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CHITIN-BINDING PROTEINS FROM *MORINGA OLEIFERA* (MO-CBPS): STRUCTURAL CHARACTERISTICS, ANTIFUNGAL MECHANISMS, AND PROSPECTS FOR APPLICATION IN PLANT IMMUNITY AND PHYTOPATHOGEN CONTROL

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Abstract *Moringa oleifera contains bioactive chitin-binding proteins (Mo-CBPs) that contribute to plant defense and possess significant biotechnological and therapeutic potential. Several isoforms, including Mo-CBP2, Mo-CBP3 and its variants, and Mo-CBP4, exhibit remarkable thermostability and strong antifungal activity. These proteins inhibit fungal growth through chitin binding, membrane disruption, oxidative stress induction, and metabolic interference. Beyond antifungal functions, Mo-CBPs also demonstrate anti-inflammatory and antinociceptive properties. Their stability, effectiveness, and low cytotoxicity make them promising candidates for crop protection, plant immune enhancement, and pharmaceutical applications. Continued research may support their development as biofungicides and therapeutic agents.*

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Introduction

Moringa oleifera, often referred to as the "*miracle tree*," thrives in tropical and subtropical climates for its tremendous nutritional, medicinal, and industrial potential. Seeds, leaves, pods, and flowers of the plant are rich sources of a variety of bioactive compounds, including phenolics, glucosinolates, vitamins, and minerals, along with a plethora of biologically active proteins (Arshad et al., 2025). Seed proteins, in particular, have gained attention as they play a dual role as both a source of nutrients and as part of the plant's natural defense mechanism. These proteins have been reported to have protective roles against microbial pathogens (Panova et al., 2025). More recently, CBPs from *M. oleifera* seed have been identified as a potential new family of antifungal proteins. Chitin, a β-(1→4)-linked polymer of N-acetylglucosamine, is a key component of fungal cell walls and a widely conserved microbial-associated molecular pattern that is recognized by the plant immune system (Gifoni et al., 2012b). CBPs are important in plant defense, as they can directly bind to and interact with fungal cell walls, leading to cell wall degradation, preventing spore germination, or disrupting hyphal growth. These attributes make CBPs promising molecules for the development of sustainable antifungal agents, especially in the face of the growing resistance to chemical fungicides (Garcia et al., 2019). *Mo*-CBPs were initially discovered during the search for antifungal proteins in seed extracts. One of these, *Mo*-CBP3, has been very well studied and is a well-known antifungal protein (Wan et al., 2008).

Amongst these, *Mo*-CBP3 has emerged as an antifungal protein and is the most well-studied chitin-binding protein from *M. oleifera* seeds (Pentecost, 2013). *Mo*-CBP3 has been shown to effectively inhibit the germination and mycelial growth of phytopathogenic fungi, whilst being highly resistant to thermal and pH denaturation. These characteristics highlight its stability and potential for use across a range of environments(Squeglia et al., 2017). A notable aspect of *Mo*-CBP3 is its classification as a member of the 2S albumin family of seed storage proteins. This classification highlights a notable case of functional diversification since 2S albumins are typically considered as storage proteins involved in nutrient storage during seed maturation (Batista et al., 2014). However, *Mo*-CBP3 shows that these proteins can take on other functions, such as plant defense. At the structural level, *Mo*-CBP3 maintains typical

characteristics of 2S albumins, such as a conserved eight-cysteine motif that forms disulfide bridges, which are important for its small size and stability. At the same time, it possesses an unusual chitin-binding activity not typically found in this family of proteins, which may have evolved to confer antifungal activity (Freire et al., 2015b).

At the molecular level, *Mo*-CBP3 is transcribed from several isoforms with high sequence similarities, which may play a role in its functional diversity (Aguiar et al., 2023). It is produced as a precursor protein with a signal peptide, suggesting its involvement in the secretory pathway, and post-translationally processed to produce two disulfide-linked polypeptide chains. These features, along with its basic nature, likely contribute to its binding to negatively charged fungal cell wall components and its antifungal property (Branco et al., 2022). This review aims to provide a comprehensive overview of *Mo*-CBPs, with particular emphasis on *Mo*-CBP3. We integrate available information about their molecular nature, gene structure, structural characteristics, and post-translational modification. Particular emphasis is placed on their antifungal activity and on postulated mechanisms of action, such as chitin binding and binding to fungal cell walls. Furthermore, we discuss recent evidence of other biological functions, including anti-inflammatory and antinociceptive properties, to illustrate their multifunctional properties. Finally, we identify current knowledge gaps and highlight future directions for research to improve the use of Mo-CBPs as antifungal agents in eco-friendly agriculture and as inducers of plant immunity.

