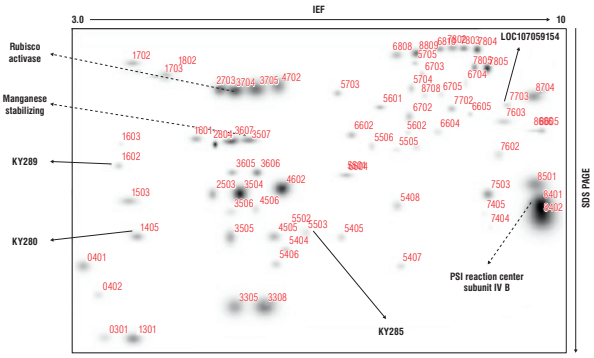
Doi: https://doi.org/10.19053/uptc.24223719.19193

Proteomics reveals an association between water stress severity in potato and photosynthetic proteins

Estudios proteómicos revelan la asociación entre la severidad del estrés hídrico en papa y las proteínas fotosintéticas



Differentially expressed proteins in potato plants under water stress conditions.

Source: Autors

ELÍAS A. SILVA-ARERO[1, 2]
ANGELA M. CASTAÑO-MARÍN[1]
LIZ P. MORENO-FONSECA[2]
MARÍA C. LONDOÑO-MÉNDEZ[1]
LAURA M. TORRES-DELGADO[2]
OSCAR D. OCAMPO-PÁEZ[1]
FABIÁN L. RUEDA-ALFONSO[1, 3]

ABSTRACT

Water stress is among the most critical factors limiting plant performance. Proteomics serves as a powerful tool for assessing changes in protein expression and identifying mechanisms underlying drought stress tolerance in plants. This study aims to identify proteins associated with water stress severity in potato (*Solanum tuberosum* L.) and to characterize their differential expression as potential defense mechanisms against stress. Potato plants at the tuber differentiation stage were subjected to mild, moderate, and severe water stress by withholding irrigation. Gas exchange measurements and proteomic analyses were conducted at each stress level. For protein extraction and proteome profiling using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), leaf samples were collected from plants under different stress conditions and from well-watered controls. A total of 74 protein spots were detected, and proteins associated with water stress were selected based on correlation analysis between their relative abundance and leaf water potential. Seven proteins were identified via mass spectrometry. Increasing water stress severity was associated with a decline in gas exchange and an increase in water use efficiency. Notably, an increase in the relative abundance of Rubisco activase, Photosystem I subunit IV B reaction center, and the manganese-stabilizing protein of Photosystem II under water stress suggests a potential protective strategy for maintaining photosynthetic function. Conversely, three isoforms of cytochrome oxidase (COX) subunit I exhibited decreased expression, which may be linked to a heat dissipation mechanism aimed at mitigating reactive oxygen species (ROS) formation during water stress. The identification of these proteins provides a baseline to further understand water stress in potatoes.

Additional key words: abiotic stress; plant proteins; chloroplasts; enzyme activity; water use efficiency; *Solanum tuberosum* L.; two-dimensional electrophoresis; metabolic pathways.

[1] Universidad Nacional de Colombia – ROR, Facultad de Ciencias Agrarias, Bogota (Colombia)
[2] Corporación Colombiana de Investigación Agropecuaria (Agrosavia) - ROR, Mosquera (Colombia)
[3] Corresponding author. frueda@agrosavia.co

RESUMEN

El estrés hídrico es uno de los factores críticos que más limitan el rendimiento de las plantas. La proteómica constituye una herramienta clave para evaluar cambios en la expresión de proteínas e identificar los mecanismos relacionados con la tolerancia al estrés por sequía en las plantas. Este estudio tiene como objetivo identificar proteínas asociadas con la severidad del estrés hídrico en la papa (*Solanum tuberosum* L.) y caracterizar su expresión diferencial como posibles mecanismos de defensa frente al estrés. Las plantas en la fase de diferenciación de tubérculos fueron sometidas a estrés hídrico leve, moderado y severo mediante la suspensión del riego. Se realizaron mediciones de intercambio gaseoso y análisis proteómicos en cada nivel de estrés. Para la extracción de proteínas y el análisis de las proteínas mediante electroforesis en gel de poliacrilamida bidimensional (2D-PAGE), se recolectaron muestras de hojas de plantas bajo diferentes condiciones de estrés y de plantas bien irrigadas como control. Se detectaron un total de 74 puntos de proteínas, y se seleccionaron aquellos puntos con una correlación entre su abundancia relativa y el potencial hídrico foliar. Siete proteínas fueron identificadas mediante espectrometría de masas. El aumento en la severidad del estrés hídrico se asoció con una reducción en el intercambio gaseoso y un incremento en la eficiencia en el uso del agua. En particular, el aumento en la abundancia relativa de Rubisco activasa, la subunidad IV B del centro de reacción del Fotosistema I y la proteína estabilizadora de manganeso del Fotosistema II, bajo condiciones de estrés hídrico, evidencia una estrategia protectora para mantener la función fotosintética. Por el contrario, tres isoformas de la subunidad I de citocromo oxidasa (COX) mostraron una disminución en su cantidad relativa, lo que podría estar relacionado con un mecanismo de disipación de calor para mitigar la formación de especies reactivas de oxígeno (ROS) durante el estrés hídrico. Estas proteínas representan posibles marcadores moleculares del estrés hídrico en la papa.

Palabras clave adicionales: estrés abiótico; proteínas vegetales; cloroplastos; actividad enzimática; eficiencia en el uso del agua; *Solanum tuberosum* L.; electroforesis bidimensional; vías metabólicas.

Received: 04-04-2025 Accepted: 27-06-2025 Published: 21-07-2025

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the fourth most important crop worldwide, following wheat, corn, and rice (FAO, 2019; Li *et al*., 2024). It serves as a vital source of energy, nutrients, and bioactive compounds (Beals, 2019; Xu *et al*., 2023a). However, potatoes are highly susceptible to drought due to their shallow root system (Zarzyńska *et al*., 2017; Fofana *et al*., 2023). Drought is a major limiting factor in agricultural production, as it disrupts physiological processes such as photosynthesis and respiration, leading to metabolic restrictions and structural damage, including impairments to photosystems (Lima-Melo *et al*., 2019; Yang *et al*., 2021; Qiao *et al*., 2024). To cope with drought stress, plants activate various mechanisms of response involving morphological adaptations, gene expression modifications, and alterations in the synthesis of key compounds such as hormones, proteins, enzymes, and antioxidants (Gervais *et al*., 2021; Yang *et al*., 2021; Haghpanah *et al*., 2024). The impact of drought stress varies depending on factors such as genotype, phenological stage, severity, and duration (Rudack *et al*., 2017; Nasir and Toth, 2022). In potatoes, water deficit during stolonization and tuber initiation restricts foliage development and reduces the number of stolons, ultimately leading to yield losses (Obidiegwu *et al*., 2015; Li *et al*., 2023a).

