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UNRAVELING THE PHOTOSYNTHETIC MECHANISMS OF SOYBEAN CULTIVARS UNDER PHOSPHORUS DEFICIENCY

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Tese apresentada a Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fisiologia Vegetal área de concentração em Fisiologia Vegetal, para obtenção do título de Doutor.

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ISADORA RODRIGUES MEDINA SANTANA

DESVENDANDO OS MECANISMOS FOTOSSINTÉTICOS DE CULTIVARES DE SOJA SOB DEFICIÊNCIA DE FÓSFORO

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RESUMO

O crescimento e o desenvolvimento das culturas dependem da fotossíntese, mas o processo fotossintético é influenciado em grande parte pela concentração de fósforo (P) na planta. No entanto, a maioria dos solos brasileiros possui baixa disponibilidade de fósforo, o que pode comprometer o rendimento das culturas. O uso de fertilizante fosfatado é uma alternativa que fornece fósforo às plantas; no entanto, trata-se de um recurso finito e caro. Por isso, compreender os ajustes fotossintéticos que diferentes genótipos apresentam sob deficiência de fósforo e como a interação com outros fatores abióticos alteram os mecanismos de eficiência no uso do fósforo é essencial para a seleção de genótipos sustentáveis. Realizamos dois experimentos distintos, um em casa de vegetação com o objetivo de elucidar como a deficiência de fósforo afeta a recuperação do estresse hídrico recorrente em cultivares de soja com diferentes níveis de tolerância à seca, e o segundo em campo, com o objetivo de compreender o mecanismo de eficiência e de resposta no uso do fósforo por diferentes genótipos de soja, relacionando características fotossintéticas e de crescimento. A deficiência de fósforo atrasou o fechamento estomático, prolongando o declínio fotossintético sob estresse hídrico entre cultivares. As plantas deficientes em fósforo apresentaram recuperação mais rápida nas taxas fotossintéticas e na condutância estomática em comparação com aquelas adequadamente supridas com fósforo. A cultivar sensível demonstrou maior recuperação fotossintética após passar por dois ciclos de déficit hídrico, atribuído à melhoria da condutância do mesofilo. Em contraste, a cultivar tolerante apresentou respostas fotossintéticas consistentes, independentemente da disponibilidade de fósforo. Esses resultados ressaltam que a plasticidade adaptativa da soja ao déficit hídrico é influenciada pela disponibilidade de fósforo. Os resultados do experimento de campo demonstraram que cultivares com alta responsividade fotossintética ao P também são responsivas na produção de grãos. Entretanto, independentemente do mecanismo de eficiência no uso do P, a deficiência de P afetou a produção dos diferentes genótipos. Cultivares responsivas apresentaram aumento na taxa de carboxilação, regeneração de RuBP e uso de trioses-P em alta disponibilidade de fósforo. O desempenho fotossintético da cultivar eficiente e não responsiva ao P foi apoiada pela maior alocação de P metabólico e decréscimo de P lipídico, além do aparato fotossintético mais robusto desde os estágios iniciais de desenvolvimento. Independente da capacidade de resposta, cultivares eficientes e ineficientes apresentaram capacidades contrastantes de dissipar o excesso de energia de forma regulada. A deficiência de P afeta a produção de grãos da soja, no entanto, a magnitude da perda é influencia pelos mecanismos de eficiência no uso do P. Além disso, há uma complexa interação entre o status de nutrientes e a tolerância à seca na produtividade das culturas

Palavras-chave: Estresse abiótico; frações P; fotossíntese; eficiência no uso do P; *Glycine max*.

ABSTRACT

Crop growth and development depend on photosynthesis, but the photosynthetic process largely depends on phosphorus (P). However, most soils have low P fertility, which can compromise crop yields. The use of phosphate fertilizer is an alternative that provides Pi to plants, however it is a finite and expensive resource. Therefore, understanding the mechanisms that different genotypes present under P deficiency and how the interaction of other abiotic factors influence the mechanisms of efficiency in the use of P are essential for the selection of sustainable genotypes. We carried out two different experiments, the objective of the first being to elucidate how P deficiency affects the recovery from recurrent water stress in soybean cultivars with different levels of drought tolerance and the second aimed to understand the efficiency and response mechanism in use of P used by different soybean genotypes, relating photosynthetic and growth parameters. The results of the first experiment indicated that phosphorus deficiency delayed stomatal closure, prolonging photosynthetic decline under drought stress among cultivars. Interestingly, phosphorus-deficient plants showed faster recovery in photosynthetic rates and stomatal conductance compared to those adequately supplied with phosphorus. The sensitive cultivar demonstrated greater photosynthetic recovery after going through two cycles of water deficit, attributed to the improvement in mesophyll conductance. In contrast, the tolerant cultivar showed consistent photosynthetic responses regardless of phosphorus availability. These findings highlight the adaptive plasticity of soybeans to water deficit, modulated by genetic factors and influenced by phosphorus availability, highlighting the complex interaction between nutrient status and drought tolerance on crop productivity. The results of the second experiment demonstrated that cultivars with high photosynthetic responsiveness to P are also responsive in grain production. Responsive cultivars showed an increase in the carboxylation rate, RuBP regeneration and use of P-trioses in high phosphorus. The photosynthetic performance of the efficient and non-P-responsive cultivar was supported by the increased allocation of metabolic P and decreased lipid P, in addition to the more robust photosynthetic apparatus from the initial stages of development. The biggest difference between efficient and inefficient cultivars, regardless of response capacity, is the ability to dissipate excess energy in a regulated manner. Regardless of the P use efficiency mechanism, P deficiency affected the production of different genotypes.

Keywords: abiotic stress; phosphorus fractions; photosynthesis; efficiency use P.

INDICADORES DE IMPACTO

A agricultura brasileira enfrenta o desafio de aumentar a produtividade das culturas de maneira sustentável, especialmente devido à grande dependência de fertilizantes, como os fosfatados, que são essenciais para o crescimento das plantas. Esses fertilizantes, que fornecem fósforo (P) – um nutriente crucial para o processo fotossintético –, são em sua maioria importados de países com instabilidade político-econômica e são derivados de fontes não-renováveis. Isso eleva os custos e torna o sistema agrícola brasileiro vulnerável a flutuações no mercado internacional. A fotossíntese, processo fundamental para o crescimento das plantas, depende diretamente da quantidade de fósforo disponível. Portanto, entender como a deficiência de fósforo afeta o desempenho das culturas, como a soja, e como diferentes genótipos respondem a essa carência, se torna um aspecto central para a busca de alternativas mais sustentáveis. Os resultados desta tese mostraram que a deficiência de fósforo afeta negativamente a produção de grãos de soja, mas a magnitude dessa perda varia conforme os mecanismos de eficiência no uso do nutriente. Além disso, observou-se que existe uma interação complexa entre o status nutricional e a tolerância à seca, ambos impactando diretamente a produtividade das culturas. Esse estudo é relevante, pois contribui para a seleção de genótipos de soja mais eficientes no uso de fósforo, permitindo práticas agrícolas mais sustentáveis e reduzindo a dependência de fertilizantes importados. A compreensão desses mecanismos também pode ajudar a mitigar os impactos econômicos e ambientais da produção agrícola no Brasil.

IMPACT INDICATORS

Brazilian agriculture faces the challenge of increasing crop productivity in a sustainable manner, particularly due to the heavy reliance on fertilizers, such as phosphate fertilizers, which are essential for plant growth. These fertilizers, which supply phosphorus (P) – a crucial nutrient for the photosynthetic process – are mostly imported from countries with political and economic instability and are derived from non-renewable sources. This raises costs and makes the Brazilian agricultural system vulnerable to fluctuations in the international market. Photosynthesis, a fundamental process for plant growth, is directly dependent on the availability of phosphorus. Therefore, understanding how phosphorus deficiency affects crop performance, such as soybean, and how different genotypes respond to this deficiency, becomes a central aspect in the search for more sustainable alternatives. The results of this thesis showed that phosphorus deficiency negatively affects soybean grain production, but the extent of this loss

varies according to the mechanisms of phosphorus use efficiency. Furthermore, it was observed that there is a complex interaction between nutritional status and drought tolerance, both of which directly impact crop productivity. This study is relevant as it contributes to the selection of soybean genotypes that are more efficient in phosphorus use, enabling more sustainable agricultural practices and reducing dependence on imported fertilizers. Understanding these mechanisms can also help mitigate the economic and environmental impacts of agricultural production in Brazil.

SUMÁRIO

Introduction

Agriculture is facing increasing challenges, such as the need to produce more affordable food, fiber and clean energy, while also facing rising fertilizer costs and adverse climate change (Rosolem & Husted, 2024).

Crop growth and development depend on photosynthesis, but the photosynthetic process is dependent on phosphorus (P) nutrition (He et al., 2017; Taliman et al., 2019). P, in turn, is not highly available to plants in most Brazilian soils. To ensure food security in the face of growing global demand for food, using phosphate fertilizers has become a common and necessary practice. However, reliance on chemical fertilizers raises concerns about the sustainability and cost of these resources.

A promising alternative to reduce dependence on phosphate fertilizers is the development and use of P-efficient genotypes. Phosphorus use efficiency (PUE) refers to the amount of biomass produced per unit of tissue P or P added to the soil. PUE is influenced by different strategies such as P remobilization, alteration of organic P fractions, vacuolar Pi release (Abbas et al., 2018; Ham et al., 2018; Mo et al., 2019). Investing in research that identifies and improves these cultivars can offer a sustainable solution for agriculture, reducing the need for fertilizers and associated costs, as well as minimizing environmental impact.

In addition to being efficient in the use of P, the genotypes can be responsive to the addition of P to the soil. Responsiveness is associated with increased growth potential with additional P inputs. A plant with a low response to P addition but efficient P use can be an alternative to crops in poor soils, as long as yields are maintained (Hammond et al., 2009). Understanding the correlation between efficiency mechanisms and plant response can open paths for the selection and management of different genotypes.

Global climate change poses an additional challenge for agriculture. Drought events are increasingly intense and frequent and can worsen nutrient deficiencies in the soil, including P. Understanding how P deficiency alters plant response to drought and rehydration as well as the underlying mechanism is useful for implementing vegetation management practices related to climate change.

Although the effects of P deficiency and water deficit separately are well elucidated (Lazali & Drevon, 2021; Malhotra et al., 2018; Seleiman et al., 2021), the effect of P deficiency on recovery from recurring water deficit is unclear considering the bidirectional impact they have on each other.

Given these problems, this study investigated the effects of P deficiency on the drought

recovery capacity of soybean cultivars during grain filling. Furthermore, we seek to elucidate the mechanisms that efficient and responsive cultivars present, correlating with photosynthetic metabolism and production.

