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RESEARCH ARTICLE

Leaf proteome analysis of maize inbred lines B73 and Mo17 infected by *Fusarium verticillioides* (Sacc.) Nirenberg

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Abstract

The maize plant (*Zea mays* L.), one of the world's most important cereals, produced 1444 million tons globally in 2022. *Fusarium* crown rot, caused by *Fusarium* spp., particularly *Fusarium verticillioides*, significantly impacts maize yield and quality. This study aimed to investigate the proteomic response of two maize inbred lines, B73 and MO17, to *F. verticillioides* infection using two-dimensional electrophoresis. Leaf proteins were extracted using the TCA-acetone method and identified based on their isoelectric points (pI) and molecular weights. A total of 99 reproducible protein spots were detected, with significant expression changes assessed using the T21 spot test. Eight spots in B73 and six in MO17 exhibited increased expression. Defence-related proteins showed the most important proportion of changes (33 %), including the BAG family molecular chaperone regulator six protein, which is involved in programmed cell death and stress response. Proteins related to energy production, photosynthesis, ion channels and signalling showed decreased expression, indicating a possible reduction in plant vigour and efforts to limit pathogen spread. Structural and defence-related proteins demonstrated increased expression, suggesting an adaptive response to fungal infection. The proteomic comparison revealed that B73 exhibited greater resistance to *F. verticillioides* than MO17, as evidenced by the distinct protein expression profiles. This study highlights the role of specific proteins in maize defence mechanisms and provides insights into potential targets for enhancing resistance to *F. verticillioides* infection.

Keywords: biotic stress; 2D-electrophoresis; *Fusarium*; maize; proteomics

Introduction

The continuous growth in global population, especially in developing nations, presents a significant challenge to food security. Maize (*Zea mays* L.) (Fig. 1), one of the world's most important cereal crops in the family *Poaceae*, has inflorescences arranged as panicles and produces caryopsis fruits with endospermic seeds. In 2022, global maize production reached 1444 million tons (1). Technological advances, including hybrid seed production, have been employed to enhance maize resilience in the face of future challenges such as climate change and population growth (2, 3). Maize is susceptible to numerous diseases caused by various pathogens, particularly fungi, which impact its roots, stems, cobs and seeds. Among these pathogens, Fusarium species-mainly *F. proliferatum*, *F. verticillioides* and *F. graminearum*-pose significant threats due to their ability to produce mycotoxins and infect maize at any growth stage, often resulting in substantial crop damage. *F. verticillioides* is the most prevalent pathogenic fungus affecting maize (4, 5).

Plants exhibit complex responses to stress, involving the activation of diverse genes and the synthesis of specific proteins. These responses can trigger a range of physiological and biochemical alterations (6). Advanced biological approaches, particularly omics technologies such as genomics and proteomics, have greatly enhanced our understanding of maize-pathogen interactions. Detailed insights into the molecular mechanisms underlying maize defence responses are crucial for clarifying the genetic foundation of resistance (7). Proteomic analysis offers a powerful tool for studying the proteins involved in maize resistance to *F. verticillioides*. Proteomics is increasingly recognized as a key method in protein study, facilitating genetic engineering and crop improvement (8). Thus, examining the maize leaf proteome in response to this fungal