CLASSIFICATION AND MOLECULAR IDENTITY OF MO-CBPS

Overview of known isoforms

The literature describes a number of chitin-binding isoforms in *M. oleifera* seeds, including *Mo*-CBP2, *Mo*-CBP3 and its sub-isoforms, and *Mo*-CBP4. Their purification typically involves salt extraction, ion-exchange, and affinity chromatography, with the latter relying on their chitin-binding ability (Gifoni et al., 2012a; Leite Pereira et al., 2011). These isoforms represent different types of chitin-binding proteins, with minor differences in molecular mass, chitin-binding affinity, and antifungal activity. The chitin-binding isoforms from *Moringa oleifera* seeds possess distinct structural and functional properties that play a role in their biological activity. They differ in their molecular mass, which affects their stability and binding to chitin. Variability in the binding affinity of the isoforms arises due to their particular ability to bind chitin, which is an important aspect in giving rise to their antifungal activity. Variation among the isoforms enables M. oleifera seeds to fight against different kinds of fungi(Freire et al., 2015a). Isolation of chitin-binding proteins is done through different methods that take advantage of the unique physical and chemical characteristics of these proteins. The first method used for this purpose is salt extraction; this method is helpful in extracting the protein from the seed. The second method used is ion-exchange chromatography, through which they are separated using their charges. Binding affinity can be used by affinity chromatography to separate the protein from others.

Sequence and structural family

Among other types, Mo-CBP3 is the best studied type of chitin-binding proteins. It belongs to the group of 2S albumins, and it is characterized by a certain number of cysteine residues, eight in particular (Freire et al., 2019). The importance of this feature lies in its ability to create disulfide bridges, which provide protein stability in various conditions. Mo-CBP3 is formed as a preproprotein, which consists of several peptides that are cleaved to form the mature protein. Even though Mo-CBP3 has some sequence similarities with classical storage proteins, its functional activity is quite different and is expressed in chitin-binding capacity and anti-fungal behavior, indicating an evolutionary shift towards giving additional protective properties to the seed besides the storing ones (de Oliveira et al., 2017). The unusual functional abilities of Mo-CBP3 when compared to traditional storage proteins indicate its multifunctionality in the process of seed development: despite their structural resemblance to traditional storage proteins, Mo-CBP3 also binds to chitin, thus actively contributing to the plant's natural defense mechanism (Wong et al., 2023). The presence of the cysteine residues in Mo-CBP3 provides its ability to create a chitin-binding pocket. Due to these peculiarities, Mo-CBP3 effectively interacts with fungi, suppressing fungal development and reproduction. This makes Mo-CBP3 a suitable subject for further biotechnological studies aimed at improving plant protection against fungi (A.Hamid et al., 2025).

Biochemical and biophysical attributes

The Mo-CBPs, generally found to be between 13 and 17 kDa in molecular weight, have a basic isoelectric point that makes it easier for them to interact electrostatically with negatively charged components of fungal cell walls, such as chitin and glucans. This is critical to their antifungal activity, as it ensures that they bind to the surface of the fungal cells, potentially leading to destruction of the cell wall and inhibition of fungal growth. The ability of the Mo-CBPs to bind to the surface of the cells is further complemented by their physical properties because they are proteins of the 2S albumin family, which are known for having relatively small and stable structures(Souza, 2020). It is evident from the stability of Mo-CBPs to both heat and pH levels that their structures are highly stable. The stability of their structure makes it possible for the Mo-CBPs to remain functional despite environmental stress.

EXPRESSION, LOCALIZATION, AND SEED-DEVELOPMENT DYNAMICS

Expression pattern during seed maturation

Mo-CBPs demonstrate unique temporal dynamics of expression during seed development as they are absent in the early developmental stages of seeds but are expressed in the mid-late stage development (Wobus and Weber, 1999). A steady rise in the number of Mo-CBPs begins approximately 60 days after anthesis (DAA), which marks the period characterized by a range of physiological and biochemical changes leading from seed growth to maturation. Thus, it may be suggested that Mo-CBPs play some role in the formation of physiological processes typical for seed maturation, such as the accumulation of reserve compounds, the emergence of the ability to endure desiccation, and the ability to enter the dormancy state until the moment when all requirements for seed germination will be met. The dynamics of Mo-CBPs expression until about 90 DAA, i.e., the point at which the maximal accumulation of these proteins occurs, underlines their involvement in later stages of development associated with seed germination processes. It may be presumed that there exist regulatory mechanisms based on both development and hormonal factors, which determine the expression dynamics of Mo-CBPs in order to ensure successful seed germination. The absence of expression of Mo-CBPs in earlier stages is caused by their absence due to the need for proper coordination of the development process and the absence of seed germination-related functions.

