

Narrow-leafed lupin (*Lupinus angustifolius* L.) β -conglutin: A multifunctional family of proteins with roles in plant defence, human health benefits, and potential uses as functional food

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Abstract

Lupinus angustifolius or narrow-leafed lupin (NLL) is an important Pulse world-wide and an outstanding alternative source of high quality proteins. NLL β -conglutin (vicilin or 7S acid globulin), a multigene family of proteins, is the main representative protein in NLL seeds exhibiting multifunctional properties and benefits recently discovered. These functions may result from specific structural features of NLL β -conglutins in comparison with most legume vicilin proteins. From the agricultural side, NLL β -conglutin proteins (1a) exert antifungal properties promoting enhanced plant resistance to necrotrophic oomycete pathogens and (1b) displaying functional interplay between storage protein mobilization and seed oxidative metabolism as a functional regulatory and signalling crossroad for the control of physiological underlying processes of the seed dormancy breakage and release, during germination, and seedling growth. NLL β -conglutins benefits go beyond nutritional properties because displayed nutraceutical functions make them an outstanding source of innovative ingredients for functional food. They have antioxidant activity and anti-inflammatory properties, which are able to improve inflammatory-related diseases such as type 2 diabetes through (a) regulation of insulin signalling pathway, (b) improving insulin sensitivity, (c) facilitating glucose uptake by cells, (d) together with their capacity for balancing the oxidative homeostasis, metabolic, and signalling pathways (e) while reducing reactive oxygen specie production and increase cellular oxidative defence capacity. Interestingly, NLL β -conglutins also display anti-nutritional properties as proteins capable to elicit allergic responses in sensitized individuals. This review outlines newly discovered knowledge of the narrow-leafed lupin β -conglutin proteins drawing on information gained from structure-functional properties, agriculture benefits, as well as nutraceuticals and molecular allergy point of views.

KEYWORDS

anti-oxidant activity, food allergy, functional food, inflammation, molecular nutraceuticals, pathogens defence, sweet lupins, vicilin

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1 | INTRODUCTION

Plants-based seed compounds, particularly proteins, are highly valuable sources of ingredients used by the food industry for the preparation of functional foods (Delgado-Andrade, Olías, Jimenez-Lopez, & Clemente, 2016; Kotchoni, Kibiti, Gachomo, Jimenez-Lopez, & Martin, 2018). Up until now, soybean has been considered the main source of plant proteins; however, other grain legumes, such as lupins with an average protein content ranging between 35–44 %wt, are fast growing sources of protein. Currently, sweet lupin group species such as *L. angustifolius* (narrow-leafed lupin, blue lupin, or NLL) and *Lupinus albus* (white lupin) are particularly used because of their increasing number of nutraceutical properties (health benefits) that are being discovered, in addition to their low content in anti-nutritional factors, that is, alkaloids (Jimenez-Lopez, Delgado-Andrade, et al., 2017). Foods supplemented with lupin seeds provide health benefits such as increased satiety and reduced energy intake helping to fight obesity, decrease blood pressure, and glucose level, promoting cholesterol- and triglyceride-lowering effects and inhibiting angiotensin converting enzyme, thus helping to tackle cardiovascular disease incidence (Arnoldi, Boschini, Zanoni, & Lammi, 2015).

Albumins and globulins are two major fractions of proteins present in lupins seeds (Foley et al., 2011). Lupin seeds globulins comprise four families such as α -conglutin (11S globulin, legumin-like protein); β -conglutin (7S-globulins or vicilin proteins), which is the main protein among lupin seed globulins; and minor components: γ -conglutin (basic 7S globulin) and δ -conglutin (2S sulphur-rich albumin; Duranti, Consonni, Magni, Sessa, & Scarafoni, 2008; Foley et al., 2011; Foley et al., 2015). All of these families contribute nutritional benefits to lupin seeds. The remaining two protein fractions typically found in seeds, prolamins and glutelins, can be considered as negligible (Foley et al., 2015). Interestingly, lupin vicilins (β -conglutins) are a multigene family integrated by eight genes, seven functional genes named from β 1 to β 7, respectively, and one non-functional pseudogene (Foley et al., 2011; Foley et al., 2015). Functional genes are identified in Uniprot database with the following accession numbers: F5B8V9 and F5B8W0 to F5B8W5, respectively. NLL β -conglutin proteins exhibit excellent technofunctional properties making them appropriate ingredients for different foodstuffs; their isolation can be achieved from seeds by salt-induced extraction followed by differential fractionation and dilutive precipitation (Muranyi et al., 2016). Recombinant methodologies have allowed us the expression and purification of five β -conglutin isoforms (β 1 to β 4 and β 6; purity >95%); however, their uses would be strictly focused in molecular studies. Theoretical (molecular weights) MWs of these five isolated and purified proteins have been calculated as 71.9, 70.7, 70.1, 68.2, and 64.4 kDa (Uniprot database, <http://www.uniprot.org/>), which are the longest currently known sequences of β -conglutins from *L. angustifolius* (<http://www.lupinexpres.org>). Recently, it has been identified two β -conglutin isoforms from *L. albus* (Uniprot accession numbers Q53HY0 and Q6EBC1) with comparable size to