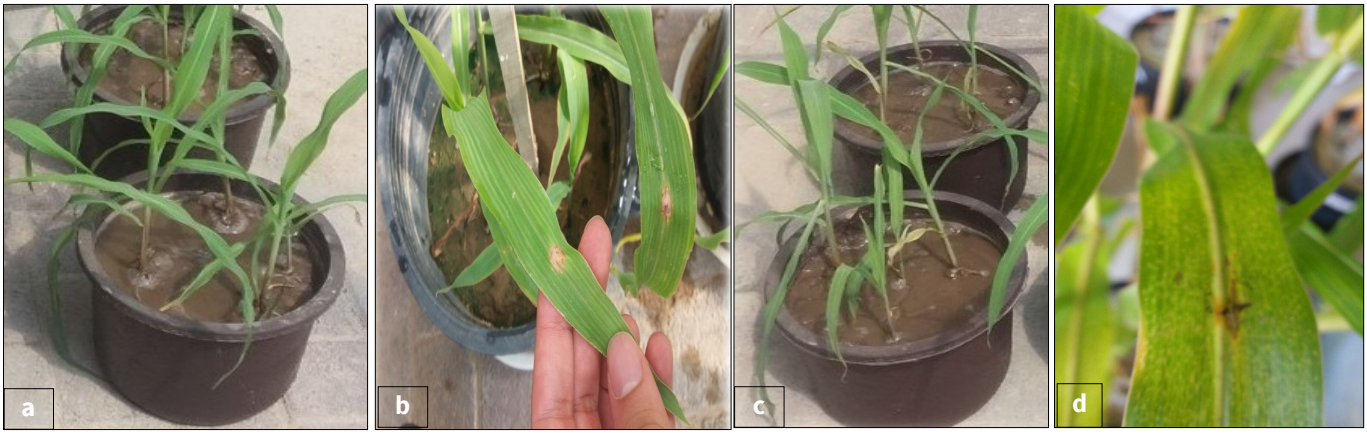

Fig. 1. Maize leaves showing: (a) a healthy leaf of inbred line B73; (b) a *Fusarium verticillioides*-infected leaf of inbred line B73; (c) a healthy leaf of inbred line Mo17; (d) a *Fusarium verticillioides*-infected leaf of inbred line Mo17.

pathogen provides valuable information on plant defence mechanisms that might be useful for developing disease-resistant maize varieties.

Materials and Methods

Plant materials

In this experiment, seeds of maize inbred lines B73 and Mo17 were first washed with distilled water and then disinfected using a 1 % sodium hypochlorite solution. After disinfection, the seeds were placed on a moist cloth to germinate for several days. Once germinated, the seeds were sown in plastic pots filled with autoclave-sterilized soil. This initial soaking and germination step facilitated uniform plant growth and accelerated seedling emergence. Four seeds were sown per pot at a depth of 5 cm, spaced evenly to avoid crowding. Plants were grown in a greenhouse using a randomized complete block design, with two treatments (fungus-infected and control) and each replicated three times. Irrigation was conducted every few days to maintain optimal soil moisture.

The process of pathogen cultivation and plant contamination

The fungal pathogen *Fusarium verticillioides* was cultured on Potato Dextrose Agar (PDA). To ensure purity, the isolate was re-cultured on a 1.5 % water-agar medium, where it developed densely within a few days. Filament tips from the culture were excised with a scalpel and transferred back onto fresh PDA to encourage dense, pure growth, as observed microscopically (9). When the maize seedlings reached the four to six leaf stage, 7 mm diameter fungal plaques were excised from the PDA culture and placed on the central leaf veins and axes of the plants. Multiple plaques were applied per leaf and small wounds were made beneath each plaque to mimic natural damage, such as insect feeding sites (10). To maintain high humidity around the inoculated plants, plastic bags were placed over them to create a moist environment. After approximately 10 days, when initial disease symptoms became visible, leaves were harvested for proteomic analysis and stored at -80 °C.

Two-dimensional electrophoresis and protein extraction

After 30 days, leaf samples were collected from each plant for protein extraction, following the method described (11). Fresh leaf tissue (300 mg) was ground to a fine powder in liquid nitrogen, then mixed with acetone containing 10 % trichloroacetic acid and 0.07 % 2-mercaptoethanol. This mixture was incubated at -20 °C for 45 min, then centrifuged at 15000 g for 25 min at 4 °C. The supernatant was discarded and the pellet was washed in 0.07 % 2-mercaptoethanol in acetone for 60 min. Following a final centrifugation at 20000 g for 5 min at 4 °C, the pellet was washed extensively in acetone with 0.07 % 2-mercaptoethanol, dried and re-suspended in a lysis buffer containing 7 M urea, 2 M thiourea, 5 % CHAPS and 2 mM tributyl phosphine.

The protein concentration was determined using the Bradford method, with Bovine Serum Albumin (BSA) as the standard (12). Protein samples were subjected to two-dimensional electrophoresis, with isoelectric focusing (IEF) in the first dimension (pH 3-10) and SDS-PAGE in the second dimension. Protein spots were visualized using Coomassie Brilliant Blue (CBB) staining and gel images were analyzed using a GS-800 densitometer (Bio-Rad).

Protein identification

The PDQuest software was utilized to analyze protein modifications. Proteins with a quantitative ratio greater than 2 (IF > 2) were classified as up-regulated, while those with a ratio below 0.5 (IF < 0.5) were classified as down-regulated, both with statistical significance ($p < 0.05$). Protein identification was conducted using the Expasy-TagIdent tool (http://web.expasy.org/tagident/) within the UniProtKB/Swiss-Prot database based on matching isoelectric points (pI) and molecular weights.

Statistical analysis

The significance of the results was assessed using a two-sample t-test with three replicates per treatment. Statistical analyses were conducted using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA), with significance set at $p < 0.05$.