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Artigo 1- Phosphorus deficiency affects memory-mediated recovery from recurrent water stress in drought-sensitive soybean

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Abstract

Phosphorus (P) deficiency and water deficit are factors that hamper soybean productivity worldwide. Although the sole effects of those stressors are well known, it is not clear how P deficiency affects the recovery capacity of soybeans from recurrent water deficit. This study investigated the effects of P deficiency on the drought-recovery capacity of soybean cultivars during grain filling. Two soybean cultivars, a sensitive and a tolerant to water stress, were grown at either adequate P conditions or P deficiency following exposition to water deficit conditions. The experiment was conducted in a randomized complete block design with a 2x3 factorial scheme, with two substrates (with high and low phosphorus availability) and three irrigation conditions (WW – well-watered condition; WS-R5 - Severe water deficit at R5 developmental stage; WS-V5+R5 - Moderate water deficit at V5 and severe at R5 stage). The P deficiency delayed the stomatal resistance, which prolonged the decline in photosynthesis under water stress in both cultivars. However, the recovery of photosynthetic rates and stomatal conductance was faster in soybean plants under P deficiency than those under adequate P conditions. The sensitive cultivar under P deficiency showed an improved memory-mediated photosynthetic recovery capacity only when experienced two cycles of water deficit. The compensatory recovery of mesophyll conductance (g_m) in the sensitive cultivar when exposed to two cycles of water stress, resulted in less mesophyll limitation and contributed positively to the greater photosynthetic recovery. However, in the tolerant cultivar, the P deficiency did not affect the photosynthetic responses to water stress, with similar recovering of the photosynthetic processes, including light saturation point and photorespiration. The quantity and quality of grains were affected by P deficiency. In both cultivars P deficiency decreased the protein content and increased the oil content in grains, especially unsaturated fatty acids. The physiological adjustments of genotypes susceptible to drought stress are affected by P deficiency.

Keywords: Drought, mesophyll conductance, *Glycine max L.*, photosynthetic limitation, fatty acid profile, phosphorus nutrition.

1- Introduction

Soybean (Glycine max. L.) is the main legume cultivated worldwide, used mainly as a source of oil and protein for animal feed (Sentelhas et al., 2015a). Brazil is the largest global producer of soybeans, with 45 million hectares of cultivated areas (Embrapa, 2024). In the 2023/2024 harvest, soybean is expected to yield 155.3 million tons. This represents a 4.2% decrease from expectations, as initial projections pointed to a harvest of 162 million tons. The recent environmental changes, mainly poorly distributed rains and high temperatures, are the main causes of yield loss in soybean cultivation especially in rainfed productions (He et al., 2020; Conab, 2024).

Water deficit affects various soybean morphophysiological characteristics in all growth stages. However, when drought occurs in vegetative stages, tolerant plants can exhibit mechanisms capable of maintaining grain production(Medina et al., 2023). The soybean water demand doubles during the reproductive phase compared to the vegetative phase (Poudel et al., 2023). When water stress occurs in the reproductive stages, it can negatively affect flower setting, seed size, seed weight and composition, as well as the final harvest yield (Du et al., 2020; Mesquita et al., 2020).

Drought impact on yield is associated with decreases in the assimilation of photosynthetic CO_2 due to stomatal or non-stomatal factors (Pilon et al., 2018; Song et al., 2020). The non-stomatal limitation of photosynthesis under drought comprises the overproduction of reactive oxygen species (ROS) (Kaur & Asthir, 2017). The damage caused by ROS to cell membranes can be observed with increased malondialdehyde (MDA) concentration (Zhou et al., 2022).

Drought impact on yield is associated with decreases in the assimilation of photosynthetic CO_2 due to stomatal or non-stomatal factors (Pilon et al., 2018; Song et al., 2020). The non-stomatal limitation of photosynthesis under drought comprises the overproduction of reactive oxygen species (ROS) (Kaur & Asthir, 2017). The damage caused by ROS to cell membranes can be observed with increased malondialdehyde (MDA) concentration (Zhou et al., 2022).

Photosynthesis and plant growth can begin to be stimulated immediately after irrigation is resumed. However, the extent of the recovery process depends on the intensity and duration of the drought (Fleta-Soriano & Munné-Bosch, 2016). Plants can have partial, total or compensatory recovery. Severe stress can cause irreversible damage to the photosynthetic system and result in only partial recovery. After a short, moderate drought event, plants may

experience compensatory growth after rehydration (Dong et al., 2019). Compensation is an essential self-regulatory mechanism plants use to compensate for damage and losses caused during drought stress (Niu et al., 2018). Recovery from a first stressful event can prepare the body for a better response to subsequent stress. These priming effects are supported by metabolic memory processes, affecting plant responses to new, potentially stressful events (Auler et al., 2021; Sintaha et al., 2022).

The memory-mediated recovery from recurrent exposure to stress is a complex mechanism presented by plants under water scarcity, that would allow improved performance and decreased yield loss (Crisp et al., 2016; Fleta-Soriano & Munné-Bosch, 2016; Medina et al., 2023). This induced memory empowers plants to more effectively resume stress response mechanisms when subjected to similar conditions in the future (Kim et al., 2020; Oberkofler & Bäurle, 2022). Medina et al., (2023) observed an increase in proline concentration in leaves and faster isohydric responses related to photosynthetic adjustments in soybean plants subjected to recurrent drought, which allowed grain production maintenance. However, nutritional deficiencies, including phosphorus (P) decrease plants' ability to respond to water deficit (Jin et al., 2007; Meena et al., 2020), which influences soybean yield and grain quality due to changes in fatty acids profile (Krueger et al., 2013; Win et al., 2010).

Understanding how plants respond to drought and rehydration, as well as the underlying mechanism, is helpful for implementing climate change crop management practices. Although the effects of P deficiency and water deficit separately are well elucidated in the literature (Lazali & Drevon, 2021a; Malhotra et al., 2018; Seleiman et al., 2021), the effect of P deficiency on recovery from recurrent water deficit is not clear, considering the bidirectional impact they have on each other. The ability to generate new molecules for post-stress homeostasis is related to the energetic metabolism with a central role of P nutrition in drought-tolerant cultivars (Gelaw et al., 2023). Therefore, it is important to elucidate how P deficiency affects the memory recruitment for the recovery capacity of the photosynthetic apparatus after consecutive cycles of water stress in soybean.

The objective of this work is to elucidate how P deficiency affects the recovery from recurrent water stress in soybean cultivars with different drought-tolerance levels, and its impacts on yield and grain fatty acid profile. We investigated if the buildup of stress memory mechanisms is linked to P deficiency, changing the drought-response time, the capacity for photosynthetic apparatus recovery, and the morphological adjustments that ensure grain quality and yield. We hypothesize that the drought-tolerant soybean cultivar subjected to two cycles of water stress, will present faster activation of stress memory mechanisms than the sensitive

cultivar. We expect that adequate P nutrition will be essential in the recovery of the photosynthetic apparatus, in order to avoid oxidative damage, and losses in grain quantity and quality.

2- Material and Methods

2.1 Plant species and growth conditions

The experiment was conducted in a greenhouse at the Federal University of Viçosa Campus Florestal (19°52'35.1" S 44°24'49.6" W). The mean temperature and relative humidity inside the greenhouse were 26.4ºC and 40.5%, respectively. We used the soybean cultivars TMG7063 and EMBRAPA048, which are known for being sensible and tolerant to water stress, respectively (E. O. Freitas et al., 2019; Winck et al., 2023). Both cultivars have an early maturity group; however, EMBRAPA048 and TMG7063 have determinate and indeterminate growth habits, respectively (Higashi et al., 1999). The soybean seeds were treated with Vitavax Thiram® fungicide (Carboxanilide + Dimethyldithiocarbamate) 250ml/100 kg and inoculated with Adhere® 60g/100 kg. Then, 5 soybean seeds were sown in each 20 dm^3 pot, with a substrate composed of soil and sand in a 3:1 (v:v) ratio.

After emergence, thinning was performed, leaving only one plant per pot. The soil used has physical characteristics similar to dystrophic red latosol, with initial values of 0.049 dag/kg total nitrogen, 2.4 mg/dm^3 phosphorus, 65 mg/dm^3 potassium, 0.62 cmolc/dm^3 calcium, 0.21 cmolc/dm^3 magnesium, 1.34 cmolc/dm^3 aluminum, 3.5 cmolc/dm^3 total acidity, 1.0 cmolc/dm^3 sum of bases, 2.34 cmolc/dm^3 effective cation exchange capacity, 4.5 cmolc/dm^3 total cation exchange capacity, 22.2% base saturation, 57.3% aluminum saturation, 7.7 mg/L remaining phosphorus, and pH 4.66. The pH was corrected with dolomitic limestone, PRNT 80%, in the quantity obtained by the base saturation method (3.78 t/ha), considering soil chemical analysis. Additionally, the limestone quantity was sufficient to meet the demands of Ca^{2+} and Mg^{2+} and stimulate nitrogen-fixing bacterial activity. Potassium oxide fertilization was applied at a dosage of 40 kg/ha. Phosphorus fertilization was only applied to control pots at a dosage of 120 kg/ha (Ribeiro et al., 1999).

Three water deficit conditions were imposed: WW - well-watered (control condition); WS-R5 - severe water deficit at R5; WS-V5+R5 - moderate water deficit at V5 and severe at R5. Soil moisture was monitored with tensiometers, maintaining it at field capacity (around 30 KPa) throughout the crop cycle in control well-watered plants (WW) and until V5 Fehr &

Caviness, (1977) in the plants exposed to water stress (WS). At the V5 stage, plants in the WS-V5+R5 treatment were subjected to moderate water stress, determined until the soil water tension, assessed by tensiometers, reached 80 KPa. Then, the soil was irrigated and maintained at field capacity. At the beginning of the grain filling stage (R5), the plants from the WS-R5 treatment, and again the WS-V5+R5 treatment, were subjected to severe water stress. Plants were kept without irrigation until net photosynthesis reached near zero, characterizing this stress as severe. Therefore, water stress was restricted to the V5 and R5 stages. After this period, irrigation was resumed, and the recovery of photosynthetic metabolism was monitored. There was no water restriction in the control treatment (WW) throughout the crop cycle.

2.2 Experimental design and statistical analysis

A randomized complete block design was adopted in a 2x3 factorial, with two substrate conditions (with high and low phosphorus availability) and three irrigation conditions (WW - control condition; WS-R5 - Severe water deficit at R5; WS-V5+R5 - Moderate water deficit at V5 and severe at R5), with five replications.

The data were considered homogeneous and normal, using the Barlett and Shapiro-Wilk tests, respectively. Subsequently, they were subjected to analysis of variance (ANOVA) and compared using the Tukey test ($p<0.05$), carried out in the statistical program RStudio (version 3.6.3).