Proteomics enables the study of changes in protein expression profiles to elucidate biological functions and uncover stress resistance mechanisms in plants (Gong *et al*., 2015; Liu *et al*., 2019). Under cold stress, potato plants exhibit alterations in the concentration of proteins associated with ribosome formation, energy metabolism, cell movement, photosynthesis, protein degradation, nitrogen metabolism, and defense responses (Li *et al*., 2021). In response to drought and heat stress, significant changes occur in the abundance of proteins involved in energy and carbohydrate metabolism (Boguszewska-Mańkowska *et al*., 2020). However, proteomic studies on water stress responses in potato remain limited. Existing analyses suggest that potato varieties may enhance drought

tolerance by regulating nitrogen and carbon transport while protecting photosynthesis and respiration (Zhang *et al*., 2013; Zhang *et al*., 2025). Furthermore, differences in protein profiles between drought-tolerant and drought-sensitive genotypes indicate distinct responses to osmotic stress, particularly in terms of reactive oxygen species (ROS) production and scavenging. This highlights the potential of comparative temporal analyses to provide deeper insights into rapid water stress-induced changes, which could aid in the development of biomarkers for plant breeding programs (Bündig *et al*., 2016; Qin *et al*., 2024). This study aimed to identify proteins associated with water stress severity in potato plants and to establish the relationship between protein profiles and potential drought defense responses. Understanding the specific proteomic responses of potato under water stress conditions is particularly relevant for developing strategies to mitigate drought stress effects in agriculturally significant species.

MATERIALS AND METHODS

Plant material and growth conditions

Certified potato tubers of the variety Diacol Capiro were planted in a loam soil under greenhouse conditions at the Research Center Tibaitatá of AGROSAVIA (4°41'27.2" N, 74°12'08.4" W), at an altitude of 2,516 m a.s.l. The planting distance was 40 cm between plants and 100 cm between rows. To avoid horizontal and vertical water flows, the rows were waterproofed from a depth of 40 cm. Water was supplied through a drip irrigation system with independent modules to regulate the volume of water applied to each treatment. Fertilization was applied according to the needs of the plants and the results of the soil analysis.

Water stress treatments

The plants were kept at field capacity until tuber differentiation was reached (66 days after planting) (Gómez *et al*., 2020) after which irrigation was withheld to impose three levels of water deficit based on leaf water potential (Ψh): mild (MI; Ψh –0.5 to –0.6 MPa), moderate (MO; Ψh –0.61 to –0.90 MPa) and severe (SE; Ψh < –0.91 MPa), while well-watered plants (WW; maintained at field capacity) served as controls. Each treatment was applied to three independent biological replicates (n=3), with six plants per replicate (total = 18 plants per treatment).

Leaf water potential (Ψh)

In physiologically active and fully expanded leaves (3rd and 4th position) the Ψh was measured with a manually pumped pressure chamber (PMS Instrument Company, Oregon, USA) at down.

Gas exchange

Photosynthesis (A), stomatal conductance (gs) and intercellular CO_2 (Ci) were measured with a LI-6800 portable photosynthesis system (LI-COR, Lincoln, NE, USA). From the above measurements, intrinsic water use efficiency (WUEi) was calculated as the ratio A/gs. Measurements were made on the terminal leaflet of the 3rd or 4th fully expanded leaf of six plants per treatment in the period from 9:00 am to 12:00 noon. A CO_2 concentration of 400 μmol m^{-2} s^{-1} and a photosynthetic flux density of 900 μmol m^{-2} s^{-1} were used.

Protein extraction and quantification

Between 9 and 12 pm, central leaflets of leaves were taken from the plant and macerated without veins in liquid nitrogen. The samples were stored at -80°C until processing. The proteins were extracted according to Cheng *et al*. (2019) with some modifications: 1.0 g of sample was mixed with 12 mL of 10% (v/v) trichloroacetic acid/cold acetone, and 0.07% (v/v) β-mercaptoethanol overnight at -20°C. Then, it was centrifuged at 4,300 g for 60 min at 4 °C, the supernatant was discarded. The precipitate was resuspended in 10 mL of cold acetone and incubated at -20°C for 20 min and centrifuged at 4,300 g for 20 min at 4°C to obtain the precipitate. Washes with acetone were made until the precipitate turned white. Pellet was air dried for 2 h and then suspended in 1.4 mL of 8 M urea, 2% (v/v) Triton X-100 and 1% (w/v) dithiothreitol for 2 h at room temperature. Then it was centrifuged at 9,800 xg for 15 min at 4°C and the supernatant was stored at -80°C until processing. Protein concentration was measured by the Bradford method, using a bovine serum albumin standard curve.

Two-dimensional electrophoresis 2D-PAGE

The pellet obtained from the extraction was hydrated with buffer, 7 M urea, 2 M thiourea, 4% CHAPS, with tributyl phosphate 2% and ampholytes 2.5% (v/v). 30 μg sample was incorporated into an isoelectrofocusing (IEF) 7 cm strip pH 3-10 from (Bio-Rad) that was hydrated for 12 h. After hydration, the strips were applied in the Protean IEF Chamber (Bio-Rad, USA). The IEF was performed following 5 steps: linear-gradient 15 min at 250 V, 30 min at 500 V, and 30 min at 800 V; fast gradient, 1 h at 1,000 V, and 1 h at 1,000 V. IEF strips were immersed for 15 min in 1 mL of equilibration solution (Tris 50 mM pH 8.8; 6 M urea, 20% glycerol, 10% SDS) supplemented with 0.020 g DTT, subsequently, the strips were transferred to 1 mL of equilibrium solution with 0.025 g of iodoacetamide for 15 min. Then, the IEF strips were placed on top of a 15 % acrylamide gel and fixed with agarose-bromophenol blue 1%. The electrophoresis gel was run at a maximum of 190 V and 30 mA per gel.

The gels were stained with a Coomassie blue R-250 (Bio-Rad) staining for 4 h and washed with 1:5 v/v methanol-acetic acid for 18 h. The gel images were acquired with the Gel doc XR (Bio Rad) and analyzed with PD-Quest software. The electrophoretic profiles of each gel were determined through a virtual reference gel, created with all the spots detected in each gel. The relative amount of each spot was obtained on three gels per treatment (MI, MO, SE, WW) and normalized with respect to the total protein amount and the background of each gel image.

From the results of the relative amount of protein (RAP) of each spot, a correlation matrix between the RAP in each treatment and the Ψh was performed. The five spots that had the highest correlation and presented significant differences among treatments were sent for identification by mass spectrometry. Additionally, two spots were selected that did not present a high correlation with Ψh but had an evident reduction or increase when comparing WW plants with stressed plants.