Results and Discussion

In this study, we investigated and analyzed the changes in the expression of leaf tissue proteins of inbred B73 and Mo17 maize lines in response to treatment with *Fusarium verticillioides* fungus, utilizing a proteomic approach to identify responsive proteins (Fig. 2). Spots with an intensity factor (IF) greater than two were considered to have increased expression. In contrast, spots with an IF of less than 0.5 were

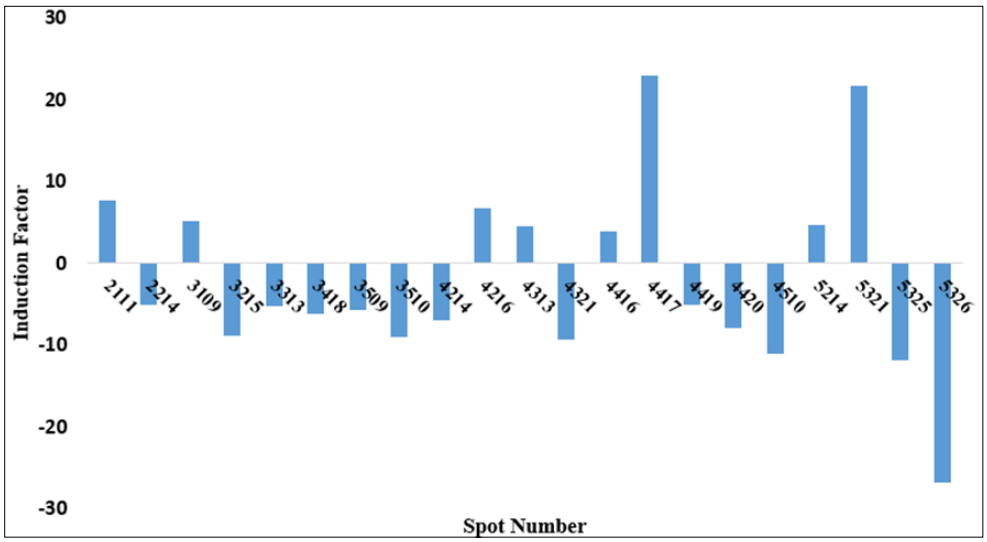

Fig. 2. Changes in the expression of inducible proteins in the leaves of the inbred B73 line as a result of treatment with *F. verticillioides*.

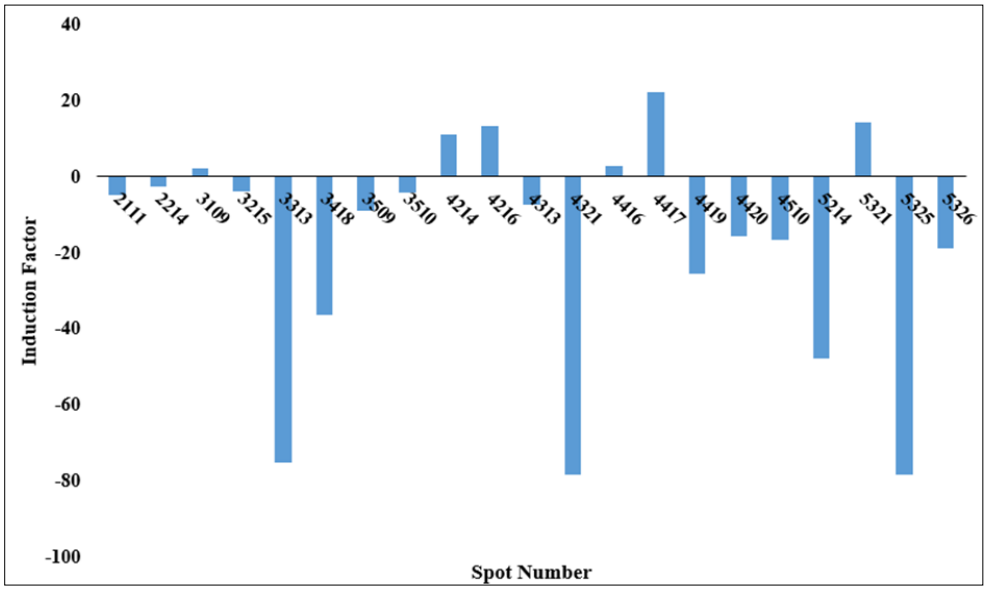

Fig. 3. Changes in the expression of inducible proteins in the leaves of Mo17 inbred line as a result of treatment with *F. verticillioides*.