Behavior during germination

In the case of Mo-CBPs expression dynamics during seed germination, the steady decrease in the concentration of these proteins may be interpreted as a consequence of their consumption. These proteins are degraded in order to provide the embryo with amino acids and other nutritional elements. Mo-CBPs accumulate in the seed tissues during the period preceding germination, as was revealed by immunolocalization studies, which demonstrated their localization in certain tissues involved in seed dormancy(Ramakrishna, 2007). The presence of Mo-CBPs in seed tissues is linked to the need to protect the seed from soil pathogens, including fungi. By interacting with chitin, Mo-CBPs presumably protect the seed from pathogens, thereby ensuring its successful germination.

Functional significance

Based on the temporal expression pattern of Mo-CBPs, the multifunctional role of the Mo-CBPs is apparent in the different physiological roles played during the process of seed development and seedling growth. In this case, as the seeds mature, Mo-CBPs serve as storage proteins that supply growing embryos with the necessary amino acid requirements and metabolic energy needed for germination and development after sprouting. This process is very important for seed survival since the reserve provides the basis for metabolism in the presence of low nutrient availability. Moreover, the temporal expression pattern of Mo-CBPs occurs at the same period as nutrients are stored in seeds and thus reflects its physiological function in nutrient accumulation as an important aspect of resource allocation (Lukasik et al., 2013). Mo-CBPs have further been shown to be involved in protecting the seeds against pathogenic attack during the periods of dormancy and germination, when seeds are most susceptible to such attacks. This approach is an important mechanism used in prioritizing seed growth while simultaneously maintaining defense against pathogens in order to conserve energy resources (Bouaziz et al., 2015).

STRUCTURAL PROPERTIES OF MO-CBPs

Secondary structure features

The investigation of Mo-CBPs using circular dichroism gives some insight into the secondary structures of Mo-CBPs. According to these studies, it is evident that Mo-CBPs have a secondary structure made up of α-helices, β-sheets, turns, and unordered residues. The existence of a secondary structure made up of different structures helps in giving the protein different physical properties that enhance its stability. The intrinsic hydrogen bond present in the secondary structures, such as α-helices and β-sheets, contributes to the stability of the structure of the protein, while turns and unordered structures make the protein flexible enough so that it can change its structure according to environmental stimuli (Bansal et al., 2016). The varied secondary structure is what makes Mo-CBPs very stable proteins because they can withstand different extreme environmental changes, such as temperature, pH, and salt concentrations (Belova et al., 2018). The existence of both flexible and stable parts of the secondary structure might be the cause of both stability and flexibility of the protein to ensure that the activity or binding function of the protein is maintained. Therefore, the varied secondary structures in the protein account for the stability of the proteins that make them active under different environments(Shaw et al., 2021).

Disulfide bonding and stability

The eight cysteines present in 2S albumins provide the basis for the formation of several intramolecular disulfide bridges that contribute to the covalent binding and shaping of the protein. The presence of these disulfide bridges confers significant structural rigidity to the protein by restricting its flexibility and thus reducing the probability of denaturation or unfolding. The additional structure of rigidity plays an important role in the stability of 2S albumins from proteolytic degradation owing to the presence of tightly packed and bound protein (Shewry et al., 1995) (Figure 1). Moreover, the formation of disulfide bonds is involved in increasing the longevity and activity of 2S albumins in harsh environmental conditions, including pH alteration, high temperatures, and oxidative stress. This way, the protein gets stabilized and retains biological activity for prolonged periods of time. Thus, eight cysteines

and disulfide bonds are critical factors behind 2S albumin stability (Martínez et al., 2020).