2.3.1 Monitoring and characterization of severe water stress

2.3.1.1 Gas Exchange

Gas exchange assessments were conducted daily, starting from the onset of severe water deficit imposition at the R5 stage. Net photosynthetic rate (A_n) and stomatal conductance (g_s) measurements were taken in the morning with the first fully expanded leaf from the apex, on the central leaflet. An infrared gas analyzer (LI-6400xt, Li-Cor Inc., Lincoln, Nebraska, USA), was used with a photosynthetic photon flux density (PPFD) of 1,200 μmol m^{-2} s^{-1}, CO_2 concentration of 400 μmol mol^{-1}, temperature of 28 ºC, and relative humidity of 44%.

2.3.1.2 Water potential

Predawn leaf water potential (ψ_w) was determined on the day of maximum stress using a digital Scholander pressure chamber, model 1400/80 (PMS Instrument Company, UK), on the day of maximum severe water stress at the R5 stage.

2.3.1.3 Proline

Proline concentration was determined in the second mature leaf collected on the day of maximum stress, following the protocol of Bates et al., (1973). For this, 100 mg of dry mass of leaf tissue was macerated in 2.5 ml of 3% sulfosalicylic acid. Then, the mixture was centrifuged for 10 minutes at 12,000 x g at 25 ℃, and the supernatant was subsequently collected and transferred to a test tube. A volume of 2.5 mL of sulfosalicylic acid was added to the tubes, which were centrifuged again under the same conditions mentioned above. After the described processes, an aliquot of 1 mL of the plant extract was added to a test tube with 1 mL of acidic ninhydrin and 1 mL of glacial acetic acid. The samples were placed in a water bath for 1 hour at 100 °C. Then, they were cooled on ice, and 2 mL of pure toluene was added for phase separation. The reading was performed with the upper aqueous phase in a spectrophotometer at 520 nm. A calibration curve was used to obtain proline concentration values.

2.3.1.4 Determination of malondialdehyde

Malondialdehyde (MDA) levels were estimated using the methodology proposed by Hodges & DeLong, (1999) on the day of maximum water stress. For this, the first mature leaf, starting from the apex, was collected and 0.1 g of fresh leaf tissue was ground in liquid nitrogen using a mortar and pestle, adding 1 mL of 80% ethanol. The sample was vortexed (Phoenix, model AP:54) and placed in a refrigerated ultrasonic bath (Elmasonic S30 H) for 15 minutes. Then, the samples were centrifuged (Thermo scientific, megafuge 16R) for 10 minutes at 10,000 x g, at 4°C. The supernatant was collected, and the extraction process was repeated two more times. From the resulting extract, 1 mL was added to screw-capped test tubes containing 1 mL of TBA+ solution (20% trichloroacetic acid, 0.01% butylated hydroxytoluene, and 0.65% thiobarbituric acid). Again, 1 mL of the sample was added to tubes containing 1 mL of TBA- solution (20% trichloroacetic acid, 0.01% butylated hydroxytoluene). The tubes were incubated in a water bath (Ethik technology, 521-3D) for 25 minutes at 95°C. After this time, the tubes were placed in an ice bath for 10 minutes. Subsequently, the samples were centrifuged for 10 minutes at 25°C and 10,000 x g, and absorbance measurements were made using a

spectrophotometer (Shimadzu, UV-1800) at wavelengths of 440, 532, and 600 nm. Calculations were performed according to the equation:

MDA= {[(A523 - A600) - (A440 - A600) (molar absorbance of sucrose at 532/ molar absorbance of sucrose at 440)] / 157000} Eqn.1

Where A is the absorbance value, MA is the molar absorbance, and 157000 is the molar extinction coefficient (Hodges et al., 1999).

2.3.1.5 Determination of hydrogen peroxide (H_2O_2)

The H_2O_2 concentrations were estimated according to the methodology proposed by Velikova et al., 2000), on the day of maximum severe water stress at the R5 stage. The first mature leaf, starting from the apex, collected on the day of maximum water stress, was used. For this, 0.1 g of each sample was ground in liquid nitrogen using a mortar and pestle, with the addition of 1 mL of 0.1% TCA (trichloroacetic acid). Then, they were centrifuged (Thermo scientific, megafuge 16R) for 15 minutes at 12,000 x g at 4°C. Subsequently, 0.250 mL of the supernatant was mixed with 0.250 mL of 0.1% TCA and 1 mL of 1M potassium iodide. Absorbance was measured using a spectrophotometer (Shimadzu, UV-1800) at a wavelength of 390 nm. The hydrogen peroxide concentration was estimated using a standard curve (Velikova et al., 2000).

2.3.2 Monitoring and characterization of water stress recovery.

2.3.2.1 Characterization of photosynthetic apparatus recovery - Light saturation curve (A_n/PPFD) *and CO_2 saturation curve (A/Ci).*

During the recovery period with resumed irrigation, the gas exchange assessments were conducted in the same way described previously by using the LI-6400xt infrared gas analyzer (Li-Cor Inc., Lincoln, Nebraska, USA), equipped with a fluorescence chamber (Li-6400-40). The recovery of the water status was considered complete only when the plants showed a net photosynthetic rate similar to that of the control plants.

After the completion of this rehydration process, light saturation (A_n/PPFD) and CO_2 saturation (A_n/*Ci*) curves were performed on the central leaflets of mature leaves. Light

saturation measurements were taken at 2000, 1500, 1250, 1000, 800, 600, 400, 200, 100, 75, 50, 25, 0 μmol photons m^{-2} s^{-1}. The minimum pre-established time for stabilization of readings at each light radiation level was 60 seconds. Atmospheric CO_2 inside the leaf chamber was kept constant during the light saturation curve determinations at 400 μmol CO_2 mol^{-1}. The data were adjusted according to Lobo et al., (2013) and Smith, E.L. (1936). according to Eqn. 2:

$$A_n = [\phi\ (Io)\ x\ I\ x\ P_gmax\ /\ (\ \phi\ (Io)^2\ x\ I^2 + P_gmax\ ^2)^{0.5}] - R_D \qquad \text{Eqn. 2}$$

Where:

A_n = net photosynthetic rate [μmol (CO_2) m^{-2} s^{-1}]

ϕ (Io) = quantum yield at light saturation point I = 0 [mmol (CO_2) $mmol^{-1}$ (photons)];

I = photosynthetic photon flux density [μmol (photons) m^{-2} s^{-1}];

$P_g$max = maximum gross photosynthesis rate [μmol (CO_2) m^{-2} s^{-1}];

R_D = dark respiration rate [μmol (CO_2) m^{-2} s^{-1}].

From this modeling, values of maximum photosynthetic rate (A_{max}), light compensation point (I_{comp}), light saturation point beyond which there is no significant change in net photosynthesis (I_{max}), effective quantum yield at the compensation point (ϕ (I_{comp})).

The CO_2 saturation curves were conducted at levels of 400, 300, 200, 100, 50, 400, 500, 600, 700, 800, 1,000, 1,200, and 1,300 μmol CO_2 mol^{-1}. The minimum time for stabilization of readings at each concentration was 60 seconds and a maximum of 120 seconds. The data obtained in the A_n/Ci curve were used to calculate the Rubisco carboxylation rate (V_{cmax}), electron transport rate (J), triose phosphate utilization (TPU) (Sharkey et al., 2007). Mesophyll conductance (g_m, mol CO_2 m^{-2} s^{-1}) and chloroplastic CO_2 concentration (C_c, μmol CO_2 mol^{-1}) were estimated using the method described by Harley et al., (1992), according to Eqn. 3,4:

$$g_m = A_n\ /\ (Ci\text{-}\ (\Gamma^*[\ J\text{+}8\ (A_n\text{+}R_I)])/\ (J\text{-}4\ (A_n\text{+}R_I))) \qquad \text{Eqn. 3}$$

$$C_c = Ci - A_n\ /g_m \qquad \text{Eqn. 4}$$

Where R_I is respiration occurring during the day and not related to photorespiration. The value of the CO_2 compensation point related to Ci (Γ^* μmol mol^{-1}) for soybean was calculated by the intersection of A_n/Ci according to Walker & Ort, (2015). From these, stomatal limitations (l_s), mesophyll limitations (l_m), and metabolic limitations (l_b) were calculated according to the

method described by Grassi & Magnani, (2005), according to Eqn. 5,6,7:

$$l_s\,(25°C) = ((g_{tot} / g_{sc}) * (\partial A_n / \partial C_c)) / g_{tot} + \partial A_n / \partial C_c \quad \text{Eqn. 5}$$

$$l_m\,(25°C) = ((g_{tot} / g_m) * (\partial A_n / \partial C_c)) / g_{tot} + \partial A_n / \partial C_c \quad \text{Eqn. 6}$$

$$l_b\,(25°C) = g_{tot} / g_{tot} + \partial A_n / \partial C_c \quad \text{Eqn. 7}$$

$$l_s + l_m + l_b = 1$$

Where g_{tot} is total conductance to CO_2 between the leaf surface and carboxylation sites ($1/g_{tot} = 1/g_{sc} + 1/g_m$) and g_{sc} is stomatal conductance to CO_2 ($g_{sc} = g_s/1.6$).
The photorespiration rate (P_r) was estimated using the following formula (Valentini et al., 1995), according to Eqn. 8:

$$Pr = \frac{1}{12}[\,\mathrm{ETR} - 4(A_n + R_D/2)] \quad \text{Eqn. 8}$$

Where ETR is apparent rate of electron transport, obtained simultaneously with gas exchange analysis.

2.3.2.2 Chlorophyll a fluorescence

The measurement of chlorophyll *a* fluorescence was performed using the Mini-PAM pulse-amplitude-modulated fluorometer (Heinz Walz, Effeltrich, Germany). The evaluation took place at the end of the water stress recovery period in the morning, on the first fully expanded leaf from the apex, on the central leaflet. Minimum fluorescence (F_0) and maximum fluorescence (F_m) were measured in the early morning after leaf acclimation in the dark for at least 30 minutes. The obtained values were used to determine the maximum quantum efficiency of photosystem II (*PSII*; F_v/F_m) according to Eqn. 9:

$$F_v/F_m = (Fm - F_0)/Fm) \quad \text{Eqn.9}$$

2.3.2.3 Chlorophyll indices

The assessment of total chlorophyll index was conducted at the end of the water stress recovery period using the portable ClorofiLOG meter (Falker, Brazil). The index was

characterized by the average of three measurements taken on the central leaflet of the first fully mature leaf from the apex.

2.3.2.4 Morphological evaluations

At stage R8 (full pod maturity) (Fehr & Caviness, 1977), the plants were sectioned into root, grain, pod, leaf, stem, and dried in a forced-air circulation oven for 72 hours at 65°C to determine dry biomass.