these from *L. angustifolius* (<http://www.uniprot.org/>). Within the NLL, β -conglutin protein sequences can be described as a variable number of micro-heterogeneities since their polymorphism ranges from 1% to 26% (Lima-Cabello, Robles-Bolivar, Alché, & Jimenez-Lopez, 2016), mainly due to their multigenic origin.

NLL β -conglutin proteins were implemented in different research aspects that are described in the following sections.

2 | STRUCTURE-FUNCTIONAL ASPECTS OF NLL β -CONGLUTIN FAMILY

β -Conglutins show extensive structural variation (Jimenez-Lopez, 2015; Lima-Cabello et al., 2016). They are integrated by over 20 polypeptide chains, covering a broad range of molecular masses (MW 10–80 kDa) resulting from proteolytic cleavage of multiple β -conglutin precursor polypeptides (Duranti, Guerrieri, Cerletti, & Vecchio, 1992; Foley et al., 2011). β -Conglutins are unique proteins among cupin superfamily; they do not contain disulfide bonds in their structure. The compact structural stability is achieved thanks to electrostatic interactions and hydrogen bond establishment (Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). In addition, this structural stability of β -conglutin is also due to the oligomerization process yielding trimeric and tetrameric forms, with the minimal individual subunit size ranging from 20 to 75 kDa, and the putative combination of different subunits defining the multimeric structure of these conglutins in different *Lupinus* cultivars and species including *L. angustifolius* (Magni et al., 2007). A heterogeneous number of up to 12 distinct frequently glycosylated polypeptides have been identified (Duranti et al., 1992, 2008). This post-translational modification was particularly abundant in the low MW range of polypeptides generated by proteolysis during seed germination, when seed storage proteins undergo mobilization (Duranti et al., 1992, 2008) to contribute as C and N sources for seedling growth and plant development.

Structural features of β -conglutins are distinctive as it includes two cupin domains forming a Rossmann fold-like structure frequently found in enzymes that use molecular oxygen as a substrate (Jimenez-Lopez, 2015; Jimenez-Lopez et al., 2015). Moreover, this is an overall similar globular structure to legume seed allergens such as peanut Ara h 1 or lentil Len c 1 sharing an average comparative superimposition value <1 Å with NLL β -conglutins (Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). This structure consists of a negatively charged surface of a globular domain constituted by two conserved β -barrels, each one comprising anti-parallel β -sheets. An additional mobile N-terminal arm constituted by 8–10 α -helices is a distinctive feature in NLL β -conglutin proteins compared with vicilins in most legume species (Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). Molecular modelling analysis has demonstrated that large structural differences between NLL β -conglutin isoforms are found mostly in this mobile arm N-terminal domain; thus, putative functional differences among NLL β -conglutins could be due to these structural differences

(Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). Molecular modelling has also shown that one of the β -barrels in the globular domain contains a semi-conserved metal binding motif (HYX ... R), typically found in oxidoreductases (Oxalate oxidase; Jimenez-Lopez, 2015; Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). This motif suggests that NLL β -conglutin structure is comparable with germins or germin-like proteins (oxalate oxidase activity, catalyzing the conversion of oxalates into CO_2 and H_2O_2), and vicilin-like glucose binding proteins, associated with functional roles exerted in the cell wall, the plasma membrane, and/or plasmodesmata (Jimenez-Lopez et al., 2016).