Mass spectrometry

Seven spots were identified by mass spectrometry, using the MALDI TOF/TOF method. The 2D spots were rehydrated in a sequencing grade modified trypsin digestion buffer. Digestion was performed at 37°C and digested tryptic peptides were desalted using C-18 Zip-tips and mixed with an alpha-cyano-4-hydroxycinnamic acid matrix. The mixture was ground in wells of a MALDI plate, to then obtain mass spectrometry (MS). 10-20 of the most abundant peptides from each sample were subjected to fragmentation analysis and tandem MS/MS. The combined MS and MS/MS spectra were used to identify homologous sequences in NCBIprot using the MASCOT search engine.

Experimental design and statistical analysis

Student t-test ($P \leq 0.05$) was used to determine differences between the water potential of well irrigated plants and those with some level of stress (mild, moderate, severe). Correlation analyses with linear and polynomial second-degree fits were performed to determine the relationship between water potential and gas exchange variables. The selection of proteins to be identified was made from a Pearson correlation matrix. To determine significant differences among treatments, an ANOVA variance analysis of the RAP each spot was performed, followed by Duncan's test ($P \leq 0.05$). For the analysis of variance, the assumptions of normality and homoscedasticity were met. The R software and the "corrplot", "agricolae" and "nortest" libraries were used for statistical analyzes.

RESULTS AND DISCUSSION

Water status

The Ψh gradually decreased until each set stress level was reached. MI (-0.52±0.03 MPa) was reached 7 days after inducing stress (DAS), MO (-0.68±0.14 MPa) was reached 10 DAS, and SE (-0.94±0.04 MPa) was reached 21 DAS. Measurements of well-watered plants at each stress level showed a significantly higher water potential than stressed plants and on average their Ψh was of -0.29±0.02 Mpa.

Gas exchange

Based on the gas exchange measurements and the calculation of the efficient use of water by plants with and without water stress, the correlations adjusted to linear and second-degree polynomial models are presented (Fig. 1). Leaf water potential reduction was negatively correlated with photosynthesis (R^2=0.86), while photosynthesis and stomatal conductance were positively correlated (R^2=0.74). As stomatal

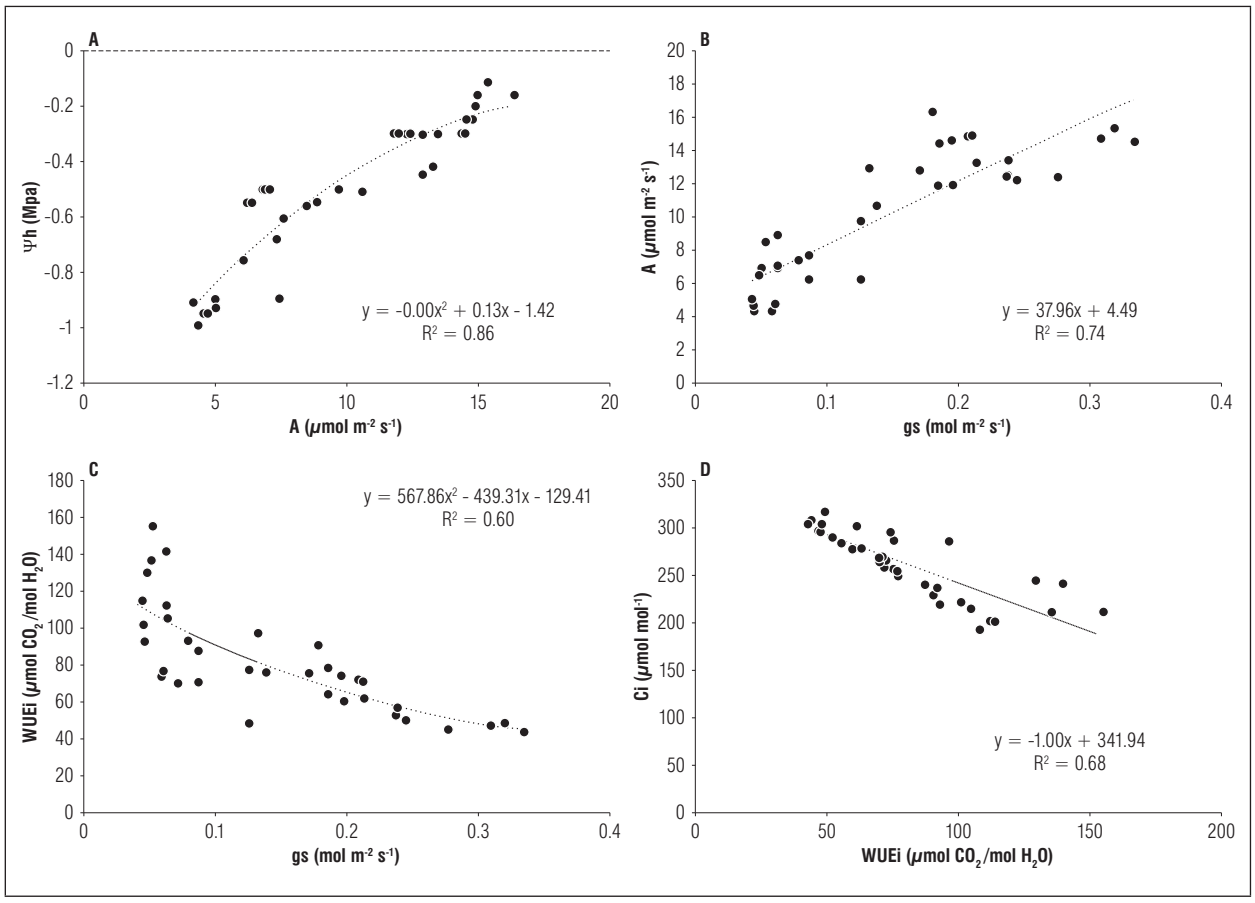


Figure 1. Relationship between gas exchange variables in plants with different levels of water stress severity. A) leaf water potential [Ψh] and photosynthesis [A], B) photosynthesis and stomatal conductance [gs], C) intrinsic water use efficiency [WUEi] and stomatal conductance, D) intercellular CO_2 [Ci] and intrinsic water use efficiency.

conductance decreased, water use efficiency increased (R^2=0.60). The intracellular CO_2 content decreased as the efficient use of water increased (R^2=0.68).

2D-PAGE profiles

The reference gel from all treatments (MI, MO, SE, and WW) included 74 protein spots detected in the leaves of potato plants (Fig. 1). Quantitative image analysis showed 51 total protein spots for WW treatment, 62 for MI, 57 for MO, and 67 for SE. The spots with the highest correlation between the RAP and the Ψh were the following: 1405 (r=0.92), 3704 (r=-0.83), 5503 (r= 0.90), 7603 (r=0.87) and 8401 (r=-0.91) (Tab. 1). 1602 and 3507 were spots that did not present a high correlation but had an evident reduction or increase when comparing stressed plants with WW plants.