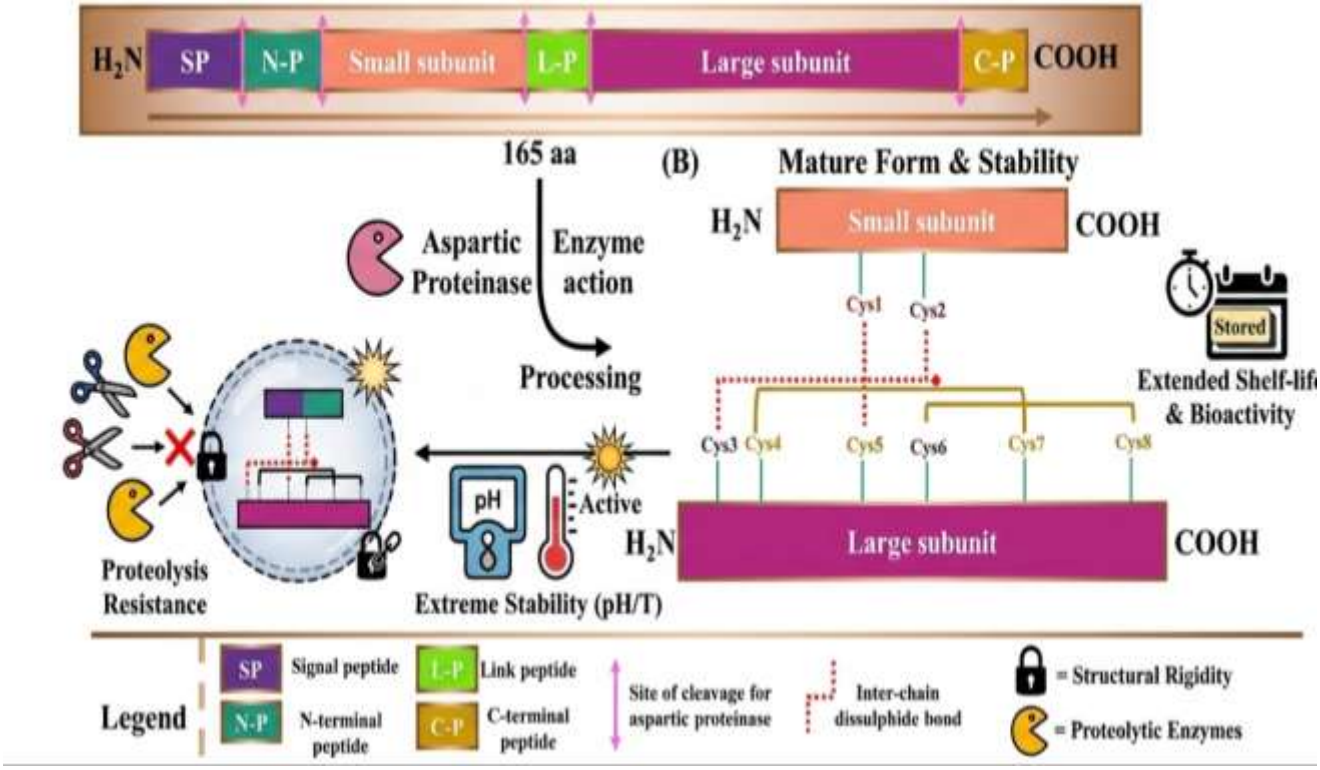

Figure 1. Schematic representation of the structural organization, proteolytic processing, and stability mechanisms of 2S albumin proteins

ANTIFUNGAL AND ANTIMICROBIAL ACTIVITY: SPECTRUM AND POTENCY

Activity against plant pathogenic fungi

Mo-CBPs have been shown to exhibit potent antifungal activity against multiple plant pathogenic fungi such as Fusarium spp. and Colletotrichum spp., with the antifungal activity being concentration dependent. In other words, high levels of Mo-CBPs will enhance the ability of the molecules to inhibit the growth of fungi. The antifungal activities of Mo-CBPs could be attributed to their interactions with the fungal cell wall or membrane, thereby affecting important physiological functions required for the growth and propagation of fungi (Terras et al., 1995). Furthermore, the wide-spectrum antifungal properties of Mo-CBPs indicate a complex mode of action. The fact that inhibition is dependent on concentration implies that the variation in the concentration of Mo-CBPs can be used to modulate antifungal properties in both agriculture and biotechnology. Similarly, the fact that Mo-CBPs retain their antifungal properties even when subjected to environmental extremes discussed above implies that they can be applied as fungicides in agriculture or for the protection of crops (Crockatt, 2012).

Fungistatic vs fungicidal effects

When the Mo-CBPs concentrations are low, the Mo-CBPs exhibit fungistatic effects on the fungi. The ability to stop fungal growth but not kill the cells at lower Mo-CBPs concentrations might interfere with vital biological functions within fungi, such as the stability of cell walls or membranes. The increased concentration of Mo-CBPs causes higher fungistatic activity, leading to fungicidal activity. At high Mo-CBPs concentrations, the substances can completely prevent the growth of the fungi or directly kill the spores. Therefore, at these concentrations, the substances will kill the pathogens effectively(Petre et al., 2015).