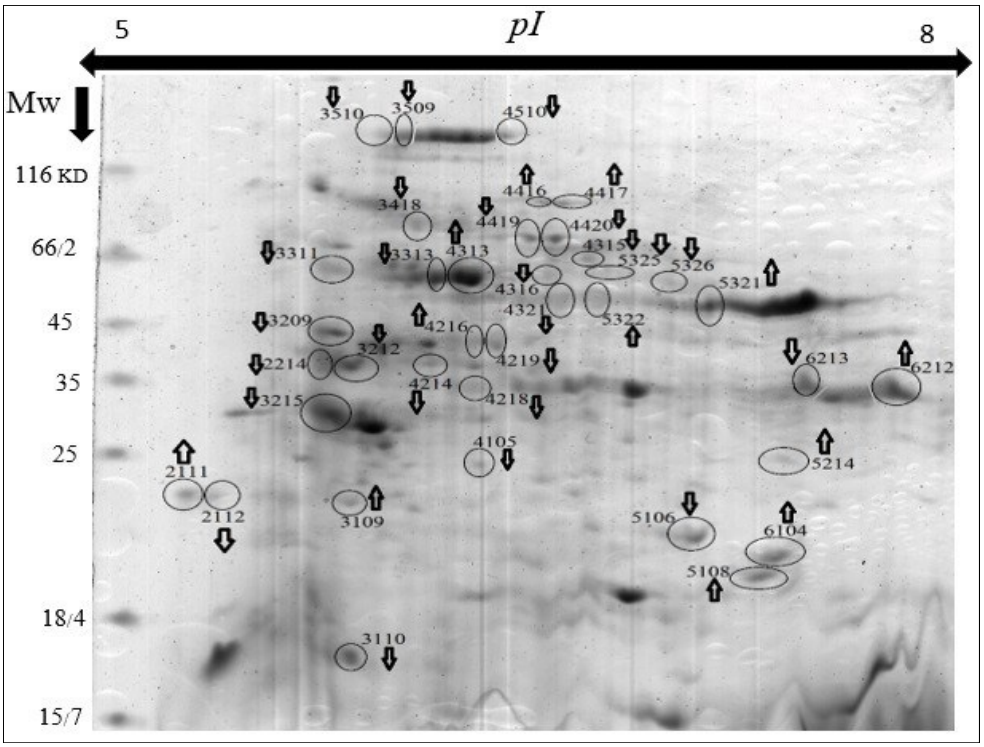

Fig. 4. Two-dimensional electrophoresis pattern of the proteome of the leaf treated with the *F. verticillium*.

categorized as having decreased expression (Fig. 3 & 4). This differential expression provides insight into the specific protein responses triggered by fungal infection in maize leaf tissues.

After thorough image analysis of the gels, 99 repeatable protein spots were identified, among which 21 protein spots exhibited significant changes in expression. This substantial number of differentially expressed proteins highlights the complexity of the plant's response to pathogenic infection. In the inbred B73 line, 8 spots showed an increase in expression, while 13 spots exhibited a decrease in expression compared to the control. Similarly, in the Mo17 inbred line, certain spots indicated an increase in expression, while the remaining proteins showed decreased expression levels (Table 1). These findings suggest that the B73 and Mo17 maize lines exhibit distinct protein expression profiles in response to fungal infection, reflecting potentially varied defence mechanisms or stress responses between these inbred lines.

The protein spots that exhibited expression changes following infection by *F. verticillioides* fungus were further analyzed for possible identification. These proteins were subsequently categorized into several functional groups, which may provide a more targeted understanding of how specific cellular processes and pathways are affected by fungal infection. By grouping the responsive proteins, this study contributes to our understanding of the functional roles these proteins may play in maize's immune response and adaptive mechanisms under biotic stress.

Protein-grouping based on function

Functional groups include proteins involved in energy production (14 %), channel and messenger proteins (28 %), proteins involved in the structure of other proteins (9 %), defence proteins (33 %) and proteins involved in photosynthesis (14 %) (Fig. 5). This categorization highlights the complexity and diversity of protein functions that are critical to sustaining plant health and adaptive responses under stress.

Proteins involved in energy production

Photosystem II CP47 reaction center protein (spot number: 4321) is a component of the central complex of photosystem II, which is connected to chlorophyll and leads to the first chemical process caused by light in photosystem II (13). This protein exhibits decreased expression in wheat plants infected with yellow rust disease, caused by the fungus *Puccinia striiformis*, consistent with the findings on both inbred lines in this research (14). This reduction in expression could suggest an early response mechanism to pathogen invasion aimed at reallocating energy resources away from growth and toward defence.

Table 1. Information on the proteins identified in the leaves of inbred lines B73 and Mo17 treated with *F. verticillioides*