Leaf area (LA; cm^2) was calculated according to Eqn.10:

$$LA = A.(C.L) \quad \text{Eqn.10}$$

Where C is the length of the leaflet (cm), L is the widest width of the leaflet (cm), A is the angular coefficient, where the value of 2.0185 was adopted(Richter et al., 2014).

2.3.2.5 Leaf P concentration

The P concentrations were estimated according to the methodology proposed by Embrapa (2017). Mature leaves were collected at the end of the water stress recovery period and also at the end of the biological cycle in R8 to quantify P concentration. Perchloric-nitric digestion was carried out on 250 mg of dry and ground material. Absorbance was measured using a spectrophotometer (Shimadzu, UV-1800) at a wavelength of 660nm. The P concentration was estimated using a standard curve, using the dye ammonium molybdate and ascorbic acid.

2.3.2.6 Oil and protein concentration in grains

Oil extraction was carried out on 2g of ground and dried grain samples using the Soxhlet method (Adolfo Lutz Institute, 2008). The solvent used was petroleum ether. The percentage of lipids was given by the Eqn. 11:

$$\text{LIPIDS}\ (\%) = (\text{PL} \times 100) / \text{P} \quad \text{Eqn. 11}$$

Where:

PL = Weight of the balloon with fat – Weight of the balloon before extraction

P = Sample weight

For the protein concentration, the methodology proposed by Kjeldahl was used, finding the value of the total nitrogen (N) of the sample and later, converting to crude protein by the factor 6.25. Four samples of 0.5g of dry and ground material was used for each cultivar in each one of the tests.

2.3.2.7 Grain fatty acid profile

Samples of 15 mg of seeds were ground and mixed with 1 mL of hexane for 16 hours. Then, the solvent was evaporated under N_2 and 0.4 mL of 1M sodium methoxide was added, and the sample was kept in a water bath at 30°C for one hour. Subsequently, 1 mL of Milli-Q water and 1 mL of hexane were added, and the sample was left to stand for another hour. Finally, 0.75 mL of the organic phase was collected, sodium sulfate was added, and chromatographic reading was performed.

Analyses were carried out using a gas chromatograph model CG Solution by SHIMADZU, equipped with a FID detector. For chromatogram recording and analysis, the device is connected to a microcomputer using the GC Solution program. Compounds were separated and identified on a Carbowax capillary column (30 m x 0.25 mm).

For chromatographic separation, 1 μL of sample was injected using a 10 μL syringe (Hamilton®) in Split = 5 system. Nitrogen gas was used as the carrier with a linear velocity programmed to 43.2 cm/s, and hydrogen and synthetic air gases formed the flame in the detector. The injector and detector temperatures were controlled isothermally at 200°C and 220°C, respectively. The initial column temperature was 100°C (maintained for 5 minutes), increasing at a rate of 4°C per minute until reaching 220°C (maintained for 20 minutes). The carrier gas flow rate in the column was 1.0 mL/min.

3 – Results

Regardless of the P nutritional condition, plants of both cultivars subjected to water deficit at the V5 stage and subsequently re-submitted to stress at the R5 stage (WS-V5+R5) showed no differences in photosynthetic responses compared to those exclusively exposed to stress at the R5 stage (Fig. 1).

The P deficiency prolonged the decline in photosynthesis (Fig. 1C, 1D) and stomatal closure (Fig. 1G, 1H) in response to water stress in both cultivars. However, the recovery of

photosynthetic rates and stomatal conductance was faster in soybean plants under P deficiency than those under adequate P conditions (Fig. 1).

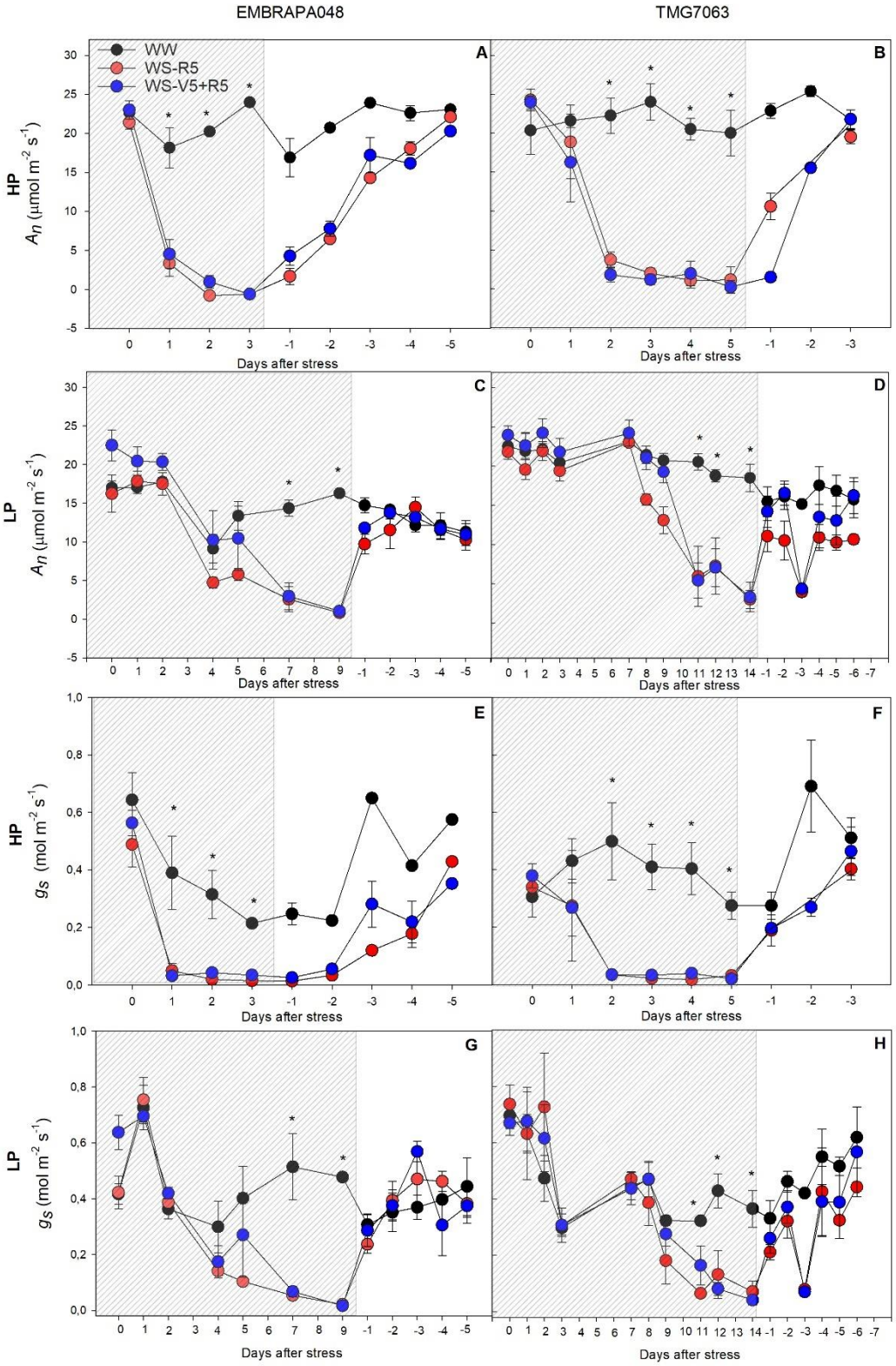

Fig. 1 Net photosynthetic rates (A_n; A, B, C, D) and stomatal conductances (g_s; E, F, G, H) of soybean

cultivars EMBRAPA048 (A, C, E, G) and TMG7063 (B, D, F, H) subjected to either adequate P conditions (HP) or P deficiency (LP), under water deficit during grain filling. The shaded area of the graph represents the period of water stress, and the white area represents the recovery period after hydration. Symbols represent the mean of four replicates, and error bars represent the standard error of the mean. Asterisks indicate statistical differences between treatments, according to Tukey's test at a 5% confidence level.

Both cultivars exhibited lower leaf water potential during maximum water stress (Fig 2A, 2B). However, the cultivar EMBRAPA048 maintained higher leaf water potential under water stress conditions concurrent with phosphorus deficiency (Fig. 2A). The drought-tolerant cultivar, EMBRAPA048, accumulated higher proline concentration in leaves when exposed to both WS-R5 and WS-V5+R5 water stress treatments (Fig. 2C). However, there was no significant difference in proline concentration in cultivar TMG7063 during maximum drought stress (Fig. 2D).

The MDA concentration in leaves was higher in plants from treatments with water stress concurrent with higher P availability in both cultivars (Fig. 2E, 2F). The cultivar EMBRAPA048 exhibited higher H_2O_2 concentration under water stress conditions and adequate P availability (Fig. 2G). In contrast, the TMG7063 cultivar showed higher H_2O_2 levels in both water stress conditions (WS-R5 and WS-V5+R5), regardless of the P nutrition (Fig. 2H).