Activity against opportunistic human pathogens

The antifungal activity of *Mo*-CBP2 against *Candida* species not only expands its antifungal spectrum but also suggests its potential use in the medical and pharmaceutical industries as well as in agriculture. *Candida* is a major group of opportunistic human pathogens, causing a variety of infections from *Mucosal candidiasis* to systemic *candidemia*, and is an important target for antifungal agents. *Mo*-CBP2's antifungal activity against these pathogens suggests it may disrupt common structures and metabolic pathways of fungal pathogens in plants and humans (Larson et al., 2011). This non-specific activity suggests *Mo*-CBP2 is a strong candidate for the development of natural antifungal agents with a range of therapeutic applications for human fungal infections. Its inherent stability suggests that it has the potential to remain active under physiological conditions, making it a potential therapeutic agent (Bernardo de Assis et al., 2013). Furthermore, Mo-CBP2's antifungal action, perhaps by disrupting the membrane or inhibiting important cellular processes in fungi, may offer an alternative means of action

compared to current anti-fungals, thus reducing chances of resistance. In addition, the increased spectrum of activity against human pathogens calls for research into the pharmacodynamics, toxicology, and administration of Mo-CBP2. Such studies will help pave the way for the use of this protein in antifungal drugs or as a template for designing new anti-fungals that are both efficacious and ecologically sound.

Cytotoxicity and hemolysis assessments

Studies have shown that *Mo-CBPs* are both non-hemolytic and non-cytotoxic to mammalian erythrocytes, thus emphasizing their safety and biocompatibility. Such a lack of toxicity is necessary for biomedical applications of the compounds as it demonstrates their safety with respect to red blood cells(Guilhelmelli et al., 2013). It is worth mentioning that the mentioned properties are favorable regarding the incorporation of the compounds into various food-based products, as it is necessary for their safety with respect to human health. Therefore, stability, lack of cytotoxicity, and antifungal activity enable the use of Mo-CBPs in both biomedical and agricultural purposes. Stability, absence of adverse effects on mammalian erythrocytes, and antifungal activity are important properties that make Mo-CBPs promising candidates for natural fungicides in agriculture as well as innovative antifungal drugs in medicine. Consequently, it is possible to investigate formulations and drug delivery methods for further enhancement of target activity and minimizing adverse effects on target organisms(Perlin et al., 2017).

MECHANISMS OF ANTIFUNGAL ACTION

Cell-surface binding

Mo-CBPs primarily attach themselves to the fungal cell wall through electrostatic attraction, where positive charges found on the amino acids in the protein bind to the negatively charged components of the fungus, particularly the polysaccharide component and the chitin molecule. Such electrostatic interactions are crucial in the recognition and attachment between Mo-CBPs and the fungi, leading to their antifungal effect (Field, 2018). Notably, these electrostatic interactions not only ensure selectivity of Mo-CBPs against fungi but also aid in the targeting of the pathogen cell wall. The reversibility and strength of the electrostatic interactions enhance the binding of the CBPs to the fungal cell wall under various environmental conditions, ensuring their stability and extended anti-fungal effects.

One mechanism through which the Mo-CBPs exhibit antifungal effects is the production of reactive oxygen species in the target cells, resulting in damage to multiple cellular components within the cells. Reactive oxygen species (ROS) disrupt metabolic activities by oxidizing important cellular compounds such as enzymes, DNA, and fats, inhibiting their respective functions(Karlgren et al., 2011). Consequently, oxidative stress ensues when ROS exceeds the antioxidant defense of the cell, leading to damage to cellular organelles, cellular membranes, mitochondrial dysfunction, and eventually death of the cell. Thus, ROS-mediated damage is a powerful strategy in destroying fungal cells, complementing the initial interactions of Mo-CBPs with the cell wall and membrane of the target cell. This ROS-mediated effect is an added benefit because it targets inaccessible intracellular components. It could be involved in fungistatic or fungicidal actions based on the concentration of the Mo-CBP, because higher concentrations would result in high ROS production. Finally, due to the stability of Mo-CBPs, ROS induction should prove successful under various physiological conditions, making Mo-CBPs effective and highly useful antifungal agents in agriculture and clinics (Vinodhini et al., 2017).

Membrane disruption and morphological damage

Observations at the microscopic level for fungi subjected to Mo-CBPs have revealed that there is severe damage to the cells, especially the instability of cell membranes, leakage of cytoplasmic contents, and destruction of cell structures. These changes imply that Mo-CBPs affect cell membranes and, therefore, make them lose selective permeability and disrupt essential functions within the cells, resulting in the loss of essential cellular constituents, thereby causing metabolic disorders and ultimately leading to cell death. These changes in cellular structure indicate that the mechanism of action of Mo-CBPs is not only through the disruption of interactions with the cell surface but also by creating structural damage(Lopes et al., 2020). Disruption of cell membrane organization may be due to the interaction of these compounds with cellular constituents. Overall, this study demonstrates the efficiency of Mo-CBPs as an antifungal compound by virtue of their multiple mechanisms against fungi through interaction with the cell surface, generation of oxidative stress, and disruption of cell membranes (Lu et al., 2025).