Proteins associated with the severity of water stress

The seven spots selected were associated with the following proteins: KY290_026389 (spot 1405) with a 6.0 isoelectric point (IP) and 20 kDa molecular weight (MW); KY289_006555 (spot 1602) with a 4.3 IP and 22 kDa MW; KY285_018371 (spot 5503) with an 8.3 IP and 25 kDa MW; manganese stabilizing protein (spot 3507) with a 6.7 IP and 34 kDa MW Rubisco activase (spot 3704) with an 8.7 PI and 40 kDa MW; PSI reaction center subunit IV B (spot 8401) with a 9.8 PI and 15 kDa MW; and an uncharacterized protein LOC107059154 (spot 7603) 9.2 IP and a 76 kDa MW (Fig. 2). All proteins had a total ion C. I. of 100%, except for the uncharacterized protein which obtained 98%, and its protein score C. I. was 77% (Tab. 2).

Table 1. Identification of reference gel spots (highlighted) and Pearson's correlation coefficient between the relative amount of each spot protein and the water potential in plant leaves (parentisis).

Identification of reference gel spots														
301	401	402	1301	1405	1503	1601	1602	1603	1702	1703	1802	2503	2604	2703
(0.29)	(0.04)	(-0.06)	(0.01)	(0.92)	(-0.17)	(0.24)	(0.77)	(0.03)	(-0.34)	(-0.77)	(-0.11)	(0.09)	(-0.17)	(-0.18)
3305	3306	3504	3505	3506	3507	3605	3606	3607	3704	3705	4505	4506	4602	4702
(0.06)	(0.17)	(-0.35)	(-0.05)	(0.41)	(-0.48)	(-0.08)	(-0.44)	(0.03)	(-0.83)	(-0.02)	(0.51)	(0.46)	(-0.54)	(-0.19)
5403	5404	5405	5406	5407	5502	5503	5504	5505	5506	5601	5602	5703	5704	5705
(0.48)	(0.27)	(-0.81)	(-0.76)	(-0.71)	(0.55)	(0.9)	(-0.32)	(-0.18)	(-0.03)	(-0.01)	(-0.71)	(0.27)	(-0.64)	(-0.69)
6602	6603	6604	6605	6702	6703	6704	6705	6706	6808	6809	6810	7404	7405	7503
(-0.13)	(0.24)	(-0.8)	(-0.69)	(0.07)	(-0.68)	(-0.71)	(0.18)	(0.18)	(-0.11)	(-0.01)	(0.00)	(-0.12)	(0.02)	(-0.43)
7602	7603	7702	7703	7802	7803	7804	7805	7806	8401	8402	8501	8605	8704	
(-0.01)	(0.87)	(0.41)	(0.21)	(0.16)	(-0.13)	(-0.22)	(0.18)	(0.12)	(-0.91)	(-0.49)	(-0.27)	(-0.01)	(-0.39)	

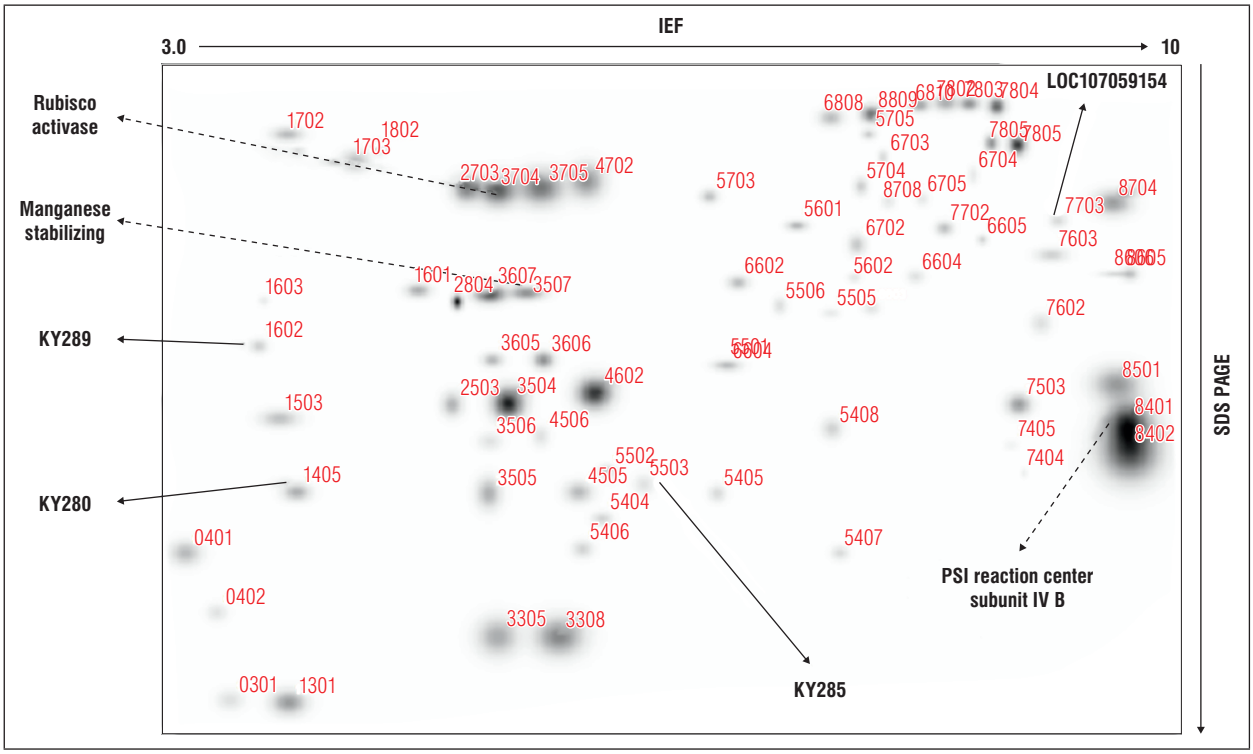


Figure 2. Differentially expressed proteins in leaves of potato plants under water stress conditions in tuber differentiation. Continuous arrows indicate retductions by water stress conditions and dashed arrows indicate increases.

Changes in proteins according to the severity of water stress

The PSI reaction center subunit IV B increased in MO and SE respect to WW, in SE the increase was greater than in the other stress treatments. Manganese-stabilizing protein (MSP) and Rubisco activase (Rca) increased only to SE (Fig. 3). Proteins KY290_026389, KY289_006555 and KY285_018371 reduced their relative amount in water stress compared to WW plants. This reduction was greater in SE than in MO or MI, especially for KY290_026389 and KY285_018371. LOC107059154 protein significantly reduced its relative amount from mild stress (Fig. 4).

Table 2. Protein identification quality parameters from the research.