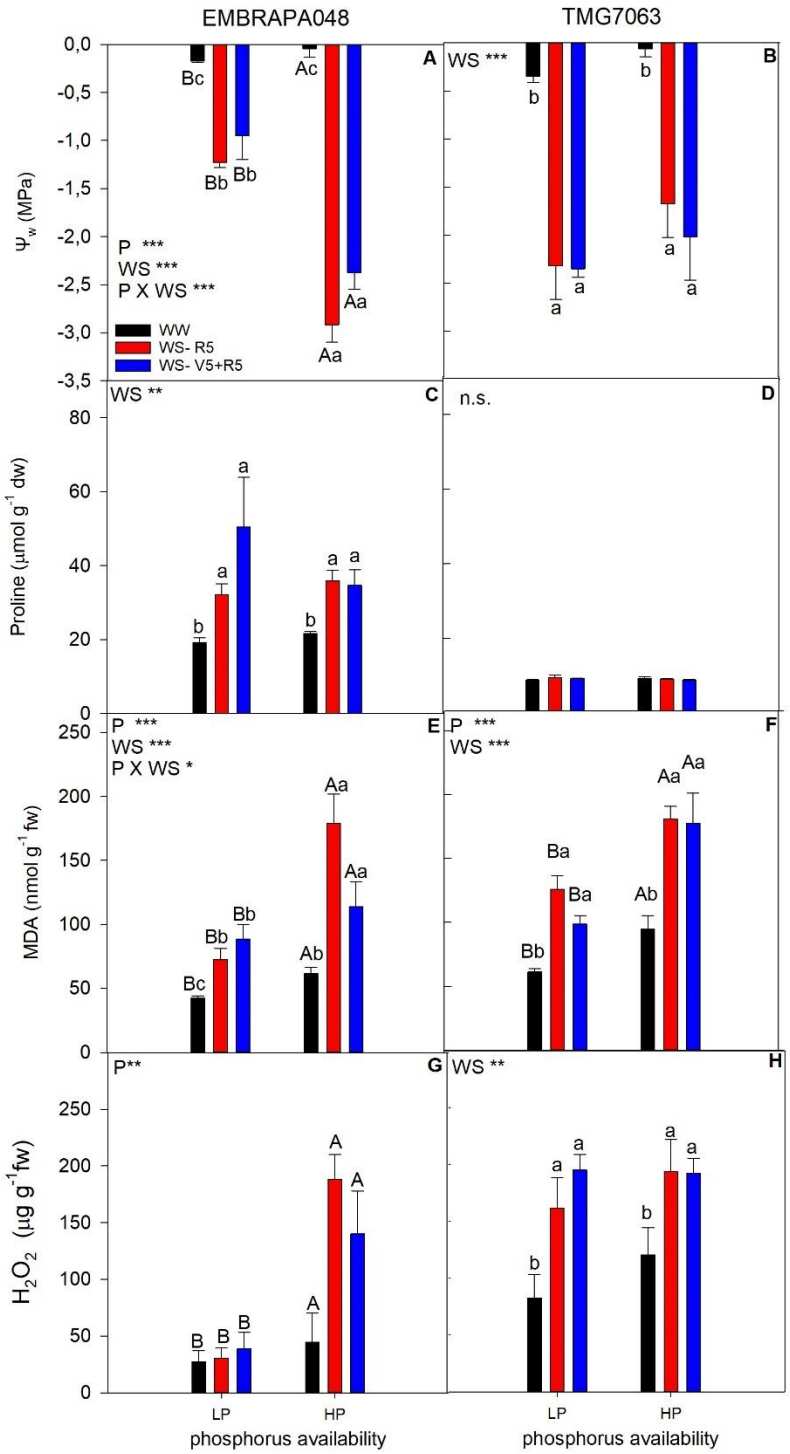

Fig. 2 Leaf Water Potential (ψ_w; A, B), proline (C, D), malondialdehyde (MDA; E, F), hydrogen peroxide concentration (H_2O_2; G, H) in soybean cultivars EMBRAPA048 (A, C, E) and TMG7063 (B, D, F) during the peak water stress period under optimal P conditions (HP) or phosphorus-deficient conditions (LP), during grain filling water deficit. Bars represent the mean of four replicates, and error bars represent the standard error of the mean. Uppercase letters represent statistical differences between nutritional conditions, and lowercase letters represent statistical differences between water conditions, by Tukey's test at 5% confidence level.

The EMBRAPA048 cultivar after recovering from both cycles of water stress (WS-V5+R5) exhibited higher photosynthetic rates in response to increased irradiance under both P

conditions (Fig. 3A, 3C). However, in the drought-sensitive cultivar TMG7063, the WS-V5+R5 plants only showed higher A_n than the WW ones with increased irradiance under P deficiency (Fig. 3B, 3D).

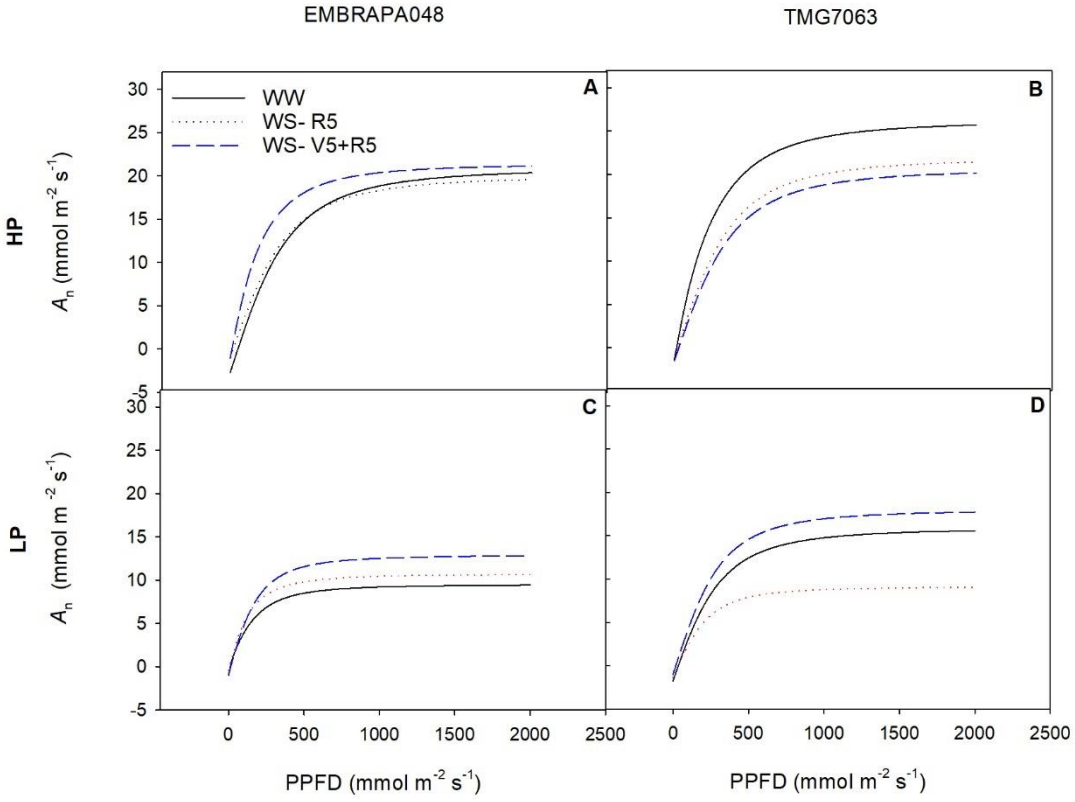

Fig. 3 Light response curves of net photosynthetic rate (A_n) in soybean cultivars EMBRAPA048 (A and C) and TMG7063 (B and D) subjected to adequate P conditions (HP) or P deficiency (LP) under grain filling water deficit.

The recovery from water deficit did not influence A_{max} and I_{max} in both cultivars, decrease in these parameters occurred only due to phosphorus deficiency (Table 1). There was no difference in the light compensation irradiance in the cultivars, regardless of water and nutritional conditions. However, the effective quantum yield at the compensation point ($\phi(I_{comp})$ was lower in soybean plants under P deficiency.

In both cultivars, F_v/F_m and also the total chlorophyll index were higher in soybean plants under adequate P conditions than those under P deficiency (Table 1).

Table 1 – Maximum photosynthetic rate (A_{max}; µmol (CO_2) m^{-2} s^{-1}), light compensation point (I_{comp}; µmol (photons) m^{-2} s^{-1}), light saturation point beyond which there is no significant change in net photosynthesis ($I_{max;}$ µmol (CO_2) µmol^{-1} (photons)), effective quantum yield at the compensation point (ϕ (I_{comp})), of soybean cultivars EMBRAPA048 and TMG7063 during

the recovery period from water stress under two phosphorus conditions: HP and LP. Different lowercase letters indicate significant differences by Tukey's analysis ($P < 0.05$) between water stress, and different uppercase letters indicate significant differences between phosphorus nutrition.

		EMBRAPA048			TMG7063		
	WW		WS-R5	WS-V5+R5	WW	WS-R5	WS-V5+R5
A_{max}	HP	20.36±0.21	19.58±0.34	21.14±0.85	25.70±1.26	21.43±0.99	20.17±1.46
(μmol (CO_2)		**Aa**	**Aa**	**Aa**	**Aa**	**Aa**	**Aa**
m^{-2} s^{-1})	LP	9.42±0.82	10.63±0.49	12.80±0.46	15.57±3.26	9.04±0,86	17.73±1.87
		Ba	**Ba**	**Ba**	**Ba**	**Ba**	**Ba**
I_{max}	HP	552.75±8.46	505±8.80	426.75±10.28	530±19.98	538±2.46	529.66±10.81
(μmol		**Aa**	**Aa**	**Aa**	**Aa**	**Aa**	**Aa**
(photons) m^{-2} s^{-1})	LP	217.25±19.57	227.25±15.71	294.66±2.78	411±21.51	267.33±9.07	438±15.78
		Ba	**Ba**	**Ba**	**Ba**	**Ba**	**Ba**
I_{comp}	HP	50.60±1.35	20.17±1.78	28.01±2.68	24.74±2.94	23.11±2.30	28.97±3.21
(μmol		**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
(photons) m^{-2} s^{-1})	LP	37.26±5.80	22.50±2.67	32.97±3.27	38.08±2.93	34.68±1.88	34.7096±1.65
		n.s.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
ϕ (I_{comp})	HP	0.053±0.002	0.050±0.002	0.058±0.006	0.061±0.004	0.054±0.003	0.050±0.002
(μmol (CO_2)		**A**	**A**	**A**	**A**	**A**	**A**
$umol^{-1}$	LP	0.084±0.032	0.066±0.008	0.061±0.016	0.049±0.006	0.038±0.001	0.052±0.003
(photons))		**B**	**B**	**B**	**B**	**B**	**B**
F_v/F_m	HP	0.846±0.002	0.841±0.001	0.845±0.0006	0.847±0.0007	0.836±0.003	0.837±0.002
		A	**A**	**A**	**Aa**	**Ab**	**Ab**
	LP	0.772±0.004	0.777±0.008	0.774±0.016	0.805±0.009	0.744±0.021	0.782±0.008
		B	**B**	**B**	**Ba**	**Bc**	**Bb**
Total	HP	47.98±0.55	35.83±2.11	40.71±1.82	44.43±0.67	34.80±1.80	36.95±1.41
chlorophyll		**Aa**	**Ab**	**Ab**	**Aa**	**Ab**	**Ab**
index	LP	39.98±0.83	35.88±1.35	36.87±3.39	40.25±1.19	29.38±1.87	33.05±3.04
		Ba	**Bb**	**Bb**	**Ba**	**Bb**	**Bb**

In both cultivars, P deficiency resulted in a decreased maximum carboxylation rate (Fig. 4A, 4B), electron transport rate (Fig. 4C, 4D), and triose phosphate utilization (Fig. 4E, 4F).

While in the drought-tolerant cultivar EMBRAPA048, the recovering from water stress did not influence the parameters, in the sensitive cultivar TMG7063, plants that underwent two cycles of water stress (WS-V5+R5) showed *J* (Fig. 4D) and *TPU* (Fig. 4F) higher than plants from WS+R5 and similar to control plants.