On the other hand, considerable structural differences have been revealed between NLL β -conglutin isoforms ($\beta 1$ to $\beta 7$), particularly affecting 2-D elements (loops and coils); and more noticeable differences in the N-terminal domain integrated by the mobile arm affecting number, length, amino acid composition, and polymorphism of their sequences. Furthermore, different microheterogeneities are presently in fundamental residues directly involved in the variability of allergy-related epitopes. This may affect the observed differences in cross-reactivity among legume vicilins (Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016).

3 | AGRICULTURAL BENEFITS OF NLL β -CONGLUTINS: FIGHTING AGAINST PATHOGENS

The functional roles of lupin vicilins in pathogen defence have remained largely unknown in comparison with other plant and crop species.

Seed germination is a key physiological process potentially affected by pathogens attacks; thus, seed defence may involve multiple compounds. Among different *Lupinus* species, β -conglutin suffers a comparable fate during germination. At the beginning of this process (between Days 3 and 5), a big change is observed in β -conglutin structure and concentration. This involves the appearance of a new set of polypeptides, including a higher molecular mass group, whose concentration progressively decays until complete disappearance after 11 days of germination. Similarly, a small molecular mass group increases from 5 to 11 days because they experience limited proteolysis during seed germination (Monteiro, Carreira, Freitas, Pinheiro, & Ferreira, 2015). Among these proteins, it was found a polypeptide of 20-kDa termed *blad* that is a stable intermediary product of β -conglutin catabolism. It accumulates exclusively in the cotyledons of *Lupinus* species and exhibited antifungal activity (Monteiro et al., 2015).

Recently, it was found that NLL β -conglutins exhibited a protective role against a wide range of necrotrophic pathogens (Jimenez-Lopez et al., 2016). The purified β -conglutin isoforms $\beta 1$ and $\beta 6$ exhibited strong *in vitro* growth inhibitory activity against necrotrophic fungal pathogens such as *Rhizoctonia solani*, *Alternaria brassicicola*, *Fusarium oxysporum*, *Phytophthora nicotianae*, and *Colletotrichum lupini*. *in vivo* antipathogenic activity was also

highlighted after $\beta 1$ and $\beta 6$ transient expression in *Nicotiana benthamiana* leaves, exhibiting a high suppression of pathogens through inhibition of both hyphal growth and elongation. Furthermore, conglutins $\beta 1$ and $\beta 6$ suppressed pathogen-induced cell death *in planta*. In addition, they inhibited pathogen-induced suppression of the plant oxidative burst as determined by protein oxidation in infected plants (Jimenez-Lopez et al., 2016), due to increased levels of pathogen- (*Sclerotinia sclerotiorum*, *P. nicotianae*) induced protein oxidation promoted by $\beta 1$ and $\beta 6$ while maintaining leaf health. This investigation provides the first demonstration for any NLL vicilin (β -conglutins) of induced protection against pathogen attack. Mechanisms to explain these functional features at the molecular level are being investigated; however, the localization of conglutin $\beta 1$ and $\beta 6$ at the cell surface including plasmodesmata constitutes an important clue. These locations are key points of highly regulated reactive oxygen specie (ROS) production by nicotinamide adenine dinucleotide phosphate oxidases; thus, NLL β -conglutins may play key signalling roles in the control of physiological processes such as cellular growth and development while providing adaptation to environmental changes as pathogen attacks (Kärkönen & Kuchitsu, 2015). Overall, NLL β -conglutin localization (Jimenez-Lopez et al., 2016) provided functional insights of their putative mediation in ROS production due to the hypersensitive reaction-related oxidative burst, providing integration of signaling responses against pathogen attack while leading otherwise to the structural strengthening of the cell wall through lignin crosslinking. This was reported previously for germin-like proteins from wheat. In addition, ROS-mediated production by β -conglutins such as H_2O_2 could play a direct antimicrobial role or acting as a signalling molecule in defence response pathways.

