

# APPLICATIONS OF LEA GENES IN BIOTECHNOLOGICAL ASPECT

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**Abstract**—LEA (Late Embryogenesis abundant) proteins in animals and plants that protect other proteins from aggregation due to desiccation. LEA group of hydrophilic proteins are mainly low molecular weight (10-30kD) proteins which are involved in protecting higher plants from damage caused by environmental stresses, especially drought (dehydration). LEA proteins are accumulated in the final stage, in contrast to storage proteins which appear earlier. LEA proteins have first been described about 25 years ago as accumulating late in plant seed development. They were later found in vegetative plant tissues following environmental stress. LEA proteins are generally classified into 6 groups (families) according to their amino acid sequence and mRNA homology, which are basically localized in cytoplasm and nuclear region. LEA proteins are variable in size, ranging from 5 to 77 KD among most groups, can be acid, neutral or basic. LEA proteins exhibit the remarkable ability to become more ordered and to develop secondary structure as dehydration proceeds. In this review article, highlighted the biotechnological applications of LEA genes, in relation to their capacity to prevent aggregation of proteins.

**Keywords:** Drought, LEA, Proteins.

## Introduction

Plants are often exposed to adverse conditions, which might cause what is called abiotic stress. It usually as a negative impact on their performance so in order to withstand such situations, plants have developed different mechanisms of tolerance. LEA proteins, found in all types of plants are thought to have a main role in abiotic stress tolerance, but their molecular mechanisms are still unknown. Plants are exposed to multiple environmental stresses along their life cycle. Abiotic stresses such as drought I salinity and freezing temperature affects more areas of the world and they impact in plants by directly reducing its survival in the natural environment and its productivity in agriculture. Abiotic stress is basically responsible for reducing average yields for most major crops to its half (Bray et al., 2000).

Among all these abiotic stresses like temperature, oxidative stress, drought, salinity, cold etc. drought is the most serious abiotic constraints to crop production all over lobe (Araus et al., 2002). Many genes have been identified to be involved in the response of drought stress in plants (Zang et al., 2012).

Numerous laboratory water stress experiments investigating dehydration induced changes in gene expression have revealed several candidate genes that may be associated with drought tolerance. LEA proteins play a special role in protecting cytoplasm from dehydration and storage of seeds and in whole plant stress resistance to drought. LEA proteins are expressed through all the developmental stages wit different expression levels and no tissue specificity (Bo. et al., 2005).

Te evolution of LEA proteins is one of these changes which play an important role in resistance to drought. This implies that studies on tolerant proteins and the isolation, identification and functional analysis of their genes will be of great benefit to the breeding of drought resistant crops. LEA protein gene expressions start at late maturation period and initiation period of drying get a maximum in progressive dehydration and sharply decrease after some hours of germination. Many reports showed that LEA protein gene expression as no tissue specificity at the levels of tissues and organs as the gene can express in cotyledons, panicles of seeds and also in stems, leaves and roots (vegetative tissues) (Shahattary et al., 2017).

Genes induced during drought stress conditions are thought to function not only in protecting cells from water deficit by the production of important metabolic proteins but also in the regulation of genes for signal transduction in the drought stress response. Four steps atleast are involved in LEA protein gene expression and regulation induced by drought and signal recognition, signal transduction, signal amplification and integration, LEA proteins gene expression responses and its product formation. Information from LEA protein gene expression shows that it is a multifunctional stress protein to maintain normal metabolism for higher plants under severe conditions and its gene can code RNA regulatory proteins which can regulate related events such as gene expression and development (Amara et al., 2016). Here, we discuss the role of LEA proteins and tries to cast some light on their possible biotechnological uses to face the impact of climate change and meet future demands.

### Late embryogenesis abundant proteins

LEA proteins were originally discovered in the late stages of embryo development. LEA proteins are accumulated in this final stage, in contrast to storage proteins which appear earlier. Moreover, their mRNA is maintained at high levels in the dehydrated mature embryos, while transcripts of storage protein genes are completely degraded during the last embryogenesis stage. Members of the LEA family seem to be ubiquitous in plant kingdom. Since their 1<sup>st</sup> description, hundreds of LEA proteins from vascular to non-vascular plants have been isolated. Their presence has been confirmed not only in angiosperms and gymnosperms (Bray et al., 1993). The correlation of LEA proteins in seed maturation stages, during water stress in vegetative plant organs and in LEA proteins represents a widespread adaptation to water deficit.

### LEA proteins Distribution

LEA proteins are formed during the last period of seed development accompanied by dehydration. LEA proteins exist mainly in higher plant seeds, but have also been found in seedlings, roots and other organs. LEA proteins exist mainly in higher plant seed, but have also been found in seedlings, roots and other organs. LEA proteins localized in cytoplasm and nuclear regions (Zhang et al., 2005). LEA proteins were initially classified in 6 subgroups on the basis of specific domains (Dure et al., 1989). Dure introduced LEA proteins from cotton were categorized by virtue of similarities in their deduced amino acid sequences.

This classification is based on the presence of specific motifs conserved across species which are unique to each family. Based on these characteristics and considering all available sequence information from different plant species. LEA proteins are grouped into 7 distinctive groups or families; groups 1, 2, 3 are considered the major LEA group containing most members of the protein family. With increasing information available on differences on expression profiles, description in organisms other than plants and especially with new bioinformatics tools, the classification has been subjected to different rearrangements. LEA proteins are basically divided into 5 groups; group 1 to 5 (Fahad et al., 2017).