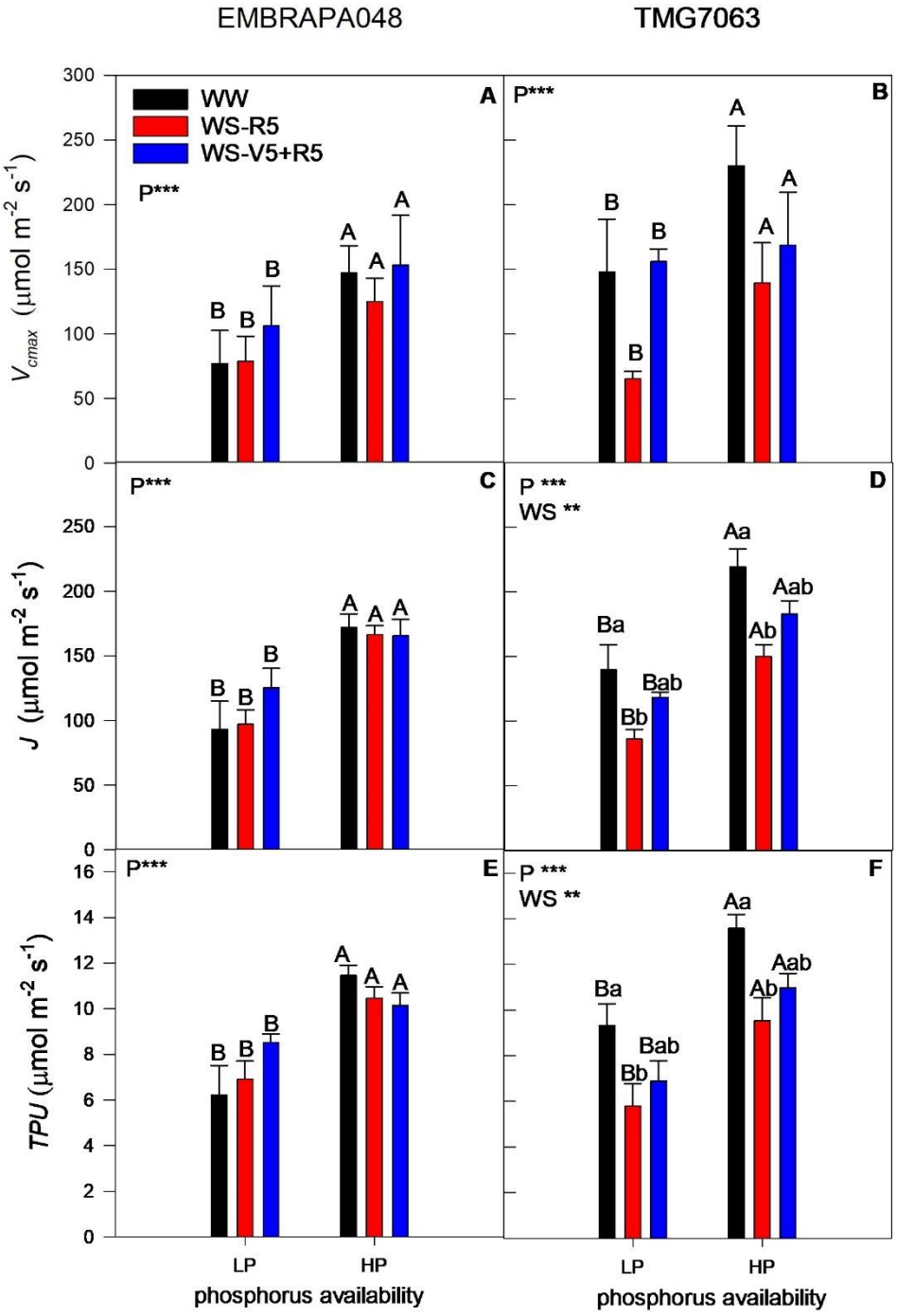

Fig. 4 Maximum carboxylation rate (V_{cmax}; A, B), electron transport capacity (*J*; C, D), triose phosphate utilization (*TPU*; E, F) in soybean cultivars, EMBRAPA048 (A, C, E) and TMG7063 (B, D, F), subjected to optimal P conditions (HP) or P deficiency (LP), under water deficit during grain filling. The bars represent the mean of four repetitions, and the error bars represent

the standard error of the mean. Uppercase letters denote statistical difference between nutritional conditions, and lowercase letters denote statistical difference between water conditions, by Tukey's test at 5% significance level.

The cultivar EMBRAPA048 showed lower mesophyll conductance under P deficiency conditions, resulting in greater mesophyll limitation (Table 2). Stomatal limitation occurred only under P deficiency conditions during the recovery period and no significant differences were observed in biochemical limitation and respiratory rates for this same cultivar.

On the other hand, cultivar TMG7063 decreased mesophyll conductance during recovering from water stress, regardless of the P nutritional condition. Plants of the cultivar TMG7063 subjected to both stress cycles (WS-V5+R5) exhibited higher mesophyll conductance and lower mesophyll limitation in the recovering phase. In contrast, plants subjected only to stress at R5 (WS-R5) showed lower mesophyll conductance and consequently higher mesophyll limitation. During the recovery from drought, the cultivar TMG7063 in the WS-V5+R5 treatment also maintained greater stomatal limitation in plants under P deficiency. Plants from the WS-R5 treatment presented full recovery, with the lowest stomatal limitation values. In summary, while the WS-R5 treatment resulted in mesophyll limitation to the soybean TMG7063 cultivar, plants from the WS-V5+R5 treatment exhibited stomatal limitation. The respiratory rate of cultivar TMG7063 was lower upon recovering from water stress, regardless of P availability in the soil (Table 2). There was no difference in respiratory rates in EMBRAPA048. The photorespiration rate was greater in plants from both cultivars under adequate P condition, regardless of the water stress exposure (Table 2).

Table 2 - Mesophyll conductance (g_m), mesophyll limitation (l_m), stomatal limitation (l_s), biochemical limitation (l_b), dark respiration (R_D), photorespiration (P_r), of soybean cultivars EMBRAPA048 and TMG7063 during the recovery period from water stress under two phosphorus conditions HP and LP. Different lowercase letters indicate significant differences by Tukey's analysis (P <0.05) between water stress conditions, and different uppercase letters indicate significant differences between phosphorus nutrition conditions.

		EMBRAPA048			TMG7063		
		WW	WS-R5	WS-V5+R5	WW	WS-R5	WS-V5+R5
g_m	HP	0.19±0.005	0.29±0.057	0.23±0.033	0.18±0.025	0.12±0.027	0.25±0.058
(mmol		**A**	**A**	**A**	**b**	**b**	**a**

$m^{-2}\ s^{-1}$)	LP	0.04±0.008	0.04±0.006	0.06±0.005	0.06±0.015	0.04±0.005	0.29±0.003
		B	**B**	**B**	**b**	**b**	**a**
l_m	HP	0.30±0.07	0.17±0.05	0.29±0.06	0.47±0.04	0.45±0.07	0.35±0.08
		B	**B**	**B**	**a**	**a**	**b**
	LP	0.61±0.02	0.59±0.03	0.60±0.06	0.66±0.03	0.60±0.03	0.17±0.007
		A	**A**	**A**	**a**	**a**	**b**
l_s	HP	0.29±0.06	0.51±0.10	0.37±0.04	0.22±0.01	0.23±0.02	0.26±0.02
		A	**A**	**A**	**c**	**c**	**b**
	LP	0.10±0.01	0.12±0.02	0.10±0.01	0.11±0.002	0.12±0.01	0.53±0.005
		B	**B**	**B**	**d**	**d**	**a**
l_b	HP	0.40±0.03	0.30±0.05	0.32±0.03	0.29±0.05	0.30±0.09	0.38±0.06
		n.s.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
	LP	0.28±0.02	0.2±0.03	0.28±0.05	0.21±0.04	0.26±0.02	0.22±0.03
		n.s.	**n.s.**	**n.s.**	**n.s.**	**n.s.**	**n.s.**
R_D	HP	1.91±0.17	0.77±0.13	1.68±0.13	1.63±0.05	0.78±0.02	1.09±0.09
(umol		**n.s**	**n.s**	**n.s**	**Aa**	**Ab**	**Ab**
(CO_2)	LP		0.73±0.02	1.33±0.04	1.68±0.07	0.94±0.03	
$m^{-2}\ s^{-1}$)		1.11±0.05	**n.s**	**n.s**	**Aa**	**Ab**	0.9±0.10
		n.s					**Ab**
P_r	HP	5.52±0.67	5.74±0.49	4.87±0.23	6.68±0.51	6.54±0.62	5.43±0.51
(umol		**A**	**A**	**A**	**A**	**A**	**A**
(CO_2)	LP	3.50±0.40	4.08±0.60	4.27±0.12	4.76±0.42	3.48±0.45	4.22±0.35
$m^{-2}\ s^{-1}$)		**B**	**B**	**B**	**B**	**B**	**B**

The grain dry mass of EMBRAPA048 cultivar was negatively impacted only by P deficiency. The grain dry mass of TMG7063 cultivar grown under adequate P conditions and subjected to WS-R5 and WS-V5+R5 treatments were lower than control plants. However, this production was still higher than that of plants with P deficiency subjected to water stress (Table 3). The dry mass of leaves, roots, total dry mass, P concentration in leaves at R5 and R8, were affected only by P deficiency in both cultivars (Table 3).

Table 3 - Dry mass data of grain, dry mass of leaves, dry mass of roots, total dry mass, leaf area (LA) of EMBRAPA048 and TMG7063 soybean cultivars during the recovery period from

water stress under two phosphorus conditions HP and LP. Different lowercase letters indicate significant differences by Tukey's analysis (P <0.05) between water stress, and different uppercase letters indicate significant differences between phosphorus nutrition.