Germins and germin-like proteins have been shown to play dual roles both in pathogen defence but also in seed germination (Cândido et al., 2011). This is due to their oxalate oxidase activity promoting H_2O_2 production that contributes to the hypersensitive reaction response but also serves as signalling molecules regulating seed physiological processes. Similarly, to germins, NLL β -conglutins displayed functional interplay between storage protein mobilization and regulation of oxidative metabolism during seed germination and seedling growth (Lima-Cabello, Robles-Bolívar, et al., 2017; Lima-Cabello, Molina-Borrego, et al., 2019). NLL β -conglutins have recently been showed to stimulate lowering nitric oxide (NO) production and down-regulating iNOS gene expression (Lima-Cabello, Morales-Santana, Foley, et al., 2018; Lima-Cabello, Morales-Santana, León, et al., 2018). They also promote increasing levels of glutathione, thus exerting antioxidative roles in the cell (Lima-Cabello, Morales-Santana, Foley, et al., 2018; Lima-Cabello, Morales-Santana, León, et al., 2018). These functions may situate NLL β -conglutin proteins as an important signalling and oxidative metabolic functional crossroad contributing to the regulation of physiological processes driving seed dormancy breakage and release, germination, and seedling growth, in addition of having a major influence on crop plants healthy development, vigor, and yield (Lima-Cabello, Robles-Bolívar, et al., 2017; Lima-Cabello, Molina-Borrego, et al., 2019).

4 | THE MOLECULAR NUTRACEUTICAL PROPERTIES OF NLL β -CONGLUTIN PROTEINS SIGNIFICANTLY CONTRIBUTES TO MAKE LUPIN AS A FUNCTIONAL FOOD

Current interest in lupin seeds is growing motivated by increasing knowledge of the health benefits that lupins provide (Arnoldi et al., 2015), as promising innovative food ingredients. NLL seed is one of the richest sources of plant proteins for humans with novel nutraceutical properties, and NLL β -conglutins particularly have emerged as outstanding proteins with potential used as functional food.

Recent research on the molecular nutraceuticals of NLL β -conglutins has revealed a plethora of functional roles as antidiabetic proteins improving insulin sensitivity, anti-inflammatory properties, with antioxidant activity.

Type 2 diabetes mellitus (T2DM) is one of the main global health concerns with deleterious complications. Molecular mechanisms recently discovered reveal how NLL β -conglutins can act as functional alternative foods to fight diabetes (Jimenez-Lopez, Delgado-Andrade, et al., 2017). Research work using *in vitro* (pancreatic PANC-1 cell-line) and *ex vivo* (mononuclear cells from whole blood and peripheral blood from diabetic patients) cell culture approaches, together with the use of recombinant purified NLL β -conglutinin isoforms β 1, β 3, and β 6, have shown different mechanistic actions of these proteins, making them strong new candidates to help fight T2DM. Mechanistic actions include their modulatory activity exerted on insulin signaling pathway activation mediated by kinases. They promote the upregulation of the expression of genes such as IRS-1, p85-P13k, AKT, and GLUT-4, as well as increasing their protein levels and activation (stimulating phosphorylation of these upstream modulators). This action was also accompanied and reinforced by decreased levels of modulatory factors such as iNOS and IL-1 β , which greatly influence the development and progression of the T2DM (Lima-Cabello, Alche, et al., 2017; Lima-Cabello, Morales-Santana, Foley, et al., 2018; Lima-Cabello, Morales-Santana, León, et al., 2018).

Moreover, insulin resistance (IR) is a main contributor and causative problem leading to the development of the long-term T2DM complications. In this regard, NLL β -conglutinin proteins are able to reverse IR through different pleiotropic effects such as (a) increasing glucose cellular uptake; (b) modulating key gene expression in the insulin-signaling pathway; (c) activation of the IRS-1/PI-3-kinase pathway through the phosphorylation of its upstream modulators, that is, IRS-1, as well as downstream mediators (Caveolin, CBL) that otherwise promote vesicular transport to the plasma membrane of glucose transporters; (d) improving insulin sensitivity; (e) regulating immune cell attraction and mobilization to the adipose tissues by controlling the expression of chemotaxis (i.e., CCL2 and CCL5) and molecular adhesion (i.e., ICAM-1 and VCAM-1) regulators; (f) decreasing cellular oxidative stress; and (g) promoting metabolic homeostasis and cell signalling (Lima-Cabello, Alche, et al., 2017; Lima-Cabello, Morales-Santana, Foley, et al., 2018; Lima-Cabello, Morales-Santana, León, et al., 2018).

It was suggested that all these mechanistic properties are due (a) to the distinctive structural features of NLL β -conglutins compared with other legume vicilin proteins (Jimenez-Lopez, 2015; Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016) and (b) in addition to their proven interaction with insulin (Lima-Cabello, Alche, et al., 2017), which may enhance insulin functional properties.