O. *pI*[e]	O. Mw[d]	T. *pI*[c]	T. Mw[b]	Induction factor Mo17	Induction factor B73	Ac. No[a]	Identified Proteins	Spot Number
5.01	18.10	5.01	17.27	0.20	7.61	Q949U7	Peroxiredoxin-2E, chloroplastic	2111
5.13	35.44	5.23	35.24	0.40	0.19	Q43349	Isoform 2 of 29 kDa ribonucleoprotein, chloroplastic	2214
5.19	15.84	5.25	15.79	2.11	5.18	Q9FDX9	Isoform 2 of Guanine nucleotide-binding protein subunit gamma 1	3109
5.17	24.43	5.16	23.42	0.26	0.11	Q9SYW8	Photosystem I chlorophyll a/b-binding protein 2, chloroplastic	3215
5.50	61.42	5.44	62.97	0.01	0.19	O24592	9-cis-epoxycarotenoid dioxygenase 1, chloroplastic	3313
5.83	61.12	5.86	61.17	0.13	4.55	C0LGL9	LRR receptor-like serine/threonine-protein kinase FEI 2	4313
5.5	77.41	5.41	78.58	0.02	0.16	Q39191	Wall-associated receptor kinase 1	3418
5.46	115.67	5.44	111.91	0.11	0.17	Q9LI74	Protein Chloroplast Unusual Positioning 1	3509
5.34	117.96	5.33	113.06	0.23	0.11	Q9FYG2	Calmodulin-binding transcription activator 4	3510
5.68	34.95	5.66	36.10	11.19	0.14	P0C1M0	ATP synthase subunit gamma, chloroplastic	4214
5.86	41.84	5.83	42.57	13.13	6.76	Q9FWQ2	Probable trehalose-phosphate phosphatase 2	4216
6.01	56.29	6.07	56.10	0.01	0.10	P05641	Photosystem II CP47 reaction center protein	4321
5.93	89.62	5.86	85.61	2.87	3.83	O82345	BAG family molecular chaperone regulator 6	4416
5.96	88.77	5.96	92.68	22.32	23.00	F4I1S7	Elongator complex protein 2	4417
5.93	75.10	5.89	76.18	0.03	0.19	A0A1D6LAB7	DEAD-box ATP-dependent RNA helicase 3B, chloroplastic	4419
5.98	74.34	5.97	74.59	0.06	0.12	O22898	Long chain acyl-CoA synthetase 1	4420
5.90	116.37	6.08	118.93	0.06	0.09	D8WUA4	Isoform 2 of Protein translocase subunit SECA2, chloroplastic	4510
6.60	24.02	6.66	24.29	0.02	4.60	Q64J17	Isoform 3 of Protein-L-isoaspartate O-methyltransferase 2	5214
6.52	55.75	6.60	57.94	14.15	21.76	Q5Z9J0	Isoform 2 of Mitogen-activated protein kinase 12	5321
6.17	62.95	6.18	66.07	0.01	0.08	Q7XJJ7	Fatty acid amide hydrolase	5325
6.33	60.65	6.33	61.58	0.05	0.03	Q84N48	CRS2-associated factor 2, chloroplastic	5326

a: access number (taken from TagIdent); b: theoretical molecular weight; c: theoretical isoelectric point; d: experimental molecular weight, e: experimental isoelectric point

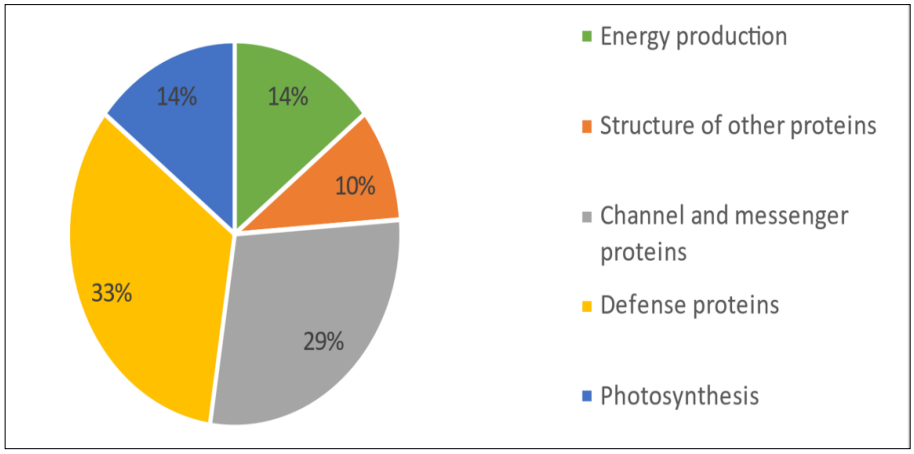

Fig. 5. Functional groups of modified proteins.

ATP synthase subunit gamma, chloroplastic protein (spot number: 4214), is an enzyme that regulates the electrochemical gradient across the thylakoid membrane and directly affects the amount of photosynthetic electron transfer; thus, it represents a key factor in regulating energy conversion in chloroplasts (15). The expression of this spot in the treatment with *Fusarium* fungus in the inbred line B73 showed a decrease in expression, whereas the opposite was true in the inbred line MO17. This differential response between lines suggests a possible genetic basis for energy modulation under stress, where each line exhibits a unique adaptation to fungal invasion.