		EMBRAPA048			TMG7063		
		WW	WS-R5	WS-V5+R5	WW	WS-R5	WS-V5+R5
Grain (g DW Plant^{-1})	HP	37.95±1.02 **A**	30.74±2.32 **A**	28.68±1.69 **A**	30.72±2.69 **Aa**	22.23±3.16 **Ab**	23.01±2.74 **Ab**
	LP	6.82±0.55 **B**	8.48±0.75 **B**	7.17±1.15 **B**	6.48±0.81 **Ba**	4.24±0.65 **Bb**	4.73±0.39 **Bb**
Leaf (g DW Plant^{-1})	HP	5.42±0.91 **A**	4.33±1.00 **A**	2.85±0.75 **A**	5.23±0.50 **A**	3.31±0.59 **A**	3.22±0.62 **A**
	LP	1.09±0.12 **B**	1.50±0.10 **B**	1.49±0.22 **B**	0.81±0.33 **B**	0.62±0.14 **B**	0.84±0.13 **B**
Root (g DW Plant^{-1})	HP	20.86±2.52 **A**	21.01±1.89 **A**	20.05±1.57 **A**	15.07±2.66 **A**	11.40±1.14 **A**	11.94±1.56 **A**
	LP	6.88±0.38 **B**	7.55±0.14 **B**	5.79±0.70 **B**	4.96±0.77 **B**	4.17±0.79 **B**	2.81±0.27 **B**
Total (g DW Plant^{-1})	HP	103.42±4.65 **A**	85.02±6.16 **A**	77.52±1.63 **A**	77.16±7.33 **A**	63.56±6.51 **A**	63.05±4.18 **A**
	LP	21.43±1.11 **B**	25.82±1.59 **B**	21.36±3.04 **B**	17.08±2.33 **B**	13.84±2.23 **B**	12.89±1.16 **B**
LA (cm²)	HP	3356.79± 206.53 **A**	2589.54± 177.99 **A**	2561.20±18 4.27 **A**	2275.75±323 .11 **A**	1624.02 ±270.98 **A**	1729.56± 238.75 **A**
	LP	824.57±43.6 3 **B**	994.36±33.35 **B**	855.98±233 .89 **B**	558.57±63.9 4 **B**	384.73±94.4 6 **B**	494.31±107. 88 **B**
[P] on leaves R5 (dag kg^{-1})	HP	0.21±0.008 **A**	0.22±0.019 **A**	0.19±0.011 **A**	0.21±0.014 **A**	0.24±0.004 **A**	0.23±0.013 **A**
	LP	0.05±0.0009 **B**	0.06±0.002 **B**	0.07±0.003 **B**	0.12±0.007 **B**	0.11±0.005 **B**	0.09±0.002 **B**
[P] on leaves R8 (dag kg^{-1})	HP	0.12±0.009 **A**	0.18±0.008 **A**	0.18±0.023 **A**	0.21±0.035 **A**	0.12±0.008 **A**	0.15±0.012 **A**
	LP	0.04±0.001 **B**	0.03±0.0008 **B**	0.05±0.001 **B**	0.08±0.001 **B**	0.06±0.002 **B**	0.07±0.006 **B**

The soybean grain oil composition was affected by P deficiency in both cultivars. Only the EMBRAPA048 cultivar showed an increase in oil content in grains under P deficiency. It was observed a decrease in protein content under P deficiency for both cultivars. The cultivar EMBRAPA048 showed no change in palmitic acid (C16), however, there was a decrease and increase in the proportion of stearic acid (C18:0) and linoleic acid (C18:2), respectively, due to P deficiency. Oleic acid (C18:1) showed the highest levels in seeds from EMBRAPA048

cultivar on the treatments subjected to water stress which received adequate P nutrition (Table 4). Saturated fatty acids (palmitic and stearic) in TMG7063 were not altered, however, the unsaturated fatty acids oleic (C18:1) and linolenic (C18:3) decreased, while linoleic (C18:2) increased due to P deficiency (Table 4).

Table 4 - The content (%) of oil, protein, and fatty acids in the grains: Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) of soybean cultivars EMBRAPA048 and TMG7063 during the recovery period of water stress under two phosphorus conditions, HP and LP. Lowercase letters indicate significant differences by Tukey's analysis ($P < 0.05$) between water stress conditions, and uppercase letters indicate significant differences between phosphorus nutrition.

		EMBRAPA048			TMG7063		
		WW	WS-R5	WS-V5+R5	WW	WS-R5	WS-V5+R5
Oil Content (%)	HP	17.21±0.51 **B**	17.34±1.25 **B**	18.09±0.62 **B**	15.71±1.95 **n.s.**	18.31±1.15 **n.s.**	18.54±0.68 **n.s.**
	LP	19.40±0.52 **A**	19.55±0.40 **A**	17.09±0.48 **A**	17.18±0.94 **n.s.**	17.66±1.02 **n.s.**	19.98±0.96 **n.s.**
Protein Content (%)	HP	33.94±0.12 **A**	33.67±1.38 **A**	35.24±0.51 **A**	33.88±1.06 **A**	32.80±0.75 **A**	32.77±0.41 **A**
	LP	29.48±0.46 **B**	29.15±0.27 **B**	30.62±0.85 **B**	31.57±0.69 **B**	28.94±1.66 **B**	28.24±1.85 **B**
C16:0	HP	12.19±0.03 **n.s.**	11.90±0.04 **n.s.**	11.71±0.19 **n.s.**	12.52±0.27 **n.s.**	12.33±0.23 **n.s.**	11.62±0.06 **n.s.**
	LP	11.35±0.16 **n.s.**	11.78±0.21 **n.s.**	11.73±0.19 **n.s.**	12.21±0.17 **n.s.**	12.14±0.21 **n.s.**	11.86±0.35 **n.s.**
C18:0	HP	3.91±0.05 **A**	3.69±0.07 **A**	4.07±0.18 **A**	3.94±0.12 **n.s.**	4.00±0.13 **n.s.**	3.97±0.08 **n.s.**
	LP	3.22±0.09 **B**	3.24±0.08 **B**	3.48±0.20 **B**	3.73±0.11 **n.s.**	4.01±0.12 **n.s.**	4.32±0.21 **n.s.**
C18:1	HP	23.73±0.10 **Bb**	27.00±0.59 **Aa**	26.11±0.47 **Aa**	24.07±1.09 **A**	25.90±0.85 **A**	27.40±0.95 **A**
	LP	23.71±0.63 **Bb**	22.35±0.31 **Bb**	22.55±0.50 **Bb**	22.79±0.46 **B**	22.89±0.33 **B**	23.29±0.28 **B**
C18:2	HP	54.15±0.16 **B**	51.70±0.64 **B**	52.39±0.40 **B**	52.97±0.89 **B**	51.07±0.85 **B**	50.28±0.48 **B**
	LP	56.13±0.58 **A**	56.65±0.49 **A**	56.57±0.73 **A**	55.08±0.15 **A**	54.96±0.28 **A**	54.65±0.31 **A**
C18:3	HP	6.00±0.10 **n.s.**	5.69±0.11 **n.s.**	5.70±0.22 **n.s.**	6.48±0.14 **A**	6.66±0.44 **A**	6.71±0.41 **A**
	LP	5.56±0.18 **n.s.**	5.94±0.04 **n.s.**	5.64±0.24 **n.s.**	6.18±0.10 **B**	5.98±0.10 **B**	5.86±0.15 **B**

4- Discussion

Phosphorus deficiency did not affect the response to water stress in the tolerant cultivar; however, it was essential to trigger a compensatory effect in the sensitive cultivar. The capacity to recover physiological processes from drought over time generally falls into three major categories: partial, complete, and compensatory recovery(Xu et al., 2010), which were influenced by P nutrition and genotype. Surprisingly, plants under P deficiency that underwent two cycles of water deficit showed a greater capacity for photosynthetic apparatus recovery. Both cultivars showed complete recovery of A_{max}, I_{max}, effective quantum yield, respiratory rates, and V_{cmax}. While the tolerant cultivar fully recovered mesophyll conductance, the sensitive cultivar showed compensatory recovery, which may have positively influenced the total recovery of *J* and *TPU*.

The cultivars showed a low capacity for synthesis of photosynthetic pigments after resuming irrigation, which resulted in a partial recovery of chlorophyll levels. Under water deficit conditions, nitrogen fixation decreases, directly affecting the synthesis of new chlorophyll molecules (Freitas et al., 2022). Furthermore, P deficiency also impaired the resumption of chlorophyll synthesis under water deficit, due to impaiments on the plant's energy metabolism.

Despite the lower levels of total chlorophyll after resuming irrigation in the cultivar EMBRAPA048, its complete recovery of F_v/F_m indicates improved reorganization of the photosynthetic apparatus even under P deficiency. The unchanged F_v/F_m under different water stress conditions demonstrates a greater resistance of PSII to water deficit in the tolerant cultivar, indicating better adjustment in energy distribution between photosystems and photoprotective energy dissipation (Kalaji et al., 2016). The sensitive cultivar under P deficit exhibited a more effective investment in recovering the integrity of PSII in the WS-V5+R5 treatment, which resulted in higher F_v/F_m values compared to plants that were exposed only to one water deficit cycle (WS-R5). The drought-sensitive soybean cultivar exposed to milder previous stresses increases its ability to resist future stress events, accelerating the recovery process and consequently mitigating its damage (Bester et al., 2024).

The diffusive phase of photosynthesis is a fundamental process regulating gas exchange between plants and the environment (Flexas & Medrano, 2002; Pilon et al., 2018). In addition to lower stomatal conductance, the decrease in mesophyll conductance contributed to the decline in chloroplastidic CO_2 concentration, causing a decrease in the photobiochemical stage parameters. The decline in density and size of the stomata is also a common effect of P

deficiency (Khan et al., 2023), and may have contributed to the decrease in CO_2 uptake. However, in our study the main cause for decreased A_n was related to the internal CO_2 concentration restriction in leaf mesophyll, which caused biochemical limitations due to lower instantaneous carboxylation efficiency, followed by stomatal limitation. Biochemical processes were compromised by P deficiency, leading to a decrease in ATP and NADPH production and lower regeneration of ribulose-1,5-bisphosphate (Medina et al., 2022). Therefore, V_{cmax}, J, and *TPU* values were even lower under P deficiency and water deficit.

The temporal dynamics of stomatal closure during drought depend on the plant's ability to establish a threshold between maintaining carbon fixation and regulating the water supply capacity of hydraulic systems to avoid embolism(Volaire, 2018). The drought-tolerant cultivar showed a greater capacity to regulate stomatal opening, so leaf water potential was higher even under P deficiency.

Proline is an amino acid that protects plants from various stresses and also helps plants recover from stress more quickly(Al-Karaki et al., 2012; Ben Ahmed et al., 2010; Medina et al., 2023). Plants continuously synthesize reactive oxygen species as byproducts of various metabolic pathways. However, under water stress conditions, ROS concentration may exceed the capacity of antioxidant metabolism to eliminate them. Excessive ROS generation results in substantial oxidative damage to proteins, DNA, and lipids, thus inhibiting plant growth. Some reports indicate that proline eliminates ROS and other free radicals (Ben Ahmed et al., 2010; Sperdouli & Moustakas, 2012; Szabados & Savouré, 2010). Maintaining high proline levels in leaf tissues post-water stress, the EMBRAPA048 cultivar possibly favored its antioxidant metabolism, confirmed by the lower H_2O_2 levels. Since the TMG7063 cultivar did not significantly increase proline concentration under water stress recovery, the H_2O_2 production was higher than in the EMBRAPA048 cultivar, especially under P deficiency.

The suppression of photosynthesis under drought altogether with P deficiency decreases ATP production, resulting in less energy for plant growth and development. Our study shows that there are losses in leaf area and biomass under P deficiency; however, the drought-tolerant cultivar managed to produce more biomass than the sensitive cultivar. This demonstrates that the drought tolerance mechanism may favor grain production under P deficiency conditions. Studying plant morphological characteristics can help researchers determine the effects of water stress and rehydration on soybeans, as well as their pattern changes (Dong et al., 2019).