Furthermore, sustained inflammation causes and promotes development of many common diseases, that is, obesity linked to metabolic disease, IR leading to T2DM, dyslipidemia, and cardiovascular diseases. On the basis of the current ongoing research, NLL β -conglutins represent an outstanding alternative to tackle inflammation and inflammatory-related diseases at the molecular level. The mechanisms involved in the anti-inflammatory properties of NLL conglutinin β 1, β 3, and β 6 isoforms have been recently discovered. They are related to their ability for (a) decreasing the production (downregulating gene expression and declining protein production) of pro-inflammatory mediators such as cytokines as TNF- α , INF- γ , IL-1 β , IL-2, IL-6, IL-8, and IL-12 and inflammation modulators as NF- κ B1; (b) while inhibiting chemotaxis and cell adhesion capacity of cells avoiding the enhancing inflammatory potential of immune cells after migration to pancreatic and adipose tissues (Lima-Cabello, Morales-Santana, Foley, et al., 2018; Lima-Cabello, Morales-Santana, León, et al., 2018); (c) in addition to promote the reduction of the NO production and the down-regulation of the iNOS gene expression and protein production.

Therefore, oxidative stress (excessive levels of ROS generation) is also a main factor contributing to the onset and progression of T2DM under obesity or hyperglycaemic conditions, also promoting the development of IR. Hyperglycaemia damages the antioxidant defence system (glutathione production, superoxide dismutase and catalase activities), enhancing ROS production that contributes to mitochondrial dysfunction and in turn aggravates IR. NLL conglutins β 1, β 3, and β 6 are newly discovered bioactive molecules with antioxidant activity (Lima-Cabello, Morales-Santana, Foley, et al., 2018; Lima-Cabello, Morales-Santana, León, et al., 2018), due to their capacity to down-regulate iNOS gene expression, and to reduce NO production. They are also able to strongly reduce protein carbonylation, a covalent oxidative modification induced by ROS, leading to alterations in proteins activity. In addition, NLL β -conglutins increased the oxidative stress defence by promoting increase of glutathione levels in the cells, while regulated the activity of superoxide dismutase, and catalase (Lima-Cabello, Morales-Santana, Foley, et al., 2018; Lima-Cabello, Morales-Santana, León, et al., 2018).

5 | THE MOLECULAR ALLERGY FEATURES OF β -CONGLUTINS AS ANTI-NUTRITIONAL FACTORS IN FOODSTUFFS

Food allergy is a serious and growing problem affecting about 1–3% in the general population and particularly between 3% to 8% among children. However, the frequency of sensitization and allergic reactions to lupin proteins in the general population is currently unknown.

Lupin allergy prevalence may depend on the daily food intake, the geographical localization, and also on the population age: Lupin allergy affects 5.2% of children aged up to 15 years and 1.7% aged between 15 to 30 years (Gayraud et al., 2009).

Allergic reactions to lupin have increased in parallel with the increasing number of food applications for this legume as alternative protein source for human consumption. These reactions can be triggered via ingestion of lupin in some peanut-allergic individuals, although triggering via ingestion by primary sensitization, inhalation, and occupational exposure in individuals without peanut allergy has also been reported (Lima-Cabello, Berral-Hens, et al., 2017). However, lupin allergies have mainly been reported in patients with allergies to other legumes such as soybean, pea, lentil, chickpea, and particularly peanut, probably due to cross-reactions to structurally similar proteins, that is, peanut Ara h1, lentil Len c 1, or pistaccio Pis s1 (Lima-Cabello et al., 2016) with comparable both linear and/or conformational epitopes to lupin β -conglutins (Jimenez-Lopez, 2015; Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016).

For these reasons, lupin is included in the list of foods that should be labelled as an allergen in pre-packaged foods as advised by the European Food Safety Authority (<http://www.efsa.europa.eu/>). The lowest eliciting dose for allergic reactions to lupin in peanut-sensitized patients was 0.5 mg of lupin flour. More recently, the Voluntary Incidental Trace Allergen Labelling programme of the Allergen Bureau of Australia and New Zealand established 4 mg of protein as the reference allergenic dose for lupin (<http://allergenbureau.net/vital/>), whereas for peanut was comparatively much lower (0.2 mg of peanut protein).