Spot number	Protein name	Molecular weight (Da)	Isoelectric point	Peptide count	Protein score	Protein score C. I. %	Total ion score	Total ion C. I. %
3704	Chloroplast Rubisco activase, partial [*Solanum tuberosum*]	39,516.9	8.65	13	660	100	588	100
7603	PREDICTED: uncharacterized protein LOC107059154 [*Solanum tuberosum*]	75,947.4	9.2	8	59	77	46	98
8401	PREDICTED: photosystem I reaction center subunit IV B, chloroplastic [*Solanum tuberosum*]	14,864.6	9.88	6	176	100	137	100
1802	Hypothetical protein KY289_006555 [*Solanum tuberosum*]	21,722.8	4.34	6	104	100	70	100
5503	Hypothetical protein KY285_018371 [*Solanum tuberosum*]	25,317.6	8.32	3	276	100	265	100
1405	Hypothetical protein KY290_026389 [*Solanum tuberosum*]	19,954.8	5.98	1	92	100	88	100
3507	Chloroplast manganese stabilizing protein, partial [*Solanum tuberosum*]	34,096.5	6.73	12	502	100	425	100

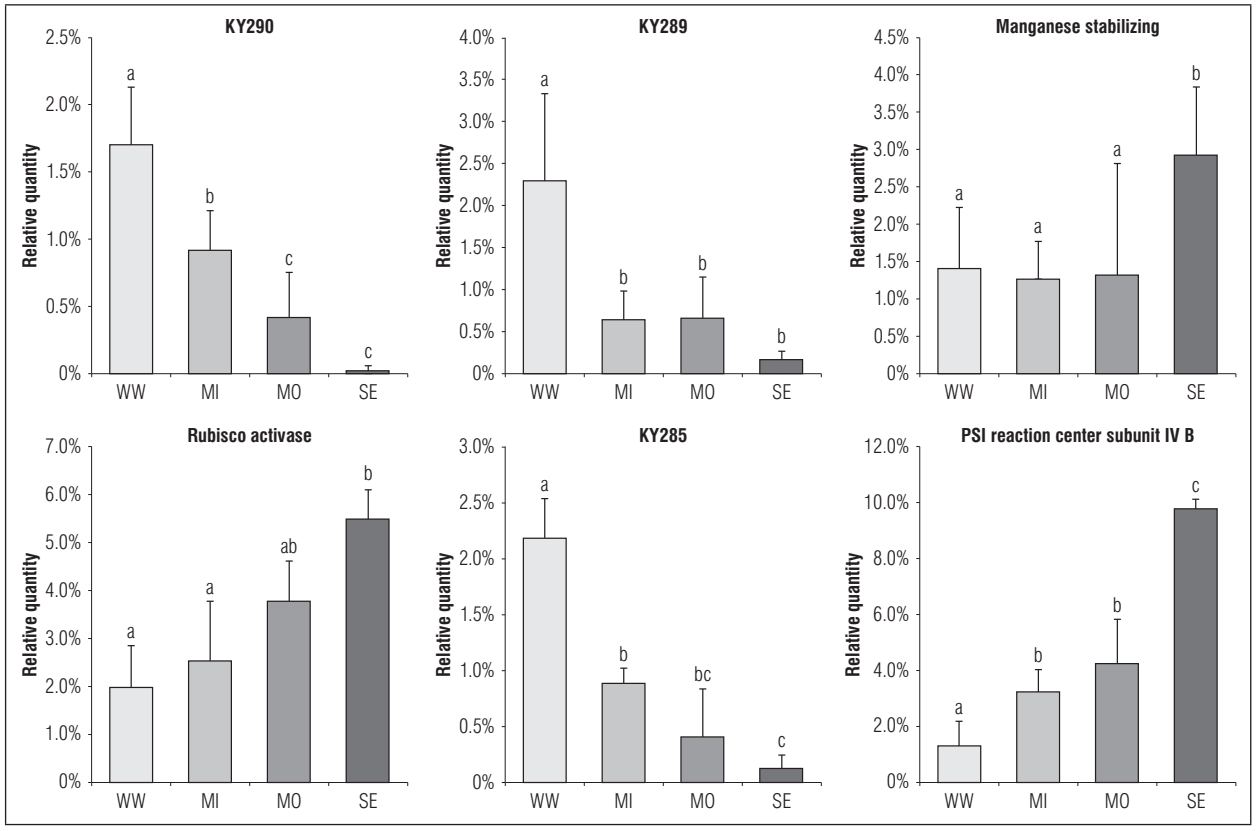


Figure 3. Relative amount of protein in leaves of potato plants subjected to three levels of water (mild: MI, moderate: MO, and severe: SE) stress and well-watered (WW) in tuber differentiation. Different letters indicate significant differences according to Duncan's test ($P \leq 0.05$).

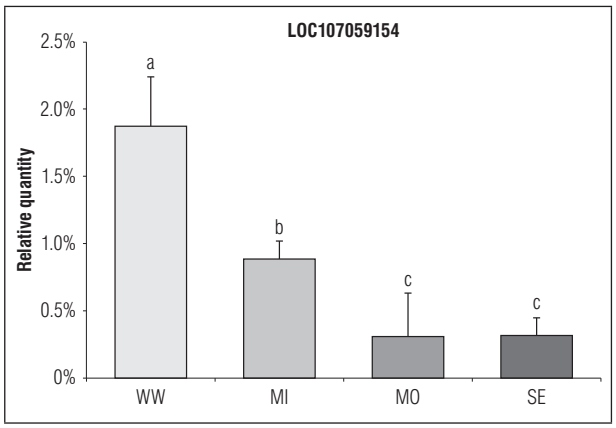


Figure 4. Relative amount of LOC107059154 protein in leaves of potato plants subjected to three levels of water (mild: MI, moderate: MO, and severe: SE) stress and well-watered (WW) in tuber differentiation. Different letters indicate significant differences according to Duncan's test ($P \leq 0.05$).

DISCUSSION

The leaf water potential at each stress level became more negative and was associated with physiological changes in water-stressed plants. Carbon fixation in potato plants decreased as leaf water potential declined (Fig. 1A), a reduction in photosynthetic activity primarily attributed to progressive stomatal closure (Fig. 1B). As stomatal closure intensified, water use efficiency improved, leading to a lower water loss per molecule of CO_2 fixed under higher water stress conditions (Fig. 1C). This increased efficiency is linked to a reduction in intracellular CO_2 concentration (Fig. 1D), indicating that under water stress, the plant minimizes both water loss and CO_2 uptake, thereby limiting carbon availability for photosynthesis. The progressive stomatal closure in response to prolonged irrigation absence reflects an adaptive strategy to cope with declining soil water content and enhance survival under extended water stress conditions This physiological behavior in response to water stress is typical of isohydric plants (Attia *et al*., 2015) and agrees with the reports of Obidiegwu *et al*. (2015). Potato plants, by reducing their photosynthetic rate in response to water stress, may experience an increased production of reactive oxygen species (ROS), leading to oxidative damage. Photosynthesis involves the conversion of light energy into chemical energy used for carbon fixation, and an excess of light energy enhances the rate of electron transport. This, in turn, promotes the overproduction of ROS, which can cause damage to the photosynthetic apparatus (Lima-Melo *et al*., 2019). The mechanisms to reduce the production or negative effects of ROS are heat dissipation and the synthesis of antioxidant metabolites (Gururani *et al*., 2015; Li *et al*., 2023b).

