L. saligna*, *L. virosa*, *L. alpina*, *L. perennis*, *L. tatarica*	study of relationships among *Cichorium* species and related genera of the tribe *Lactuceae.*	Vermeulen et al. [27]
	L. sativa (Calmar × Kordaat cross)	a construction of genetic map of *L. sativa.*	Kesseli et al. [30]
RAPD	*L. sativa*	differentiation of nearly identical lettuce germplasm accessions.	Waycott and Fort [29]
	L. sativa (Calmar × Kordaat cross)	a construction of genetic map of *L. sativa.*	Kesseli et al. [30]
	L. sativa	identification of DNA polymorphisms in 12 lettuce (*L. sativa*) varieties.	Yamamoto et al. [34]
	Lactuca sativa var. capitata	intraspecific relationships of *L. sativa* var. capitata cultivars.	Yoo and Jang [35]
	L. sativa, *L. serriola*, *L. saligna*, *L. virosa*, *L. indica, L. altaica*, *L. dregeana*, *L. indica*, *L. perennis*, *L. tatarica*.	genomic characterization and genetic improvement of some *Lactuca* spp.	El-Esawi [6]
AFLP	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*, *L. perennis*, *L. indica*	study genetic relationships in *Lactuca* species.	Hill et al. [38]
	L. sativa, *L. saligna*	integrated interspecific AFLP map of lettuce based on 2 *L. sativa* × *L. saligna* F2 populations.	Jeuken et al. [40]
	L. sativa, *L. serriola*, *L. dregeana*, *L. altaica*,*L. aculeata*, *L. saligna*, *L. virosa*, *L. tenerrima*,*L. perennis*, *L. tatarica*, *L. sibirica*, *L. quercina*, *L. viminea*, *L. indica*	study of species relationships in *Lactuca* species.	Koopman et al. [37]
	L. serriola	evolution and genetic structure of prickly lettuce (*L. serriola*) and its RGC2 resistance gene cluster.	Kuang et al. [42]
	L. serriola	Insight into the genetic polymorphism among European populations of *Lactuca serriola*.	Lebeda et al. [43]
Microstaellites	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*, *L. perennis*, *L. indica*	identification, genetic localization, and allelic diversity SAMPL in lettuce and wild relatives (*Lactuca* spp.).	Witsenboer et al. [50]

Technique	*Lactuca* species studied	Aim of the work	Reference of study
	L. *sativa*, *L*. *serriola*, *L*. *saligna*, *L*. *virosa*	variety identification in lettuce cultivars (*L. sativa*) and discriminate between cultivated lettuce and wild relatives.	Van de Wiel et al. [48]
	L. sativa, L. serriola, L. saligna, L. virosa	distinguishing lettuce cultivars and screening diversity of genetic resources.	Van de Wiel et al. [51]
	L. sativa, L. serriola, L. saligna	molecular diversity at major cluster of disease resist-ance genes in cultivated and wild *Lactuca* species.	Sicard et al. [49]
	L. sativa	molecular characterisation of lettuce germplasm collection. In: Hintum Th.J.L. van, Lebeda A., Pink D.A., Schut J.W. (eds.): Eucarpia leafy vegetables.	Hintum van [52]
	L. serriola	EST-SSR development from 5 *Lactuca species* and their use in studying genetic diversity among *L. serriola* biotypes.	Riar et al. [44]
ITS-1 DNAsequence	*L. sativa*, *L. serriola*, *L. aculeata*, *L. dregeana*,*L. saligna*, *L. altaica*, *L. virosa*, *L. tenerrima*,*L. perennis*, *L. tatarica*, *L. sibirica*, *L. quercina*,*L. viminea*, *L. indica*Other related species:*Mycelis muralis*, *Steptorhampus tuberosus*, *Cicerbita plumieri*, *C. alpina*, *Prenanthes purpurea*,*Chondrilla juncea*, *Taraxacum officinale*, *Cichorium intybus.*	phylogenetic relationships among *Lactuca* species and related genera; specification position of *L. altaica;* delimitation of genus *Lactuca*; the taxonomic position of *Cichorium.*	Koopman et al. [45]
SNPs	*L. sativa*	Genome-wide association of 10 horticultural traits, fingerprinting of lettuce germplasm.	Kwon et al. [58,59]