Photosystem I chlorophyll a/b-binding protein 2, chloroplastic protein (spot number: 3215), traps light energy, necessitating adjustments to light-receiving receptors in response to physiological state and environmental signals. Chlorophyll acts as a photoreceptor in algae and higher plants by binding to the proteins of the light-receiving complexes (LHC) and carotenoids (16, 17). This protein, the most crucial membrane protein in nature, is coded by nuclear genes, synthesized on cytoplasmic ribosomes, traverses the double-layer membrane of the chloroplast and is situated within the thylakoid membrane (18, 19). This protein displayed decreased expression in both B73 and MO17 lines infected with the fungus. The downregulation of such a crucial protein further suggests an inhibition of photosynthetic activity, likely a defensive adaptation by the plant to conserve resources. Given that most proteins involved in metabolism and energy exhibit decreased expression, this is likely due to the pathogen's impact, resulting in reduced energy production within the plant. This reduction may be a strategic response by the plant to slow down growth processes, preserving energy for immune response and potentially enhancing survival under pathogen stress.

Channel and signaling proteins

Peroxiredoxin-2E, a chloroplastic protein (spot number: 2111), is part of a recently identified group of H_2O_2-decomposing antioxidant enzymes. These proteins not only reduce H_2O_2 but also detoxify alkyl hydroperoxides and peroxynitrite, even though they exhibit significant differences in substrate specificity and kinetic characteristics. Due to these functions, peroxiredoxins are likely involved in regulating exolipid-dependent signalling, contributing to broader stress response mechanisms within the plant (20, 21). In this study, the expression of Peroxiredoxin-2E increased in the B73 line compared to the control, while a decrease was observed in the MO17 line. This suggests that genotypic differences may influence the regulation of antioxidant mechanisms under varying conditions.

Isoform 2 of Guanine nucleotide-binding protein subunit gamma 1 (spot number: 3109) plays a critical role in eukaryotic cells by detecting extracellular stimuli. These stimuli are recognized and activated by receptor proteins located in the plasma membrane, such as ion channels and enzymes and the signal is then transmitted to intracellular components. Among the proteins involved in these processes, guanine nucleotide-binding proteins (G proteins) are well studied due to their importance in a range of biological functions, including sensory processes such as sight and smell, as well as the nervous system (22). In *Arabidopsis thaliana*, only a few G protein components have been identified, including one Gα (GPA1; At2g26300), one Gβ (AGB1; At4g34460) and three Gγ (AGG1; At3g63420, AGG2; At3g22942 and AGG3; At5g20635) genes (23). In this study, the expression of this protein increased in both the B73 and MO17 lines, suggesting a potential role in defence signalling, likely facilitating the transmission of defence-related proteins.

The Calmodulin 4-binding transcription activator protein (spot number: 3510) is involved in the expression of CAMTA genes, which respond to hormonal stimuli, including auxin, ethylene, abscisic acid and salicylic acid (24). In *Arabidopsis*, the calmodulin-related transcriptional activator 1 is crucial for the response to auxin signalling, with its expression pattern significantly affected by external auxin (25). In the present research, the expression of this protein was reduced in both the B73 and MO17 lines. This reduction may indicate a specific reaction to pathogenic stress, suggesting a downregulation in signalling pathways associated with stress adaptation.

Chloroplast Unusual Positioning 1 protein (spot number: 3509) plays a key role in chloroplast movement in response to variations in light intensity. The Chloroplast Unusual Positioning Protein (CHUP1) is anchored in the outer chloroplast membrane. It interacts with actin and profilin, coordinating the restructuring of actin filaments to facilitate the relocation of chloroplasts (26). In this study, the expression of CHUP1 was reduced in both lines compared to the control, suggesting downregulation in response to specific

environmental or developmental cues affecting light adaptation.

LRR receptor-like serine/threonine-protein kinase FEI 2 (spot number: 4313) is known to regulate cell wall signaling pathways and cellulose biosynthesis. It may be regulated through a signalling cascade involving 1-aminocyclopropane-1-carboxylic acid (ACC), which is a precursor of ethylene (27). In the current study, the expression of this protein increased in the B73 line, while it decreased in the MO17 line. Interestingly, similar trends have been observed in tomato plants exposed to stress from *Fusarium oxysporum* fungus, where FEI 2 expression decreased (28). This pattern aligns with the findings in MO17, suggesting that the expression of this kinase may be tightly linked to genotype-specific responses to biotic stress.

Isoform 2 of Protein translocase subunit SECA2, chloroplastic (spot number: 4510), is involved in transporting proteins out of the chloroplast. It likely interacts with other proteins to ensure that, after being transported into the inner membrane of plastids, proteins are directed along the correct biochemical pathways. ATP hydrolysis is essential for SECA2's function, facilitating the passage of proteins across the membrane (29). In a study conducted, SECA2 showed increased expression in sugar beet plants infected with the necrotic yellow vein virus (30). This finding contrasts with the present results, where SECA2 expression decreased in both inbred lines. Such discrepancies could be attributed to genotypic variations, emphasizing the complexity of chloroplast-related stress responses. Further research into the specific mechanisms regulating SECA2 expression may clarify its role in different genotypes.