Of the seven proteins that were related to the severity of water stress, three of them are part of the photosynthesis process (Rubisco activase, PSI subunit IV B reaction center, and manganese-stabilizing protein). Other identified proteins were: KY290_026389, KY289_006555, and KY285_018371 reported in the NCBI for different species such as subunit I of cytochrome oxidase (COX SI) that is part of the respiration process in the mitochondria, these spots could represent isoforms of COX S1 in leaves of potato. Proteins involved in photosynthesis showed an increase under water stress conditions, especially in SE (Fig. 3). Chen *et al*. (2015) found that under different types of abiotic stress, the *rca* gene (*Ribulose-1,5-bisphosphate carboxylase/oxygenase activase*), which codes for Rca, modulates photosynthetic acclimatization and could balance the relationship between photosynthesis and stress tolerance through changes in the ratio of Rca protein isoforms. The increase in the relative amount of Rca protein during SE (Fig. 3) could suggest that water stress in 'Diacol Capiro' stimulates the Rca synthesis pathway to protect photosynthesis proteins such as Rubisco, and favoring the repair function when Rubisco is inhibited, as reported by Flecken *et al*. (2020) and Wellpott *et al*. (2023).

In this study, MSP increased in SE. This protein is involved in the water photolysis of the PSII reaction center. This reaction is catalyzed in a tetranuclear Mn cluster in which MSP increases the rate of O_2 reaction and stabilizes the Mn cluster (Svensson *et al*., 2004). The reduction of MSP is related to a decrease in the accumulation of PSII reaction center components and a reduction of variable fluorescence in autotrophic organisms, including higher plants (Yi *et al*., 2005). Therefore, MSP proteins are necessary for assembly and PSII stability. The increase in MSP as well as Rca during ES (Fig. 3) would suggest that these proteins are involved in protecting the functionality of the photosynthetic apparatus during stress in potato plants (Chauhan *et al*., 2023).

Subunit IV B of the PSI reaction center increased in potato plants subjected to water stress (Fig. 3). PSI is involved in NADP+ reduction and cyclic photophosphorylation in photosynthesis. It consists of a multisubunit membrane protein complex that catalyzes

electron transfer from reduced plastocyanin in the thylakoid lumen to oxidized ferredoxin in the chloroplast stroma (Ozakca, 2013). Under drought stress conditions, stomatal closure limits CO_2 fixation and causes light stress by photoinhibition. Therefore, the ROS production leads to limited availability of oxidized ferredoxin for degradation of PSI subunits (Rodriguez-Heredia *et al*., 2022). The PSI core is composed of 13 to 14 subunits known as PSI-A to PSI-N and a decrease in PSI reaction center subunit IV (PSI-E) limits the reduction of ferredoxin and causes an excessive reduction of the iron and sulfur centers of FA/FB, leading to ROS generation and degradation of PSI-D and PSI-C (Haldrup *et al*., 2000). The increase of PSI subunit IV B (Fig. 3) in 'Diacol Capiro' under SE conditions indicates a possible mechanism of protection and promotion of photosystem component synthesis, in which carotenoids could be involved. The above is supported by the fact that carotenoids promote the synthesis of PSI components (Dall'Osto *et al*., 2013) and that 'Diacol Capiro' does not reduce photosystem components such as chlorophylls and increases carotenoids under severe water stress (Rodríguez-Pérez *et al*., 2017; Hu *et al*., 2023).

The protein changes reported in this study suggest that 'Diacol Capiro' potato has a defense strategy against SE that involves protection of the photosynthetic complex through Rca and MSP synthesis and possibly carotenoids, potentially triggering the synthesis of PSI IVB subunit. Carotenoids are pigments that protect chloroplasts as they act as antioxidants under abiotic stress conditions (Uarrota *et al*., 2018). Rodríguez-Pérez *et al*. (2017) found that in 'Diacol Capiro' that carotenoid concentration increases, and that chlorophyll concentration is not affected even when Ψh was reduced to -1.99 MPa. Therefore, the protective strategy of the photosynthetic complex is possibly maintained at a higher water stress level than that achieved in this study (Ψh = -0.94 MPa) (Xu *et al*., 2023b).

On the other hand, we found that the relative amount of the three possible COX SI isoforms in potato decreases, when the severity of stress increases, possibly linked to heat dissipation strategies. In mitochondria, during the respiratory process, the electron transport process can take two pathways, the cytochrome oxidase (COX) pathway sensitive to cyanide/antimycin A, and the alternative oxidase (AOX) pathway sensitive to salicylhydroxamic acid. In the COX pathway electron flow is coupled to ATP production, whereas in the AOX pathway the energy produced is dissipated as heat, which prevents ROS formation under stress conditions (Vishwakarma *et al*., 2015). While the AOX pathway can increase by 70% under osmotic stress conditions, the COX pathway drastically decreases by 77% (Dinakar *et al*., 2016). The above, together with the results of this study, would indicate that 'Diacol Capiro' COX reduction under water stress may be associated with an increase in the AOX pathway, as a possible strategy to prevent ROS formation. However, more experimental work is needed to support this hypothesis.

The relative amount of the LOC107059154 protein decreased with MI; however, its functionality has not been characterized. This protein becomes a possible molecular marker of water stress in potatoes, and further studies are required to characterize it and confirm its relationship with water stress.

CONCLUSION

The increase in the severity of water stress was related to a reduction of the gas exchange and an increase in water use efficiency, Rubisco activase, PSI subunit IV B reaction center, and manganese stabilizer. This relation would indicate a protective response of the potato photosynthetic complex under water stress conditions. In addition, three possible isoforms of the cytochrome oxidase 1 subunit (KY290_026389, KY289_006555, and KY285_018371) decreased in potato leaves, which could indicate a reduction in the COX pathway, and an increase in the AOX pathway as a possible strategy to dissipate heat and avoid the formation of ROS. The high correlation found between the expression of these proteins and water stress sheds light on possible strategies related to the tolerance of the potato variety Diacol Capiro to water stress, which had not been previously described and should be studied in greater depth.

Finally, it is remarkably valuable in future studies on the specific characterization of LOC107059154, which is related to the severity of water stress detected in our study. However, the role of this sequence in *Solanum tuberosum* has not been clearly defined, warranting deeper functional exploration.

Conflict of interest: The authors declare no conflict of interest.

Author's contributions: EASA, conceived and designed this research, investigation, acquisition of

data, formal analysis, analysis and interpretation of the data, writing - original draft. AMCM and LPM, conceived and designed this research, analysis, and interpretation of the data, writing – review and editing. MCLM, LMTD, and ODOP, acquisition of data, analysis, and interpretation of data, writing - original draft. FLRA, coordinated the proteomic analysis, software, methodology, writing – review and editing. All authors have read and agreed to the published version of the manuscript.