Metabolic interference

The potential of the Mo-CBPs to inhibit metabolic processes involved in the fungal cells, such as the acidification of the environment, indicates the inhibition of critical functions in the fungal cell membranes, which include inhibiting proton transport pathways essential in the maintenance of the cellular energy and pH levels. The Mo-CBP protein can inhibit proton gradients or proton pumps found in the fungal plasma membrane. These processes are critical in fungal energy production and therefore limit the growth and vitality of fungi (Gassen et al., 1990).

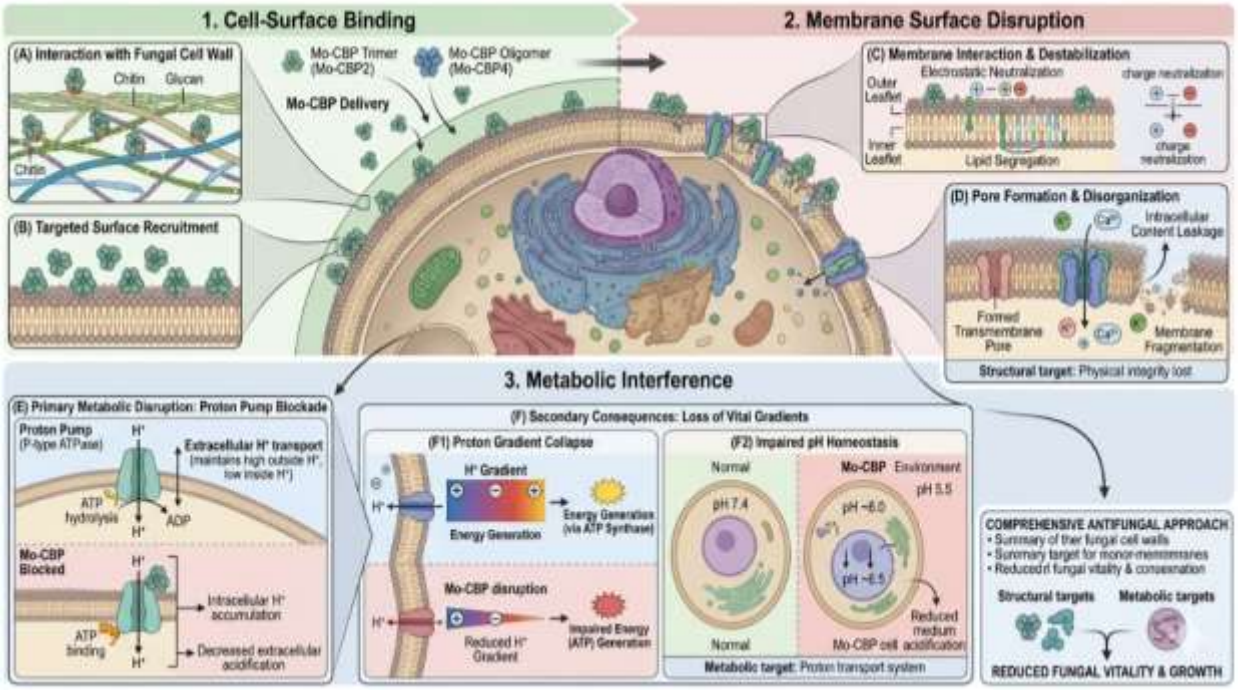

Figure 2. Proposed antifungal mechanism of *Moringa oleifera* coagulant-binding proteins (Mo-CBPs) against fungal cells

The absence of chitinase or glucanase activity further demonstrates that the Mo-CBPs do not degrade cell walls of fungi using enzymatic actions. The antifungal properties of the Mo-CBPs are based on the non-enzymatic inhibition of fungal cells, making the fungal cells unstable. It is important to note that resistance has been a problem with enzymes, but this protein offers an efficient means of inhibiting fungi without the limitation of enzymatic action.

