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# Biochemical evaluation of *Lactuca* L. germplasm

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## ABSTRACT

The genus *Lactuca* L. has an economic, edible and medicinal importance. However, it faces many biotic and a biotic problems that limited its yield. Several viral, bacterial and fungal pathogens infect the genus and inflect a devastating effect. Also, some insect pathogens cause damage to leaves of *Lactuca serriola* and *Lactuca sativa*. The use of pesticides to control these devastated diseases is harmful for human and environment. The most important abiotic factors that limited the growth of lettuce are low and high temperature, high soil salinity, soil acidity, high soil moisture and humidity. Therefore, to overcome the harmful effect of biotic and a biotic factors, the scientists pay attention to genetic resources to find the genes conferring resistance, and the genes conferring high and good agronomic traits. The first step in exploiting genetic resources for human interest is the collection of genetic resources and the assessment of genetic diversity within and among species. There are many techniques used in evaluation of genetic diversity within and among plant species. They are extends from morphological traits to molecular markers passing with biochemical markers. In this review, we surveyed all the data on using biochemical markers in the genetic assessment of *Lactuca* spp. © 2014 Trade Science Inc. - INDIA

## INTRODUCTION

The genus *Lactuca* L. belongs to one of the largest plant families, Asteraceae, in the tribe Lactuceae of subfamily Cichorioideae. The Lactuceae comprises 70 genera and 2300 species[1], while Bremer *et al*.[2] reported 98 genera and more than 1550 species. The genus *Lactuca* L. includes 97 wild species (16 in Europe, 12 in America, 43 in Africa, 51 in Asia)[3,4] confined mainly to temperate and warm regions of Europe, Asia, North America, further Africa and Indonesia. Some of them are naturalized in Australia[4].

Several species of the genus *Lactuca* L. have been cultivated since ancient times for their economic and medicinal importance. Cultivated lettuce (*Lactuca sativa*) is the most important leafy salad vegetable in the world[5] and rich in vitamins B and C. The oil of the Oilseed group, with a high vitamin E content, is used for human consumption. *Lactuca serriola* and other wild *Lactuca* species can be eaten as a salad, although they have something of a bitter taste. Several species of the genus *Lactuca* L. are rich in a milky sap that flows freely from any wounds in the plant. This sap contains lactucarium which is used in medicine for its anodyne, antispasmodic, digestive, diuretic, narcotic, aphrodisiac, soporific and sedative properties. It is taken internally in the treatment of insomnia, anxiety, neuroses, hyperactivity in children, dry coughs, whooping cough, rheumatic pain[6]. The sap has also been applied externally in the treatments of warts.

Several viral, bacterial and fungal pathogens, such as yellows virus, turnip mosaic potyvirus, *Microdochium*, *Rhizomonas* (corky root disease), *Bremia* (downy mildew), and *Erysiphe*[7] are one of the most important problems affecting lettuce. Also, some insect pathogens such as *Bremisia* spp. and

*Trichoplusiani* are the only insects reported as causing damage to leaves of *Lactuca serriola* and *Lactuca sativa*[8,9]. The use of pesticides to control these devastated diseases is harmful for human and environment. Therefore, attention has been paid to genetic resources to find the genes conferring resistance, and the genes conferring high and good agronomic traits, including oil content in oil producing species.

## GEOGRAPHICAL DISTRIBUTION AND ECOLOGY

Detailed survey of available literature data have shown that the genus *Lactuca* L. confined mainly to temperate and warm regions of Europe, Asia, North America, further Africa and Indonesia. Some of them are naturalized in Australia[4]. Most of *Lactuca* species are xerophytes well adapted to dry climatic conditions, except for some endemic liana-like species from East Africa rain forests and Madagascar[10]. The northern limits of occurrence of many of Eurasiatic species is between 50 and 55°N. *L. sibirica* has the northernmost distribution, Some localities reach at 70°N. The Eurasiatic species *L. tatarica* extends further to the west to 9°W. Boundary of distribution of the most common species *L. serriola* reaches 65°N and 5°W in Europe. The optimal elevations for the majority of *Lactuca* species are between 200 and 600 m but representatives of this genus can be found from the sea level to more than 2,000 m[4,11].