Funding statement: The authors would like to thank Ministry of Agriculture and Rural Development of Colombia (MADR), through the Administrative Department of Science, Technology, and Innovation – Colciencias (MINCIENCIAS) for funding and supporting this research.

Open-access data repository: The dataset is available upon request.

IA-assisted content generation or analysis: not declared.

BIBLIOGRAPHIC REFERENCES

Attia, Z., J.C. Domec, R. Oren, D.A. Way, and M. Moshelion. 2015. Growth and physiological responses of isohydric and anisohydric poplars to drought. J. Exp. Bot. 66(14), 4373-4381. Doi: https://doi.org/10.1093/JXB/ERV195

Beals, K.A. 2019. Potatoes, nutrition and health. Am. J. Potato Res. 96, 102-110. Doi: https://doi.org/10.1007/S12230-018-09705-4

Boguszewska-Mańkowska, D., M. Gietler, and M. Nykiel. 2020. Comparative proteomic analysis of drought and high temperature response in roots of two potato cultivars. Plant Growth Regul. 92, 345-363. Doi: https://doi.org/10.1007/s10725-020-00643-y

Bündig, C., A.M. Jozefowicz, H.-P. Mock, and T. Winkelmann. 2016. Proteomic analysis of two divergently responding potato genotypes (*Solanum tuberosum* L.) following osmotic stress treatment in vitro. J. Proteomics 143, 227-241. Doi: https://doi.org/10.1016/J.JPROT.2016.04.048

Chauhan, J., M.D. Prathibha, P. Singh, P. Choyal, U.N. Mishra, D. Saha, R. Kumar, H. Anuragi, S. Pandey, B. Bose, B. Mehta, P. Dey, K.K. Dwivedi, N.K. Gupta, and R.K. Singhal. 2023. Plant photosynthesis under abiotic stresses: damages, adaptive, and signaling mechanisms. Plant Stress 10, 100296. Doi: https://doi.org/10.1016/j.stress.2023.100296

Chen, Y., X.M. Wang, L. Zhou, Y. He, D. Wang, Y.H. Qi, and D.A. Jiang. 2015. Rubisco activase is also a multiple responder to abiotic stresses in rice. PLoS One 10(10), e0140934. Doi: https://doi.org/10.1371/JOURNAL.PONE.0140934

Cheng, L., S. Zhang, L. Yang, Y. Wang, B. Yu, and F. Zhang. 2019. Comparative proteomics illustrates the complexity of Fe, Mn and Zn deficiency-responsive mechanisms of potato (*Solanum tuberosum* L.) plants in vitro. Planta 250, 199-217. Doi: https://doi.org/10.1007/S00425-019-03163-W

Dall'Osto, L., M. Piques, M. Ronzani, B. Molesini, A. Alboresi, S. Cazzaniga, and R. Bassia. 2013. The *Arabidopsis* nox mutant lacking carotene hydroxylase activity reveals a critical role for xanthophylls in photosystem I biogenesis. Plant Cell 25(2), 591-608. Doi: https://doi.org/10.1105/tpc.112.108621

Dinakar, C., A. Vishwakarma, A.S. Raghavendra, and K. Padmasree. 2016. Alternative oxidase pathway optimizes photosynthesis during osmotic and temperature stress by regulating cellular ROS, malate valve and antioxidative systems. Front. Plant Sci. 7, 68. Doi: https://doi.org/10.3389/FPLS.2016.00068

FAO, 2019. FAOSTAT. In: https://www.fao.org/faostat/en/#data/QC; consulted, February, 2022.

Flecken, M., H. Wang, L. Popilka, F.U. Hartl, A. Bracher, and M. Hayer-Hartl. 2020. Dual functions of a Rubisco Activase in metabolic repair and recruitment to carboxysomes. Cell 183(2), 457-473. Doi: https://doi.org/10.1016/J.CELL.2020.09.010

Fofana, B., B. Soto-Cerda, M. Zaidi, D. Main, and S. Fillmore. 2023. Genome-wide genetic architecture for plant maturity and drought tolerance in diploid potatoes. Front. Genet. 14, 1306519. Doi: https://doi.org/10.3389/fgene.2023.1306519

Gervais, T., A. Creelman, X.Q. Li, B. Bizimungu, D. De Koeyer, and K. Dahal. 2021. Potato response to drought stress: physiological and growth basis. Front. Plant Sci. 12, 698060. Doi: https://doi.org/10.3389/fpls.2021.698060

Gómez, M.I., A. Barragán, S. Magnitskiy, and L.E. Rodríguez. 2020. Normalized difference vegetation index, $N–NO^{-}_{3}$ and K^{+} in stem sap of potato plants (Group Andigenum) as affected by fertilization - Corrigendum. Exp. Agric. 56(1), 159-159. Doi: https://doi.org/10.1017/S0014479719000176

Gong, F., X. Hu, and W. Wang. 2015. Proteomic analysis of crop plants under abiotic stress conditions: where to focus our research? Front. Plant Sci. 6, 418. Doi: https://doi.org/10.3389/FPLS.2015.00418

Gururani, M.A., J. Venkatesh, and L.S.P. Tran. 2015. Regulation of photosynthesis during abiotic stress-induced photoinhibition. Mol. Plant 8(9), 1304-1320. Doi: https://doi.org/10.1016/J.MOLP.2015.05.005

Haghpanah, M., S. Hashemipetroudi, A. Arzani, and F. Araniti. 2024. Drought tolerance in plants: physiological and molecular responses. Plants 13(21), 2962. Doi: https://doi.org/10.3390/plants13212962

Haldrup, A., D.J. Simpson, and H.V. Scheller. 2000. Down-regulation of the PSI-F subunit of Photosystem I (PSI) in *Arabidopsis thaliana*: the PSI-F subunit is essential for photoautotrophic growth and contributes to antenna function. J. Biol. Chem. 275(40), 31211-31218. Doi: https://doi.org/10.1074/JBC.M002933200

Hu, F., Y. Zhang, and J. Guo. 2023. Effects of drought stress on photosynthetic physiological characteristics, leaf microstructure, and related gene expression of yellow horn. Plant Signal. Behav. 18(1), 2215025. Doi: https://doi.org/10.1080/15592324.2023.2215025

Li, X.-C., C. Chang, and Z.-M. Pei. 2023b. Reactive oxygen species in drought-induced stomatal closure: the potential roles of NPR1. Plants 12(18), 3194. Doi: https://doi.org/10.3390/plants12183194

Li, H., W. Luo, R. Ji, Y. Xu, G. Xu, S. Qiu, and H. Tang. 2021. A comparative proteomic study of cold responses in potato leaves. Heliyon 7(2), e06002. Doi: https://doi.org/10.1016/J.HELIYON.2021.E06002

Li, S., Y. Kupriyanovich, C. Wagg, F. Zheng, and S. Hann. 2023a. Water deficit duration affects potato plant growth, yield and tuber quality. Agriculture 13(10), 2007. Doi: https://doi.org/10.3390/agriculture13102007