Proteins involved in the structure and function of other proteins

The 9-cis-epoxy carotenoid dioxygenase 1, chloroplastic protein (spot number: 3313) is a key enzyme identified through molecular genetic studies focusing on mutants with impaired abscisic acid (ABA) production in various plant species (31). ABA, a critical phytohormone in plants, is synthesized from zeaxanthin, a 40-carbon carotenoid and plays a pivotal role in the conversion of zeaxanthin to xanthoxin within plastids (32). In the present study, there was a notable decrease in the expression of this protein in the B73 and MO17 maize lines. This reduction suggests a potential downregulation of ABA synthesis pathways in these lines, which may impact their stress response and adaptation mechanisms.

Additionally, Isoform 3 of Protein-L-isoaspartate O-methyltransferase 2 (spot number: 5214) plays a significant role in protein maintenance and repair. This enzyme catalyzes the esterification of methyl L-isoaspartate residues in peptides and proteins, which results in the spontaneous degradation of damaged L-aspartyl and L-asparaginyl residues (33). Such degradation is crucial for the plant's ability to either repair or remove damaged proteins, ensuring cellular homeostasis. Previous studies have shown decreased expression of this protein in wheat subjected to salt stress conditions (34). In contrast, our research observed an increase in the expression of this protein in the B73 line, potentially indicating an enhanced capacity for managing protein damage in response to *Fusarium verticillioides* infection. This upregulation might suggest an adaptive defence mechanism that improves resistance to the pathogen. However, in the MO17 inbred line, the same protein exhibited reduced expression, which may imply a lower defensive capacity and resistance to fungal infection compared to B73.

The differential expression patterns observed between B73 and MO17 lines suggest a distinct genetic basis for stress tolerance and protein maintenance in these maize varieties. This could have significant implications for breeding programs aimed at improving pathogen resistance and stress adaptability. Future research could focus on further elucidating the regulatory networks associated with these proteins to develop targeted strategies for enhancing crop resilience under various stress conditions.

Defense proteins

The Wall-associated receptor kinase 1 protein (spot number: 3418) may serve as a receptor for extracellular matrix signals. Its binding to pectin is likely crucial for regulating cell proliferation, morphogenesis and development. These processes are essential during plant responses to pathogen invasion and in the defence mechanisms against heavy metal toxicity (35). In this study, both examined lines have exhibited decreased expression of this protein, suggesting a potential compromise in their defensive capability.

The Long-chain acyl-CoA synthetase 1 protein (spot number: 4420) plays a key role in activating long-chain fatty acids, leading to the synthesis and breakdown of cellular fats via beta-oxidation. This protein is active in wax and cutin synthesis pathways, which are critical for the plant's protective barriers (36). It appears to have a specific activity towards very long-chain fatty acids (VLCFA class), which are fatty acids containing more than 24 carbons (37). In the current study, a decrease in expression of this protein was observed in both maize lines, potentially impacting the integrity of the plant's surface wax and cuticle.

The Fatty acid amide hydrolase protein (spot number: 5325) degrades the bioactive amides of fatty acids into their constituent acids, terminating the functional signalling activities of these molecules (38). This enzyme converts N-acetyl ethanolamine (NAE) to ethanolamine, which may play a role in abscisic acid signalling and other plant defence mechanisms, aside from its catalytic activities (39). Previous research reported an increase in this protein's expression in *Arabidopsis* under stress from various strains of *Pseudomonas syringae* (40). However, contrary to those findings, the data in this study show a decrease in expression in both B73 and MO17 lines, suggesting a potential vulnerability to pathogen attack. This observed reduction in expression across the studied defence proteins may be linked to the increased sensitivity of both inbred maize lines, B73 and MO17, to the fungal pathogen *Fusarium verticillioides*.

The Isoform 2 of Mitogen-activated protein kinase 12 protein (spot number: 5321) is implicated in the defense signaling pathway. It activates transcription factors, enhancing their DNA-binding affinity to GCC box promoters in pathogen-related genes under in vitro conditions (41). According to research (42), expression of this protein increased in wheat

exposed to stress from the *Septoria tritici* fungus, even before deep fungal penetration. This observation aligns with the results of this study, which noted an increase in expression in both B73 and MO17 lines, indicating a potential early response mechanism against pathogen attack.

The BAG family molecular chaperone regulator 6 protein (spot number: 4416) is involved in regulating multiple cellular pathways, including programmed cell death and stress response (43). It plays a crucial role in basal plant resistance (44). The observed increase in its expression in both lines might be attributed to its role in bolstering the plant's resistance to disease. This suggests that the enhanced expression of this protein is a key element in activating the plant's defense pathways.