The genus *Lactuca* L. is variable from the ecological viewpoint and its species occupy various habitats[4]. The most common species *L. serriola, L. saligna* and *L. virosa* are weedy and occur on waste places and ruderal habitats, mainly along roads, highways, ditches[11]. *L. aurea, L. quercina, L. biennis* and partly also *L. sibirica* occur in woodland communities[12]. The majority of *Lactuca* species are calciphilous plants and settle limestone and dolomite areas, mostly rocky slopes (*L. perennis, L. Viminea, L. graeca, L. tenerrima*)[13]. *L. tatarica* and *L. acanthifolia* grow on cliffs at the seashore, but *L. tatarica* expands to Asia and Central and North Europe as a weedy species occupying unfertile salt substrata as well[12,14].

## ORIGIN

Zohary[15] assumed that *L. dregeana* DC. originated and spreaded in the region from Eurasia to South Africa. Also, he pointed out that much less attention has been paid to the Southwest Asian species in relation to the origin of cultivated lettuce. *Lactuca aculeata* Bois. & Kotschy ex Bois., *L. scarioloides* Boiss. and *L. azerbaijanica* Rech. appeared in the centre of origin (Southwest Asia) of lettuce. *L. serriola* is generally believed native to Europe, western Asia and northern Africa[12]. It is likely that it originated on the Mediterranean rim on rocky wasteland or woodland clearings. In general European *Lactuca* species have centre of origin in the Mediterranean region[11].

There are several different opinions about the centre of origin of cultivated lettuce (*Lactuca sativa*). According to Lindqvist[16], it probably originated from Egypt.. According to Vavilov, cultivated lettuce originated in the Mediterranean area[17]. This is also the view of[18]. Boukema *et al*.[19] stated that domestication of lettuce took place in Southwest Asia in the region between Egypt and Iran. Rulkens[20] stated that cultivated lettuce has its origin in the Kurdistan-Mesopotamia area and not in Egypt. Kesseli *et al*.[21] reported that Lindqvist[16], Durst[22], De Candolle[23] and Sturtevant[24] expressed several views on the origin of cultivated lettuce. These were that: (1) lettuce arose from wild forms of *L. sativa*; (2) it is originated directly from wild *L. serriola*; and (3) lettuce is product of by hybridizations between different species. The generally held view was hypothesis 2; there are no extant, truly wild *L. sativa* populations and *L. serriola* was thought to encompass fully the variation of cultivated lettuce[25]. However, Lindqvist[16] concluded that hypothesis 3 was the most plausible, since he found two morphological characters of *L. sativa* (pointed leaf apex and spotted anthocyanin on leaves) not known in *L. serriola* but present in *L. saligna* and *L. virosa*, respectively. Ryder and Whitaker[25] further elaborated on the hybridization hypothesis of Lindqvist, pointing out that relationships between progenitor and derivative species may be difficult to define.

## GENE POOL AND BREEDING SYSTEM

The primary gene pool of *Lactuca sativa* L. is represented by its numerous cultivars, primitive landraces and wild species without crossing barriers - a worldwide spread *L. serriola*, further *L. aculeata, L. scarioloides, L. azerbaijancia, L. georgica, L.*

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*altaica* occurring in Asia and by *L. dregeana* from the South Africa[15]. *L. saligna* belongs to the secondary gene pool. The tertiary gene pool includes *L. virosa* and some other wild species which can be crossed with *L. sativa* with difficulties[4].

Modern breeding methods of cultivated lettuce are based on utilization of wild related species and progenitors. Although cultivars are 99% self-pollinating[26], some cross pollination occurs and inter-specific hybrids among different members of the subsection *Lactuca* can be made. The species *L. sativa* is cross compatible with *L. serriola*, *L. altaica* and *L. aculeata*, and with some difficulties with *L. saligna* and *L. virosa*[4]. *L. serriola*, *L. Saligna* and *L. virosa* have been used in *L. sativa* breeding[27]. Close relationships of wild species from section *Lactuca* demonstrate occurrence of spontaneous interspecific hybrids *L. serriola* x *L. aculeata*, *L. serriola* x *L. dregeana*, *L. serriola* x *L. altaica*, *L. serriola* x *L. saligna* and *L. saligna* x *L. altaica* in natural populations[4]. Modern approaches e.g. somatic hybridization[28] and a creation of transgenic plants[29] are explored in lettuce breeding programs as well. *L. sativa* can be somatically hybridized with *L. tatarica* to produce fertile hybrids[30]. *L. viminea* and *L. sibirica* can be hybridized with *L. virosa* and with *L. tatarica*[31]. Since the majority of the wild *Lactuca* spp. is obligate self-fertilizing species, the accessions are not isolated during their regeneration[4]. The accessions of out-breeding species (*L. perennis* and *L. viminea* subsp. chondrilliflora) are isolated and manually pollinated.