β -Conglutins have been positioned as the main allergen proteins in lupin seeds (Jimenez-Lopez, Foley, et al., 2017; Jimenez-Lopez et al., 2018). They have been named as Lup an 1/Lup a 1, respectively, in blue and white lupins, by the International Union of Immunological Societies allergen nomenclature subcommittee (<http://www.allergen.org/>). This may potentially limit the usage of β -conglutin proteins as a functional and nutritional ingredient in food production. Processed Foodstuffs under different conditions, that is, fermentation, extrusion, soaking, cooking, microwave heating, and boiling, has revealed that these cooking processes do not affect the allergenic potential of lupin seed protein extracts. However, the autoclaving is the only process that drastically reduces the binding capacity of Immunoglobulin E (IgE) antibodies tested with serum from atopic patients (Alvarez-Alvarez et al., 2005). This may be due to the disappearing of the lupin proteins conformational epitopes under this processed condition.

β -Conglutins have showed to be the main proteins responsible for the IgE reactivity of lupin species and cultivars with potential to trigger immune responses leading to allergy symptoms in atopic patients. Newly identified polypeptides between 15 and 80 kDa from "sweet lupin" group, constitute a potential new source of primary or cross-allergenicity sensitization to lupins (Jimenez-Lopez et al., 2018). Several of them exhibited differential and variable expression among lupins species and cultivars (Foley et al., 2011, 2015), as well as qualitative and quantitative IgE-binding differences (Jimenez-Lopez et al., 2018).

The study of β -conglutins allergy at molecular level is complicated because this family of proteins (a) is composed of different isoforms with high structural variation compared with other lupin protein families as α , γ , or δ -conglutins or other species of vicilin-like proteins, which may increase the number and potential types of epitopes (Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). On the other hand, this feature makes β -conglutin family outstanding as biomarkers (Lima-Cabello, Mrani-Alaoui, et al., 2017) for species/cultivar identification and for screening parental lines of low allergenicity in breeding programs; (b) exhibits a complex polypeptide composition, with over 25 polypeptide chains with no disulphide bridges, covering a broad range of molecular masses (Mr 15–80 kDa; Foley et al., 2011, 2015; Jimenez-Lopez et al., 2018). Many of these polypeptides were not previously described as potential allergens in species from the sweet lupin group until recently (Jimenez-Lopez, Foley, et al., 2017; Jimenez-Lopez et al., 2018); (c) the putative combinations of different subunits defining trimeric or quaternary (multimeric) structures of β -conglutin proteins in different *Lupinus* cultivars and species (Magni et al., 2007) multiply the allergenic potential and possibilities.

Therefore, epitome characterization of β -conglutin isoforms has been achieved for the first time by using experimental and computational biology-based approaches, data, and databases (vectorial and machine learning algorithms) making possible to identify several specific, commonly shared and cross-reactive potential allergy-related epitopes in lupin seed proteins as β -conglutin family (Jimenez-Lopez, 2015; Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). Lineal and conformational B- and T-cell epitopes variability has been revealed, along with microheterogeneities present in fundamental residues directly involved in IgE-binding allergenic epitopes (Jimenez-Lopez et al., 2015; Lima-Cabello et al., 2016). This may be one of the main contributors to the observed differences in cross-reactivity among legumes analyzed.

β -Conglutin proteins are difficult to purify from natural sources such as species/cultivars lupin seeds; furthermore, isolations are often contaminated with other globulins (α , γ , and/or δ). Separation methods can hardly isolate all members of individual families and/or individual isoforms from each family of conglutins, that is, individual isoforms of the seven β -conglutins. For these reasons, high-purity individual recombinant β -conglutins proteins are an invaluable tool for studying lupin molecular allergy. This constitutes a reliable and accurate molecular tool helping to develop immunological test systems able to identify and quantify these allergens in foodstuffs (Lima-Cabello, Alché, & Jimenez-Lopez, 2019), and for diagnosis and alternative therapies development for food allergies.