Yamamoto et al. [34] identified twelve lettuce (*L. sativa*) varieties based on RAPD markers. Out of the amplified fragments of *L. sativa*, 47% was polymorphic. Some of the PCR fragments were specific and could be used as indicators for morphotype- selection. The dendrogram constructed showed differentiated clusters of crisphead, leaf and butterhead types. Kesseli et al. [30] compared the levels of polymorphism of 2 types of molecular markers, RFLP and RAPD, as detected between 2 cultivars of lettuce. Of 1008 probes derived from cDNA, 10% were polymorphic and 9% could be mapped. Similar results were obtained with 180 probes derived from genomic DNA, 11% were polymorphic, which could all be mapped. RFLP and RAPD markers identified similar levels of polymorphism. However, RAPD loci were identified more rapidly. Furthermore, Yoo and Jang [35] used RAPD markers to investigate the variation and relationships among 39 cultivars of *Lactuca sativa* var. capitata. Results revealed that 55 (78.6%) of the 70 bands derived from the 12 primers showed polymorphism. El-Esawi [6] also estimated the amount of the genetic variation and assessed the phylogenetic relationships of 40 accessions of cultivated and wild *Lactuca* species using 5 RAPD primers.

The above studies demonstrated that RAPD markers could be used in the genetic studies. However, the major disadvantage of RAPD markers is that they are less reproducible.

3.3 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a DNA fingerprinting technique, which detects DNA restriction fragments by PCR amplification [3]. AFLP includes the restriction of genomic DNA, followed by ligation of adaptors complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments can be viewed on denaturing polyacrylamide gels either through autoradiographic or fluorescence methodologies [28,36]. AFLP markers have major advantages such as the effectively unlimited number of loci assayed using different combinations of oligonucleotide primers, the time efficiency (short time required to assay large numbers of DNA loci), and the enhanced performance in terms of reproducibility, sensitivity and resolution efficiency. AFLP markers have been successfully used to study the genetic variation and phylogenetic relationships of *Lactuca* species [3,37-43] (Table 2). The results of those studies recommended some *Lactuca* accessions to be included in the promising breeding programmes.

Hill et al. [38] used AFLP markers to assess the diversity and phylogenetic relationships of 44 different cultivars of *Lactuca sativa* and 13 accessions of the wild *Lactuca* species. A total of 320 polymorphic AFLP loci were recorded using 3 pairs of AFLP primers. The cluster analysis revealed that all the analyzed species were clustered as discrete units (*L. serriola*, *L. virosa*, *L. saligna*, *L. indica*, and *L. perennis*). *Lactuca serriola* accessions formed a cluster on the sister branch of the *Lactuca sativa* complex. The 44 accessions of *Lactuca sativa* were also subdivided as distinct branches according to their morphotypes. The AFLP data were compared to RFLP data resulted from analysis of the same 56 accessions of *Lactuca* species [26]. However, RFLP markers showed a higher overall genetic distance among taxa.

Koopman et al. [37] used AFLP markers to determine the phylogenetic relationships of 95 accessions from 20 species of *Lactuca* and related genera. The results did not reveal a discrimination among the *serriola*-like species (*L. serriola*, *L. sativa*, *L. altaica*, and *L. dregeana*), assuming that these species are conspecific. The subsection *Lactuca* is well defined by this data, however the positions of *L. virosa* and *L. saligna* as related species to the *serriola*-like species remain unclear. Jeuken et al. [40] used AFLP markers to construct an integrated interspecific AFLP map of cultivated *Lactuca sativa* based on two *L. saligna* × *L. sativa* F2 populations.