## GENETIC DIVERSITY

Genetic diversity is the raw material permitting species to adjust to a changing world, whether these changes are due to natural or human factors. The genetic profile of whole populations typically varies from place to place across a species range. These differences may arise as the result of chance occurrences, such as the genetic composition of dispersing individuals that create a new population (founder effect), or changes in allele frequencies that result from chance mating in very small populations (genetic drift)[32]. Differences among populations can also arise systematically, especially if the environment in various places exposes individuals to different optima for survival and reproduction (fitness). For these and other reasons, populations often diverge from one to another in their genetic composition. Such divergence is especially strong and rapid when there is little gene flow among populations (e.g., limited dispersal of seeds or pollen, or limited movement of animals across physiographic barriers). Over evolutionary time, such among-population genetic differences can accumulate and result in the development of a new species (allopatric speciation). Knowledge of the amount and distribution of genetic variability within a species is vital to plant breeders because it is an important consideration when selecting germplasm to be included in a breeding program. Also, it is helpful to geneticists managing plant genetic resources and provides information for designing sampling protocols[33]. So, genetic diversity studies are essential for providing information for propagation, domestication, and breeding programs as well as conservation of genetic resources for plant species. The biochemical traits (storage proteins and isozymes) have been used by geneticists and evolutionists to describe genetic variation within and among populations of the same species.

## GENETIC DIVERSITY BASED ON BIOCHEMICAL TRAITS

### Storage proteins

Proteins are the post-transcriptional and translational products of an organism's DNA[34], and form structural and enzymatic components of cells[35-38]. Their size and amino acids sequence are the direct results of transcription and translation of the nucleotide sequences of the genes[39,40]. Hence, any observed variation in protein systems is considered as a mirror for genetic variations.

Proteins have been separated and characterized by different methods e.g., ultracentrifugation, chromatography, serology and electrophoresis[37,41,42] of these methods only, electrophoresis provided data for gene-ecological studies[43,44]. This method is the most appropriate for the separation and equivocal comparison of proteins [45-49]. Electrophoretic techniques have been widely used as a rapid and accurate test to identify and characterize different cultivars and genotypes of plants[50-52]. Genotype identification by electrophoretic protein fingerprinting was used to assess the uniformity, purity and agronomic merits[53-58]

Electrophoretic analysis of native or denatured seed storage proteins was used to provide information concerning the genetic variability, which represent a source of information for assessing genetic and taxonomic relationships at the species level and below[59], for example, *Vicia faba*[51](Sammour 1990a), *Cicer*[60], *Panicum*[61], *Triticum*[62], *Hordeum*[63], *Lotus*[47], *Lens*[64], *Phaseolus*[65], *Trifolium*[66], *Astragalus*[67], *Arachis hypogaea*[56], *Lathyrus sativus*[68,69] and *Lactuca*[70-71].

Mejia and McDaniel[70] used electrophoretic techniques to study characterization of lettuce cultivars. Some, but not all, of the two autumn desert, three winter desert and three coastal cultivars studied could be distinguished from one another on the basis of differences in seed soluble protein and esterase isozyme contents, as revealed by polyacrylamide gel electrophoresis.

Vries[71] used SDS-electrophoresis method to study characterization and identification of *Lactuca sativa* cultivars and wild relatives. Patterns of achene proteins of *L. sativa* cultivars are compared with those of *L. saligna, L. serriola* and *L. virosa*. The study revealed that *L. virosa* and *L. saligna* are easy to identify and are characterized by typical banding patterns. *L. sativa* and *L. serriola* share the same banding pattern. They differ clearly from *L. saligna* and *L. virosa*. *L. sativa* and *L. serriola* are closely related (in terms of similarity), forming a common gene pool and *L. serriola* has plainly been involved in the domestication process of *L. sativa*. So, SDS-PAGE of seed storage proteins has proven a simple and effective method for distinguishing among plant accessions.