The successful purification of these recombinant β -conglutin proteins has allowed studying their IgE-binding capacities compared with the natural proteins from lupin in both species and varieties (Jimenez-Lopez, Foley, et al., 2017; Jimenez-Lopez et al., 2018; Lima-Cabello, Alché, & Jimenez-Lopez, 2019). Furthermore, these comparisons provide basic knowledge for further research in diagnosis and immunotherapy in food allergy, which is just emerging. In this regard, current diagnosis of food allergy among atopic subjects is currently being developed by skin prick tests with general seed extracts (including

lupin) or using ImmunoCAP monospecific for the detection and quantification of IgEs raised to a limited number of seed storage proteins from legumes, cereals, and nuts. Another more plausible alternative still to be developed is a multiplexing ELISA that would substitute for the monospecific ImmunoCAP, on the basis of recombinant proteins.

In this regard, recombinant β -conglutins could contribute to develop a molecular tool to be implemented in the identification and quantification of specific allergy IgEs-related in lupin-sensitized subjects while constituting a new method for the identification and quantification of Lup an/Lup a 1 main lupin allergen in foodstuffs in both natural and processed (roasted, fermented, boiled, cooked, pickled, toasted, pasteurized, etc.) as it was recently demonstrated by Lima-Cabello, Alché, and Jimenez-Lopez (2019). This development using purified β -conglutin proteins would also make possible to study the complicated pathology that is food allergy by identifying new patients' individual sensitization patterns of lupin proteins and to understand geographical differences in clinical reactivity and cross-reactions to seemingly distant allergens. This recombinant protein (β -conglutin) could also constitute to an accessible development for the quantification of food allergens; thus, it should be approved as (certified) reference material for food allergen identification (Lima-Cabello, Alché, & Jimenez-Lopez, 2019), and helping to implement the European Union mandatory labelling (Directive 2006/142/EC).

Furthermore, β -conglutins may be implemented as possible sources of desensitization in food allergy therapies whether meeting the appropriate prerequisites, allowing these recombinant allergens to be used for these procedures (a) to identify possible food allergens (the main family or group of allergens) and (b) to have them available in recombinant forms, which could be facilitated by genome sequencing projects, that is, *L. angustifolius* (<http://www.lupinexpres.org>), and (c) these recombinant peptides will exhibit the same IgE-binding capacity as the natural analogues (d) and a homogeneously IgE response on a large population.

Two recently published studies on allergic responses to recombinant NLL β -conglutin proteins (Jimenez-Lopez et al., 2018; Lima-Cabello, Alché, & Jimenez-Lopez, 2019) would contribute to a more reliable and operative control of allergens by the food industry, the regulatory agencies and clinicians, thus helping in protecting the health of sensitized/allergic consumers.

6 | CONCLUSION AND PERSPECTIVE

NLL β -conglutins are demonstrated multifunctional proteins with special structural features that make them unique proteins among Cupin superfamily: They are not only involved in defence against pathogens of fungal nature but also acting as signaling molecules and functional regulations of underlying processes driving seed germination.

NLL β -conglutins may be a new source of nutraceutical proteins with health benefits making them excellent candidates as functional food; however, their allergenic potential has to be considered for this purpose. In this sense, recombinant NLL β -conglutins have

demonstrated to be an innovative molecular tool for the identification and quantification of lupin allergen proteins in natural and processed food.

Much work is still ahead to be accomplished to unveil the domain(s) of NLL β -conglutin proteins implicated in their multifunctionality, which may benefit from molecular docking developments, direct mutagenesis, and recombinant technology. Furthermore, it is still to be discovered the type of control exerted by NLL β -conglutins over the molecular mechanisms implicated in the defence against diverse pathogens, and in the oxidative metabolism driving seed germination.

A big effort has been done in the last few years in functional nutraceuticals and molecular allergy research on NLL β -conglutins. The next level of research in these fields has to be achieved on in vivo models in order (a) to check whether NLL β -conglutin proteins are able to improve health properties at molecular and physiological level and (b) to investigate the potential allergenic reactions of individual conglutin proteins and how to manage the safety improvement of them. All this knowledge could be used in the implementation of these proteins to make functional foodstuffs without consumers risks.

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CONFLICTS OF INTEREST

The author has declared that no competing interests exist.

AUTHOR CONTRIBUTION

JCJ-L is responsible for the conceptualization; investigation; data analysis; resources; writing—original draft preparation, review, and editing—of the manuscript; and funding acquisition.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in NCBI (<https://www.ncbi.nlm.nih.gov/>) and Uniprot (<https://www.uniprot.org/>) databases, and references Foley et al., 2011 and 2015.

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