ADDITIONAL BIOLOGICAL ACTIVITIES AND THERAPEUTIC POTENTIAL

Anti-inflammatory effects

The anti-inflammatory and analgesic activities displayed by Mo-CBP4 in animals are indicators of the multitalented nature of Mo-CBPs in addition to their antifungal functions and suggest that Mo-CBPs are capable of undertaking several biological functions. Mo-CBP4 works through the inhibition of vascular permeability, which prevents the leakage of fluids and immune cells into damaged areas while decreasing tissue swelling and injury. Additionally, Mo-CBP4 prevents leukocyte infiltration, which is an important step in the process of inflammation. The protein may do this through interfering with mechanisms involved in guiding immune cells to areas of damage or infection (El Bilali et al., 2024). These findings indicate that Mo-CBP4 may play an important role in controlling excess immune response to prevent further damage to tissue. Anti-inflammatory properties are important considerations when discussing therapeutic uses of Mo-CBP4 to treat conditions related to pain and inflammation, including autoimmune diseases, inflammation resulting from tissue damage, or inflammatory diseases. With such versatile roles, Mo-CBPs are promising candidates for future bioactive product development in the fields of agriculture, biotechnology, and medicine. Future research efforts towards understanding specific target sites and mechanisms of Mo-CBP4 will provide insights that can be used to create Mo-CBP-based treatments for inflammation and pain-related disorders.

Broader pharmaceutical implications

The excellent antifungal efficacy, broad spectrum of activity, stability, and nontoxicity linked to the Mo-CBPs suggest that it is highly likely for these proteins to be used as excellent starting points in developing novel antifungals. The stability of Mo-CBPs means that these proteins will be able to remain active under various environmental conditions, including differences in temperature and pH levels. This stability property means that the Mo-CBPs can be incorporated into a drug formulation that may involve thermal treatment (Abdull Razis et al., 2014). Apart from the broad spectrum of activity and the high efficacy of these proteins, the non-toxicity of Mo-CBPs towards mammalian cells also provides an added advantage. This characteristic, coupled with other properties of Mo-CBPs, suggests that these proteins have good potential for therapeutic application. The stability and durability of the Mo-CBPs against various environmental factors provide an indication that they can be used for different methods of delivery, such as topical, systemic, or agricultural applications. As a result, exploiting the features of these proteins may help develop new antifungals.

Agricultural applications

Due to their antifungal properties, stability, and safety, Mo-CBPs could be used as natural

biofungicides in agriculture. Their ability to inhibit a broad spectrum of phytopathogenic fungi like *Fusarium spp*. and *Colletotrichum spp.* makes Mo-CBPs good candidates as biological control agents in agricultural ecosystems. The utilization of Mo-CBPs as biofungicides would minimize the utilization of conventional fungicides, thereby minimizing the risk of fungal resistance and possible environmental contamination (Montesinos, 2007). In addition, the genetic modification of crops with Mo-CBPs gene encoding sequences is a viable way of improving the plants' antifungal defense system. The expression of Mo-CBPs within the plant would provide in vivo and in situ antifungal activity through direct action on the invading pathogen due to their multifactorial antifungal activity while remaining non-toxic to the host plant and other nontargets. This technology will take advantage of the proteins' stability and high level of bioactivity, irrespective of the environmental conditions.

Comparison with Other Plant CBPs

CBPs are commonly found in various plants such as wheat, barley, maize, and rubber tree; they belong to families such as hevein-like lectins and chitinases, which have carbohydrate-binding domains and catalytic function. Consequently, they actively participate in the physiological activities of these plants. Mo-CBPs, however, stand out because they are seed storage proteins that have acquired the ability of strong antifungal activity, thereby distinguishing them from these classical CBPs. They differ from the other CBPs in that they are stable and have nonenzymatic chitin-binding activity, contrary to the other CBPs, which may require enzymatic activities to hydrolyze the cell wall of fungi. As a result, Mo-CBPs will be able to maintain their structural stability and antifungal activity under challenging environments; also, they will be able to effectively counteract the fungal pathogen using the nonenzymatic mechanism (Moulin, 2019). Their unique nature indicates that they have undergone evolution, whereby seed storage proteins acquired defense capabilities and consequently developed into the current multifunctional protein – Mo-CBP. In summary, this is a good example of biochemical and ecological adaptation, which makes it possible for plants to maximize their resource utilization and improve their survival rate against the fungal pathogens. In this regard, Mo-CBPs may serve as models for multifunctional protein studies and sources of antifungal proteins (Neto et al., 2017).

LIMITATIONS, GAPS, AND CHALLENGES

Limited structural and mechanistic detail

Although significant progress has been made in the study of *Mo*-CBPs, several key gaps limit the full understanding of their structure-function relationships and applications. The absence of structural information, like from X-ray crystallography or cryo-electron microscopy techniques, limits the ability to map the structural aspects underlying the binding and antifungal activity of Mo-CBPs, which would give insights into how different amino acids orient themselves spatially, the arrangement of the disulfide bridges, and dynamics, contributing to structural stability and function of the proteins. With such knowledge, it would be easier to engineer Mo-CBPs to have improved specificity and stability in their bioactivities (Orisawayi et al., 2026). Additionally, current research seems to be focused on in vitro studies of Mo-CBPs' functionality. There is no sufficient insight into their in-planta function, as well as their stability and their interaction with the innate immune system of the transgenic plants. Understanding their role in biological pathways as well as in systemic resistance, and their stability in planta, will give insights into their utility in biotechnology. Moreover, their interaction in a complex environment, such as a cell where protein degradation occurs, and physical properties of the environment vary, is still unknown(Masarkar et al., 2025a).