Jeuken and Lindhout [41] developed an F2 population based on a resistant *L. saligna* × susceptible *L. sativa* cross. This F2 population was characterized using AFLP markers and tested for resistance to 2 *Bremia lactucae* races (NL 14 and NL 16). QTL mapping showed a qualitative gene (*R39*) included in the race-specific resistance and 3 QTLs (*RBQ1*, *RBQ2* and *RBQ3*) included in the quantitative resistance to this pathogen. Johnson et al. [39] used F2 and F3 families of *Lactuca sativa* and *Lactuca serriola* for genotyping using AFLP markers. Composite interval mapping was used to characterize marker-trait associations. Quantitative trait loci were recorded for differences between wild and cultivated lettuce in root architectural traits and water acquisition.

Kuang et al. [42] studied the genetic structure and diversity of 696 accessions from 41 populations of *Lactuca serriola* using 319 AFLP markers. The results showed that Armenian and eastern Turkish populations were the most diverse and might be located in the center of diversity of *Lactuca serriola*. Screening 709 accessions using the microsatellite MSATE6 located in the coding region of most *RGC2* homologs revealed 366 different haplotypes. There was a significant variation of copy number of *RGC2* homologs in different populations. The authors hypothesized that the high genome wide diversity and diversity of the *RGC2* cluster in Armenian and eastern Turkish populations resulted from high biotic and abiotic stresses in the origin regions of *Lactuca serriola*.

Lebeda et al. [43] assessed the genetic variation and relationships among 50 populations of *Lactuca serriola* using AFLP and isozyme markers. The population clustering corresponded approximately to their geographical distribution in Europe. This study revealed that accessions originating in various eco-geographical conditions of Europe differ significantly in their morphology and genetic and protein polymorphism.

The above summary of studies demonstrates that AFLP markers exhibit a high level of polymorphism and has proven to be useful for assessing the genetic diversity, phylogenetic relationships and genotyping of *Lactuca* germplasm. The availability of many different restriction enzymes enables the direct manipulation of AFLP fragment generation for defined applications such as QTL analysis, polymorphism screening and genetic mapping. However, the major disadvantage of AFLP markers is that these are dominant markers [3].

3.4 Microsatellites (SSRs) and Internal Transcribed Spacers (ITS-1)

Microsatellites, known as simple sequence repeats (SSRs) are DNA sections which consist of tandemly repeating mono-, di-, tri-, tetra-or penta-nucleotide units arranged throughout the eukaryotic genomes [3]. Because of their high degree of variability, microsatellites have been successful for studying plant genetic diversity and distinguishing closely related *Lactuca* genotypes [9,44-52] (Table 2). Microsatellites polymorphism could be observed using Southern hybridisation or PCR.

Van de Wiel et al. [48] used SSRs in Southern hybridisation to assess the variation among different cultivars of *Lactuca sativa* as well as among the accessions of *Lactuca* virosa, *Lactuca serriola* and *Lactuca saligna*. The majority of this plant material has been characterized morphologically [53]. The remainder of this material has also been characterized morphologically and analysed using ITS1 sequencing [45]. Fourteen microsatellite and 3 minisatellite motifs were tested for diversity studies in *Lactuca* species. The microsatellite array TCT was the best for fingerprinting in cultivated lettuce and some of wild *Lactuca* species. The TCT fingerprinting was also useful for testing the cultivars homogeneity, but it was not suitable for assessing the relationships among accessions. The polymorphism level observed with this probe was very high.

Van de Wiel et al. [51] isolated microsatellite-containing sequences from cultivated *Lactuca sativa* using enriched genomic libraries. Those isolated microsatellite loci will be useful for distinguishing lettuce cultivars and for assessing the genetic diversity of plant genetic resources. Witsenboer et al. [50] assessed the potential of SAMPL (Selectively Amplified Microsatellite Polymorphic Locus) analysis in *Lactuca sativa* in order to detect PCR-based codominant microsatellite markers. Fifty-eight SAMPLs were recorded and placed on the genetic map of lettuce [3]. Seventeen were codominant. Forty-five cultivars of lettuce and five wild species of *Lactuca* were characterized to determine the allelic diversity for codominant SAMPLs. Around 2–6 alleles were found within *Lactuca sativa* and 1–3 alleles were found among the crisphead genotypes. This allelic variation is higher than that was reported for RFLP markers. Therefore, SAMPL analysis is more applicable to intraspecific comparisons. Sicard et al. [49] assessed the genetic diversity in the cultivated and wild *Lactuca* species using molecular genetic markers derived from resistance genes of the NBS-LRR type. Three molecular markers, one microsatellite and two SCAR markers amplifying LRR-encoding regions, were developed from the resistance genes sequences at the main resistance gene cluster in lettuce. Hintum van [52] also used microsatellites and AFLP markers to describe the entire lettuce collection of the Centre for Genetic Resources in Wageningen. The above studies demonstrated that SSR markers have a great potential for producing large numbers of informative characters for phylogenetic studies of closely