Li, L., T. Zhu, L. Wen, T. Zhang, and M. Ren. 2024. Biofortification of potato nutrition. J. Adv. Res. 75, 23-34. Doi: https://doi.org/10.1016/j.jare.2024.10.033

Lima-Melo, Y., V.T.C.B. Alencar, A.K.M. Lobo, R.H.V. Sousa, M. Tikkanen, E.M. Aro, J.A.G. Silveira, and P.J. Gollan. 2019. Photoinhibition of photosystem I provides oxidative protection during imbalanced photosynthetic electron transport in *Arabidopsis thaliana*. Front. Plant Sci. 10, 916. Doi: https://doi.org/10.3389/FPLS.2019.00916

Liu, Y., S. Lu, K. Liu, S. Wang, L. Huang, and L. Guo. 2019. Proteomics: a powerful tool to study plant responses to biotic stress. Plant Methods 15, 135. Doi: https://doi.org/10.1186/S13007-019-0515-8

Nasir, M.W. and Z. Toth. 2022. Effect of drought stress on potato production: a review. Agronomy 12(3), 635. Doi: https://doi.org/10.3390/agronomy12030635

Obidiegwu, J.E., G.J. Bryan, H.G. Jones, and A. Prashar. 2015. Coping with drought: stress and adaptive responses in potato and perspectives for improvement. Front. Plant Sci. 6, 542. Doi: https://doi.org/10.3389/FPLS.2015.00542

Ozakca, D.U. 2013. Effect of abiotic stress on photosystem I-related gene transcription in photosynthetic organisms. In: Dubinsky, Z. (ed.). Photosynthesis. IntechOpen. Doi: https://doi.org/10.5772/55350

Qiao, M., C. Hong, Y. Jiao, S. Hou, and H. Gao. 2024. Impacts of drought on photosynthesis in major food crops and the related mechanisms of plant responses to drought. Plants 13(13), 1808. Doi: https://doi.org/10.3390/plants13131808

Qin, T., Y. Wang, Z. Pu, N. Shi, R. Dormatey, H. Wang, and C. Sun. 2024. Comprehensive transcriptome and proteome analyses reveal the drought responsive gene network in potato roots. Plants 13(11), 1530. Doi: https://doi.org/10.3390/plants13111530

Rodriguez-Heredia, M., F. Saccon, S. Wilson, G. Finazzi, A. Ruban, and G.T. Hanke. 2022. Protection of photosystem I during sudden light stress depends on ferredoxin:NADP(H) reductase abundance and interactions. Plant Physiol. 188(2), 1028-1042. Doi: https://doi.org/10.1093/PLPHYS/KIAB550

Rodríguez-Pérez, L., C.E. Ñústez, and L.P. Moreno. 2017. Drought stress affects physiological parameters but not tuber yield in three Andean potato (*Solanum tuberosum* L.) cultivars. Agron. Colomb. 35(2), 158-170. Doi: https://doi.org/10.15446/agron.colomb.v35n2.65901

Rudack, K., S. Seddig, H. Sprenger, K. Köhl, R. Uptmoor, and F. Ordon. 2017. Drought stress-induced changes in starch yield and physiological traits in potato. J. Agron. Crop. Sci. 203(6), 494-505. Doi: https://doi.org/10.1111/JAC.12224

Svensson, B., D.M. Tiede, D.R. Nelson, and B.A. Barry. 2004. Structural studies of the manganese stabilizing subunit in photosystem II. Biophys. J. 86(3), 1807-1812. Doi: https://doi.org/10.1016/S0006-3495(04)74247-2

Uarrota, V.G., D.L.V. Stefen, L.S. Leolato, L. D.M. Gindri, and D. Nerling. 2018. Revisiting carotenoids and their role in plant stress responses: from biosynthesis to plant signaling mechanisms during stress. pp. 207-232. In: Gupta, D., J. Palma, and F. Corpas (eds.). Antioxidants and antioxidant enzymes in higher plants. Springer, Cham Doi: https://doi.org/10.1007/978-3-319-75088-0_10

Vishwakarma, A., S.D. Tetali, J. Selinski, R. Scheibe, and K. Padmasree. 2015. Importance of the alternative oxidase (AOX) pathway in regulating cellular redox and ROS homeostasis to optimize photosynthesis during restriction of the cytochrome oxidase pathway in *Arabidopsis thaliana*. Ann. Bot. 116(4), 555-569. Doi: https://doi.org/10.1093/aob/mcv122

Wellpott, K., A.M. Jozefowicz, P. Meise, A. Schum, S. Seddig, H.P. Mock, T. Winkelmann, and C. Bündig. 2023. Combined nitrogen and drought stress leads to overlapping and unique proteomic responses in potato. Planta 257, 58. Doi: https://doi.org/10.1007/s00425-023-04085-4

Xu, J., Y. Li, L. Kaur, J. Singh, and F. Zeng. 2023a. Functional food based on potato. Foods 12(11), 2145. Doi: https://doi.org/10.3390/foods12112145

Xu, X., C.F.F. Lima, L.D. Vu, and I. De Smet. 2023b. When drought meets heat – a plant omics perspective. Front. Plant Sci. 14, 1250878. Doi: https://doi.org/10.3389/fpls.2023.1250878

Yang, X., M. Lu, Y. Wang, Y. Wang, Z. Liu, and S. Chen. 2021. Response mechanism of plants to drought stress. Horticulturae 7, 50. Doi: https://doi.org/10.3390/HORTICULTURAE7030050

Yi, X., M. McChargue, S. Laborde, L.K. Frankel, and T.M. Bricker. 2005. The manganese-stabilizing protein is required for photosystem II assembly/stability and photoautotrophy in higher plants. J. Biol. Chem. 280(16), 16170-16174. Doi: https://doi.org/10.1074/JBC.M501550200

Zarzyńska, K., D. Boguszewska-Mańkowska, and A. Nosalewicz. 2017. Differences in size and architecture of the potato cultivars root system and their tolerance to drought stress. Plant Soil Environ. 63(4), 159-164. Doi: https://doi.org/10.17221/4/2017-PSE

Zhang, Y.T., D.Q. Zhou, Y. Su, P. Yu, X.G. Zhou, and C.X. Yao. 2013. Proteome analysis of potato drought resistance variety in Ninglang 182 leaves under drought stress. Yi Chuan 35(5), 666-672. Doi: https://doi.org/10.3724/SP.J.1005.2013.00666

Zhang, R., Y. Wang, Y. Kang, Y. Du, X. Wang, S. Jiao, X. Yang, Y. Liu, S. Qin, and W. Zhang. 2025. Transcriptomics–proteomics analysis reveals *StCOMT1* regulates drought, alkali and combined stresses in potato. Plant Cell Rep. 44(5), 109. Doi: https://doi.org/10.1007/s00299-025-03496-9