The Probable trehalose-phosphate phosphatase 2 protein (spot number: 4216) is linked to the biosynthesis of the non-reducing disaccharide trehalose. This pathway involves T6P synthetase (TPS), which synthesizes T6P from UDP-glucose and glucose-6-phosphate (45). An increase in the expression of this protein was noted in both studied lines, potentially indicating an elevated capacity for carbohydrate management under stress conditions.

The Elongator complex protein 2 (spot number: 4417) functions as a subunit of the RNA polymerase II elongation complex, a histone acetyltransferase, associating with RNA polymerase II during the elongation phase of transcription. This protein also participates in oxidative stress signalling and is involved in preventing abscisic acid (ABA) signalling, which would otherwise cause stomatal closure, thereby inhibiting germination and growth (46). Furthermore, it plays a role in suppressing anthocyanin accumulation and accelerates the induction of defence-related genes, thereby contributing to both basal and induced resistance mechanisms (47). The increase in expression observed in this study across both maize lines likely reflects an active response to oxidative stress and pathogen presence, enhancing the plant's defensive capabilities.

Proteins involved in photosynthesis

Isoform 2 of the 29 kDa ribonucleoprotein, chloroplastic protein (spot number: 2214), plays a crucial role in the normal development of the chloroplast under low-temperature stress conditions by stabilizing specific mRNAs (48). In the current study, this protein exhibited decreased expression in both tested lines, indicating a potential impairment in chloroplast development under stress. Another protein expressed in both the MO17 and B73 lines is the DEAD-box ATP-dependent RNA helicase 3B, chloroplastic (spot number: 4419). This protein serves as a nuclear-encoded genomic factor involved in the production of chloroplast ribosomes. It is essential for the maturation of rRNA in plastids and likely cooperates with the large subunit of the ribosome (50S). Its role is critical for normal chloroplast growth (49). Interestingly, this protein has been shown to exhibit increased expression in banana plants under infection with *Fusarium oxysporum*, suggesting a possible protective mechanism or adaptation in response to pathogen attack (50).

Additionally, the CRS2-associated factor 2, chloroplastic protein (spot number: 5326), plays an essential role in connecting group IIB introns in the chloroplast. This factor performs this function by shaping connecting parts (introns) and establishing interactions with RNA, thereby enhancing the binding properties of introns (51). In the present study, this protein also exhibited decreased expression in both lines. This reduction in expression may be attributed to a decline in photosynthetic pigments, including chlorophyll, during the infection process. Such a decrease in chlorophyll could lead to impaired photosynthesis, further exacerbating the stress on the plants. Overall, the changes in expression levels of these proteins highlight their critical roles in photosynthesis and stress responses, suggesting avenues for further research into the molecular mechanisms underlying these processes.

Conclusion

Proteome analysis of the leaf tissue of inbred B73 and MO17 maize lines through two-dimensional electrophoresis and staining with Coomassie blue led to the discovery of 99 reproducible protein spots that had significant expression based on the T21 spot test. Eight spots in inbred line B73 and six spots in inbred line MO17 showed increased expression. Most of the proteins involved in energy production showed a decrease in expression due to the treatment with *Fusarium verticillioides*, which is a direct effect of the infection with the pathogen. Among the defence proteins, three proteins exhibited decreased expression, including the wall-associated receptor kinase one protein, while four other proteins showed increased expression. The BAG family molecular chaperone regulator six protein is a member of this group and is involved in programmed cell death and stress response. The highest amount of expression (33 %) occurred in this group of proteins. Among the channel and signalling proteins, three proteins had decreased expression, which may prevent the spread of pathogenic fungal proteins in cells or organs by reducing their expression. In the proteins involved in photosynthesis, all the proteins displayed decreased expression, which is likely due to the pathogenic effect on the plant. Furthermore, the decline in photosynthetic proteins may result in reduced overall plant vigour. According to the comparison of the increase in expression between the two inbred lines, it seems that the B73 line has shown more resistance to *F. verticillioides* than the MO17 line.

Authors' contributions

AB conceptualized and designed the study, conducted experiments, performed formal and data analysis, edited tables and figures, contributed to drafting and revising the manuscript, provided supervision and co-managed the project. MAH co-conceptualized and designed the study, assisted in experimentation, contributed to figure and table editing and participated in manuscript writing and review. PRO took part in experimentation, contributed to formal analysis, drafted sections of the manuscript and participated in manuscript review. SM was involved in experimentation and helped draft the manuscript. SA assisted in experimentation data analysis and contributed to manuscript writing. RHG carried out disease analysis and

contributed to treatment-related work. FBM performed disease diagnosis and treatment procedures, participated in drafting the manuscript and managed project administration. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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