related species, especially when ITS-1 diversity is insufficient.

3.5 Genomic Technologies and Their Promising Advances in *Lactuca* Germplasm

Genomics applies recombinant DNA, DNA sequencing approaches, and bioinformatics to sequence, assemble, and analyze the structure and function of genomes [54]. New algorithms and approaches are being used to generate genome, epigenome and transcriptome datasets for model and highly agronomic crop species [55]. Sequencing means identifying the exact order of the bases in a strand of DNA. DNA sequencing can be used to evaluate genetic variations and mutations that may play a vital role in the progression or development of a disease. The mutations may include the substitution (SNPs) or insertion and deletion (INDELs) of a single base pair or a deletion of thousands of bases. Single nucleotide polymorphisms (SNPs) are the most abundant type of DNA sequence variation found in the genome, and are of co-dominant nature allowing quick and efficient genotyping of very large numbers of samples [56]. Therefore, genome sequencing and SNP markers have successfully been used in *Lactuca* genetic analyses, such as phylogenetic studies, evaluation of genetic variation and population structure, genome-wide association studies, and construction of genetic linkage maps [57-60] (Table 2).

Recently, the genetic variation and population structure of 380 cultivated lettuce (*Lactuca sativa* L.) accessions were studied based on SNPs markers [58]. High-quality genotype data revealed a high level of heterogeneity within accessions**.** This study proved to be useful for rapid evaluation of genetic variation and population structure in the lettuce germplasm collection. Kwon et al. [59] assessed the genetic variation, population structure, and genome-wide marker-trait association analyses in lettuce based on 322 high-quality SNP markers. Only 258 unique genotypes were revealed among the 298 lines. Nine significant marker-trait associations (SMTAs) were detected at $P < 0.0001$, with 5 SMTAs for seed coat color, 1 for leaf undulation, 2 for leaf anthocyanin, and 1 for stem anthocyanin. These markers may be useful in marker-assisted selection.

Lavelle et al. [60] has recently sequenced the genome of *Lactuca sativa* cv. Salinas. Approximately 2.5 Gb (93% of the entire genome) was assembled into 13,352 scaffolds. A genetic map comprising over 11,000 transcript-derived loci was used to evaluate the quality of the assembly. These loci revealed 3200 scaffolds. Of these, 3100 scaffolds were genetically validated and assigned to 9 linkage groups. Additional scaffolds are being assigned to genetic bins on chromosomal linkage groups via mapping by sequencing the gene space of RILs from the reference *L. sativa* cv. Salinas x *L. serriola* mapping population. The lettuce genome contains 73% repeated sequences, mostly retrotransposons and uncharacterised repeats. Over 45,000 genes have been predicted; 79% of these have some levels of functional annotation.

4. CONCLUSION

This article summarized recent knowledge of the application of biochemical and molecular genetic technologies. It is highly expected that sequencing platforms will continue to improve the length and quality of output, and that the algorithms and bioinformatic software required tohandle large, repetitive genomes will be enhanced. The future is bright for a better understanding of conservation and utilization of *Lactuca* germplasm. Molecular genetic methods and studies discussed in this paper should be applied together in the broadest context of a complicated view of biodiversity, taxonomy, population genetics and management of *Lactuca* and related crops. This is in order to better understand and improve breeding programmes and biology of crops for future use in agriculture and food security [60-64].

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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