## Isozymes

Isozymes were defined as structurally different molecular forms of an enzyme with, qualitatively, the same catalytic function. Isozymes originate through amino acid alteration, which cause changes in net charge, or the spatial structure (conformation) of the enzyme molecules and also, therefore, their electrophoretic mobility. After specific staining the isozyme profile of individual samples can be observed[72]. Data derived from electrophoretic gels consists of the number and relative motilities of various enzyme forms, which with appropriate genetic analyses, become transformed into single or multi loci genotypes for each individual[59]. Reasons are many for the popularity of electrophoretic data[59]., but foremost among these is that isozymes provide a series of readily scored, single-gene markers. Enzymes that are coded by different alleles of a distinct locus or those coded by separate loci frequently show different electrophoretic motilities[73-74]..

Allele frequency data are used to obtain a number of measures which include average level of heterozygosity (which estimates the probability that two alleles taken at random from the population are different), average level of polymorphism (which is the condition of polymorphic gene and characters, where the polymorphic gene has at least two alleles and polymorphic character has two or more qualitatively distinct morphs) and mean number of alleles per locus[75].

Isozyme analysis has been used for over 60 years for various purposes in biology, e.g., to delineate phylogenetic relationships, to estimate genetic variability and taxonomy, to study population genetics and developmental biology and, to direct utilization in plant genetic resources management and plant breeding[72]), for example, *Phlomis mergaritae*[76], *Pereskia guamacho*[77], red clover[78], *Ballota*[79], *Kirengeshoma palmata*[80], *Lespedeza*[81] and *Lactuca*[60,70,82-88].

A few articles have been published, focusing on the study of *Lactuca* spp. using isozymes analysis. Three of these works focused on *Lactuca* species[82,85,86], one on *L. sativa* (cultivated lettuce)[70], one on *L. serriola*[88] and four studying the genetic variability of cultivated lettuce and wild *Lactuca* species[60,83,84, 87-90]. These publications summarized the application of isozyme techniques for the identification of genetic variability among cultivars and wild populations of *Lactuca* spp. (*L. aculeata, L. serriola, L. saligna, L. virosa*) and the determination of the genetic and phylogenetic relationships of *Lactuca* spp. The results showed a lower level of intra-species than inter-species diversity.

Isozyme variation was used to characterize levels of variation and the systematic relationships of wild and cultivated *Lactuca* populations by Kesseli and Michelmore[83]. *L. sativa* is generally assumed to have a progenitor similar to *L. serriola*[16]. Isozyme data suggested a polyphyletic origin of *L. sativa*[60]. Roux *et al.*[82] used isozyme data to show that *L. aculeata* is a part of the *L. serriola* complex, confirming their genetic closeness with *L. sativa*, and also reported that

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*L. saligna* and *L. virosa* are very distinct from the others.

Dziechciarková *et al*.[91] used the isozyme technique to study genetic variation in European *Lactuca serriola* germplasm. The study included two different forms of *Lactuca serriola*. They are described as *L. serriola* f. *serriola* and *L. serriola* f. *integrifolia*. Altogether 56 samples (accessions) of both forms of *L. serriola* originating from twelve European countries were analyzed for isozyme polymorphism. Eleven enzymatic systems were used for the characterization of variation and nine of them showed polymorphism. From 66 bands (isoforms) observed, 42 displayed polymorphism. According to isozyme polymorphism, the studied set was divided into two groups. The first group comprised mostly samples from Austria, Czech Republic, Slovenia and Sweden; the second group contained mostly samples from France, Germany, Great Britain, Italy, Netherlands and Switzerland. A good relationship was recorded between isozyme polymorphism and taxonomic status of both *L. serriola* forms.

From the above summary of studies, it was demonstrated that isozyme markers display a high level of polymorphism and are useful for the characterization of variability and the determination of taxonomic relationships and species identity.

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