There is also a safety concern regarding Mo-CBPs. Since they belong to the 2S albumin protein family and have allergenic members, it is important to conduct exhaustive evaluations of allergenicity as well as biosafety. Biosafety assessments must not only ensure human safety but also environmental safety by checking whether they will have harmful effects on the ecology if released into the environment (Masarkar et al., 2025b). Concerning the issue of production, scaling up the production of Mo-CBPs poses problems in ensuring optimal yields, proper protein folding, and post-translational modification that would make them functionally active. In addition, purification of Mo-CBPs can be costly, and their stability in their intended applications might be hard to achieve. These challenges need to be overcome if we wish to apply our research in the agriculture and medical fields (Bekele, 2013).

Conclusion

The Mo-CBPs have unique characteristics such as extraordinary stability and versatility of biological activities, which make them suitable to stimulate plant immunity as well as inhibit plant pathogens. First of all, the high stability of Mo-CBPs in diverse environments, such as different temperatures, pH, and oxidative stresses, is due to the structural properties of Mo-CBPs, like their secondary structure and multiple disulfide bonds, which are features of the 2S albumin family. This unique structural property guarantees not only the high level of biological activity of Mo-CBPs, but also their antifungal activity under harsh conditions in agriculture and in clinical settings. Notably, Mo-CBPs not only possess excellent structural properties but also demonstrate great multifunctionality, which might be utilized. The antifungal property of Mo-CBPs is capable of being employed for producing biofungicides or genetic modification of plants by transferring genes of antifungal Mo-CBPs to produce genetically modified

crops, thus reducing the application of synthetic fungicides to prevent the potential emergence of resistance in fungi. Regarding health and food preservation, the low toxicity as well as antifungal activities of Mo-CBPs against both plant and human fungal pathogens, such as Candida species, make them suitable to be alternative drugs to antifungal medicines. Clearly, such multifunctionalities of Mo-CBPs fit the current world trend towards developing environment-friendly antifungal treatments, considering the high risks associated with drug resistance and environmental problems.

Future directions

The further advancement of studies concerning Mo-CBPs depends on applying advanced methods for analyzing high-resolution structures, such as X-ray crystallography and cryo-electron microscopy. They will provide insights into the complex three-dimensional structure of amino acid residues, disulfide bonds, and conformational alterations involved in chitin binding and antifungal properties. The gathered molecular-level information will contribute to the development of protein engineering technologies capable of enhancing the specificity, stability, and functionality of Mo-CBPs, leading to the rational design of customized variants for industrial and medicinal applications. In addition, more genomic and proteomics studies are required to investigate the diversity of Mo-CBPs in plants and the molecular processes associated with their functioning during pathogen attack. They will provide insights into the functional role of Mo-CBPs in plant immunity and facilitate the identification of suitable candidate genes that could be used in gene transformation experiments to develop transgenic plants expressing Mo-CBPs. Transgenic research will provide valuable information on the efficiency, stability, and potential adverse effects of Mo-CBPs in crops, which will be helpful for translating them into practical uses in plant protection. The safety assessment represents an integral component of translational studies, including allergenicity, cytotoxicity, and ecotoxicity tests. The potential allergenicity of the 2S albumin protein family requires rigorous testing to ensure biosafety for its application in agriculture and medicine. The efficient production of Mo-CBPs is crucial for practical use. It requires the establishment of reliable heterologous expression systems providing optimal conditions for protein folding and purification procedures, preserving antifungal activity along with appropriate formulations stabilizing Mo-CBPs against environmental factors such as photolysis, proteolysis, and denaturation. Furthermore, it is essential to perform studies regarding Mo-CBP delivery systems capable of ensuring their stable presence within the crop and biological system for a prolonged period of time.

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Statements and Declarations

Data Availability statement

All relevant data are within the manuscript file.

Author's Contribution Statement

MZS, MJA, MN, and GZJ collected data and wrote manuscript equally. KH, SARS, SM, NH, SY make final editing. All authors have read the final manuscript and approve its submission.

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The investigation was undertaken without any financial conflicts of interest or any other commercial relationships that could be seen as such by any of the authors.