#### LEA Group 1

This LEA group originally represented by the D-19 and D-132 proteins from cotton seeds contains an internal 20-mer sequence. This motif may be present in several copies arranged in tandem, from one to four in plant species and upto eight in other organisms.

#### LEA Group 2

This group of LEA protein also known as “dehydrins” was originally identified as D-11 family in cotton. Group 2 LEA proteins are the most characterized group of LEA proteins. Many proteins of this group contain a tract of ser residues called the S-segment, acting as a site for protein

phosphorylation. The presence and arrangements of these different motifs in a single polypeptide allow the classification of group 2 LEA protein into 5 subgroups.

#### LEA Group 3

This group of LEA proteins is characterized by a repeating motif of 11 amino acids. Differences found in the molecular mass in this group of proteins are usually consequences of the number of repetitions of this 11-mer motif leads to a sub-classification of the group 3 proteins into 2 sub groups: 3A, represented by cotton D-7 LEA proteins; 3B, represented by cotton D-29 LEA proteins.

#### LEA Group 4

Group 4 proteins of LEA are of widespread occurrence in plant kingdom, including non-vascular plants and vascular plants. 4 additional motifs can be distinguished in many group 4 LEA proteins. The presence or absence of motif defines 2 subgroups within the family. The presence or absence of motif defines 2 subgroups within the family. The 1<sup>st</sup> subgroup (group 4A) consists of small proteins (80-124 residues long). The other subgroup (group 4B) has longer representatives (108-180 residues). D-113 protein from cotton, the 1<sup>st</sup> discovered of this group.

#### LEA Group 5

The 1<sup>st</sup> proteins described for this group were D-34, D-73 and D-95 from cotton. They represent a typical LEA subgroup because they contain a higher proportion of hydrophobic residues. These proteins are not soluble after boiling suggesting that they adopt a globular confirmation.

#### LEA Group 6

1<sup>st</sup> described protein of this group is LEA 18 from bean. 36 genes of this family have been described from vascular plants. The proteins in this group are characterized by small size (7-14 kD). A distinctive characteristic of the group 6 LEA proteins characterized is its accumulation in the elongation or growing

#### Structure of LEA proteins

LEA proteins are variable in size, ranging from 5 to 77 kD among most groups. They can be acid, neutral or basic. LEA proteins from group 1, 2, and 3 are predicted to be atleast 50% unfolded. Since the discovery that group 1 protein from wheat was flexible in confirmation with little secondary structure (i.e.;  $\alpha$ -helix or  $\beta$ -sheet) and 70% of the protein behaving as random coil most LEA proteins have been found to have an unfolded structure in the hydrated state. Environmental conditions can also affect folding and several LEA proteins become more structured when dried. On the basis of structure, it is predicted that group 5 proteins play roles in seed maturation, dehydration and combining concentrated ions (Savitri et al., 2013).

Water was removed from 83.5 wt% to 2.4 wt%. As water is removed, the protein assumes progressively a more folded confirmation. At less than 20% water the protein becomes more dehydrated and begins to adopt a significant amount of secondary structure.  $\alpha$ -helical structure is apparent and hairpin like structures are very similar to that in the complete absence of water. The propensity of some LEA protein to gain structure under some conditions may be a general property of these proteins and may have important functional implications in their physiological roles (Kumar et al., 2014).

In plants, LEA proteins have been found localized in cytoplasm, nucleus, mitochondria, chloroplast, endoplasmic reticulum, peroxisomes and plasma membrane. In plants, LEA group 1 are found mostly in seeds and they are not induced by stress conditions in vegetative tissues. Group 2 LEA proteins accumulate during seed desiccation and in response to water deficit induced by drought, low temperature or salinity. Most of them accumulate in all tissues upon water deficit although there are those that preferentially respond to particular stress conditions.

#### LEA proteins function

LEA proteins have the capacity to protect target proteins from inactivation and aggregation during water stress. A role in protein stabilization is supported by the fact that some LEA proteins preserve enzyme activity *in vitro* after partial dehydration, desiccation or freezing. One mechanism of protection is the prevention of water stress induced aggregation of proteins. Many studies show the protection conferred by LEA protein into plants and microorganisms results in an enhanced stress tolerance and storage of seeds and in whole plant stress resistance to drought, salt and cold.

Numerous transgenic studies revealed a positive effect of LEA gene expression on plant stress tolerance. In general the phenotypes of the transgenic plants show enhanced stress tolerance, often related to drought or salt stress. Most studies report enhanced growth rates and reduced wilting of the aerial parts under stress under laboratory conditions and in some field trials, demonstrating a real potential of LEA proteins could be useful for other biotechnological applications in relation to their capacity to prevent aggregation of proteins.

#### Conclusion

Information from LEA protein shows that it is a multi-functional stress protein to maintain normal metabolism for higher plants under severe conditions, and its gene can code RNA-regulatory proteins, which can regulate related events, such as gene expression and development. The relevant progress made toward understanding the role of LEA, the molecular mechanisms through which they can enhance stress tolerance is unknown.

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