Soybean is an important source of protein and vegetable oil. Only P deficiency adversely affected grain composition, because P is directly related to the formation of energy molecules, which will be used in N metabolism (Jin et al., 2006). This also explains the decrease in protein

content in both soybean cultivars under P deficiency. In addition to the decrease in protein content in EMBRAPA048, there was an increase in oil content. Fatty acid metabolism was stimulated under P deficiency. Linoleic acid (C18:2) contributed to this increase, compromising grain quality. With the increasing interest in eliminating trans fatty acids in diets, soybean breeders need to emphasize the development of varieties that can produce seeds with high levels of desired fatty acids, such as oleic acid (Yin et al., 2016).

5- Conclusion

P deficiency prolonged the decline in photosynthesis in response to drought stress in both cultivars. However, photosynthetic rates recovered faster under P deficiency than under adequate P conditions.

P deficiency did not affect the response pattern to water stress in the tolerant cultivar; however, it was essential to trigger a compensatory effect on the sensitive cultivar.

Plants in the sensitive cultivar that went through two cycles of stress showed compensatory effects in the recovery of mesophyll conductance and F_v/F_m, which was reflected in higher rates of V_{cmax}, J and TPU. However, the compensatory photosynthetic adjustments presented did not result in better grain production.

The quantity and quality of grains were affected by P deficiency in both cultivars P deficiency reduced the protein content and increased the oil content in grains, especially unsaturated fatty acids.

The physiological plasticity of genotypes susceptible to drought stress is affected by P deficiency.

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Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

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Artigo 2 - Phosphorus use efficiency and responsiveness are associated with the differential allocation of organic P fractions and improved photoprotection in soybean genotypes (*Glycine max* L.)

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Abstract

Soybeans have a high demand for phosphate since its production is strongly suppressed in phosphorus (P)-deficient soils. Increasing the P use efficiency (PUE) is one of the approaches to improving the sustainability of agricultural production. However, the mechanism between genotype PUE in poor soils and its responsiveness to P addition is poorly understood. Therefore, this work sought to understand the efficiency and response mechanism in the use of P used by different soybean genotypes, relating photosynthetic and growth traits. For this, seven soybean genotypes were cultivated in field conditions with high (HP) and low phosphorus (LP) supply in the soil. The cultivars were classified as efficient and responsive to P addition (ER), efficient and non-responsive (ENR), non-efficient and responsive (NER) and non-efficient and non-responsive (NENR) based on photosynthetic yield and grain yield. The cultivars with high photosynthetic responsiveness to P were also responsive in grain production. However, regardless of the P-efficiency mechanism, P deficiency affected the production of different genotypes. P-responsive cultivars showed increased carboxylation rate, RuBP regeneration and use of P-trioses in HP. The photosynthetic efficiency of the ENR cultivar was supported by the greater allocation of metabolic P and reduction of lipid P, in addition to the more robust photosynthetic apparatus from the initial stages of development. The most significant difference between efficient and inefficient cultivars, regardless of response capacity, was the photoprotection mechanism to dissipate excess energy in a regulated manner. We conclude that cultivars that are efficient in the use of P have a greater allocation of P-metabolic and an efficient mechanism for dissipating excess energy.

Keywords: Metabolic-P, photosynthetic efficiency, photosynthetic responsiveness, rubisco carboxylation rate, triose phosphate utilization, growth.

1- Introduction

Phosphorus (P) is an essential nutrient for biological processes in plants (He et al., 2017), but the low availability of this element in most soils is a limiting factor for the growth and crop yield (Ham et al., 2018). The use of phosphate fertilizer is an alternative solution that provides P to plants, but only 10–25% of the applied Pi can be absorbed by plants (Johnston et al., 2014) and is costly. Management practices with P-responsive genotypes for increasing the P use efficiency in crops is a practical and environmentally friendly solution to boost agricultural production and maintain global food security (Abbas et al., 2018). Furthermore, improved P efficiency and responsiveness are of interest for the sustainability of production systems, especially in tropical regions with soils of low fertility, high acidity and high phosphorus adsorption capacity (L.-W. Xu et al., 2024).

The soybean (*Glycine max*. L.) is the main legume cultivated in the world, used as a source of oil and protein for animal feed (Sentelhas et al., 2015). Soybean is a crop that requires large amounts of phosphate fertilizers; its production is strongly suppressed in the soil when Pi is deficient. Pi deficiency is more problematic than other nutritional deficiencies for soybeans (Valentine et al., 2018). Considering the large area of soybean cultivation in the world, selecting cultivars that present strategies to ballance Pi absorption and production, will have great economic and environmental impact.

Phosphorus deficiency can limit photosynthesis through stomatal resistance, mesophilic resistance and restriction in photobiochemical processes (Chu et al., 2018a). Under P deficiency plants present morphophysiological acclimative strategies that contribute to overcome these limitations. The changes can includes the replacement of phospholipids by sulfolipids in membranes, accumulation of anthocyanins in leaves, increased thickness of the epidermis (Chu et al., 2018a; Iqbal et al., 2019; Mehra et al., 2017). Understanding the acclimative strategies to low P availability of different soybean cultivars is essential for developing plants that are more efficient in using P (Lazali & Drevon, 2021).

Phosphorus use efficiency (PUE) is the result of P absorption efficiency and internal P use efficiency (Huang et al., 2011). P absorption use efficiency (PUEa) refers to the plant's ability to solubilize P from poorly available sources and/or absorb available soluble Pi from the soil solution. Internal P use efficiency (PUEi) refers to the amount of biomass produced per unit of tissue P concentration. Strategies contributing to increasing PUEi in crops includes P remobilization, alteration of organic P fractions, higher release of vacuolar Pi (Abbas et al.,

2018; Ham et al., 2018; Mo et al., 2019a). Variation between cultivars for PUEa and PUEi may be due to differential P uptake characteristics or P distribution patterns within plants, respectively (Irf et al., 2020), which affects yield.

In addition to being efficient in using phosphorus, plants can be responsive (RE) to the addition of this nutrient to the soil. P efficiency is related to the ability to grow with low P inputs, while responsiveness is associated with increased growth potential with additional P inputs. Plants with high RE may have efficient absorption mechanisms—high PUEa, resulting in increased [P] in tissues. In the global context, with finite and limited phosphate fertilizer resources, exclusively responsive cultivars may be unsustainable. However, the combination of both characteristics—responsiveness and efficiency in the use of P—may be the path to genetic improvement aiming at a more efficient use of fertilizers.

Grain yield is the final output of production, which is strongly affected by P deficiency. The magnitude of production loss caused by P deficiency can vary depending on PUE. Some genotypes are able to avoid large yield losses under P deficiency conditions, while other genotypes do not avoid losses but show high response capacity after phosphate fertilizer applications. Some research has demonstrated the responsiveness of different soybean cultivars to P deficiency (Soares et al., 2020; Xu et al., 2024). However, the relationship between the physiological mechanisms for increasing PUE and RE is unclear.

Therefore, this work sought to understand the efficiency and response mechanism in the use of P by different soybean genotypes, relating photosynthetic and growth parameters. Efficient and responsive soybean cultivars to phosphorus use are expected to present higher photosynthetic rates under P deficiency, supported by greater P allocation in the P metabolic fraction and efficient mechanisms for dissipating excess energy. As a result, they will show similar growth and grain production under conditions of high and low P availability.

2- Material and methods

2.1 Plant species and growth conditions

The experiment was conducted under field conditions in the horticulture sector at the Federal University of Viçosa, Campus Florestal (19°52’35.1”W 44°24’49.6”W), in the 2020/2021 agricultural harvest. Local temperature and precipitation data were monitored using INMET data (Fig. 1). Sprinkler irrigation was carried out using the conventional permanent system, when the soil moisture tension reached 40 kPa.

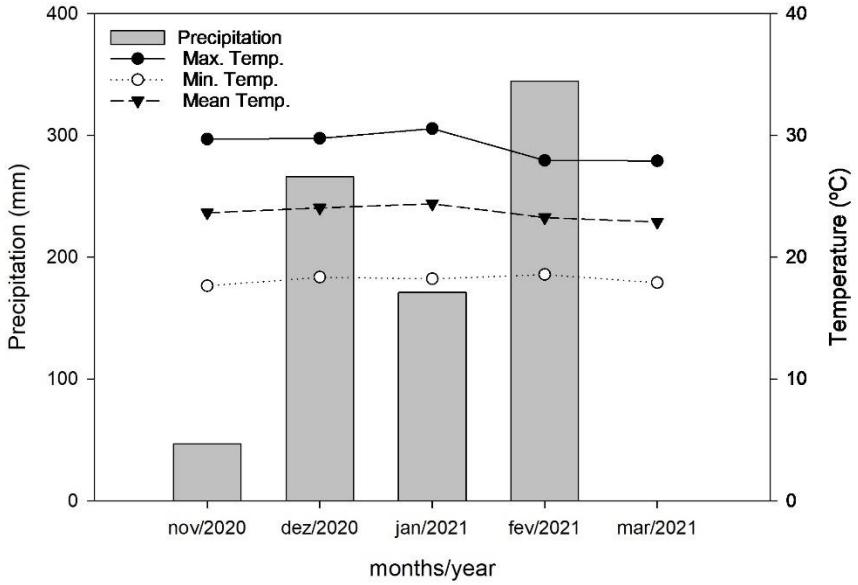

Fig. 1 Characterization of the soybean growing environment. The bars represent the sum of monthly precipitation (mm) and the lines represent the maximum, mean and minimum temperatures (ºC).

Seeds of seven soybean cultivars (Table 1) were sown within the appropriate planting window (October/November). The seeds were treated with the fungicide Vitavax Thiram® (Carboxanilide + Dimethyldithiocarbamate) 250ml/100kg and inoculated with Adhere® 60g/100kg.

The local soil has physical characteristics similar to Dystrophic Red Argisol. The pH was corrected with dolomitic limestone, PRNT 80%, at the quantity obtained (0.169 t/ha) using the base saturation method, considering the soil chemical analysis. The chemical characteristics of the soil were: 1.56 mg/dm³ P, 59.7 mg/dm³ K, 1.2 cmolc/dm³ Ca^{2+}, 0.89 cmolc/dm³ Mg^{2+}, 0 cmolc/dm³ Al^{3+}, 3.79 cmolc/dm³ H+Al, 2.24 cmolc/dm³ SB, 2.24 cmolc/dm³ t, 6.03 cmolc/dm³ T, 37% V, 0 % m and pH 6.48. Potassium oxide fertilization was applied at a dosage of 40 kg/ha. Phosphate fertilization was applied only in the control plots at a dose of 120 kg/ha. (Ribeiro et al., 1999). The total P concentration in the soil after fertilization was 26.56 mg/dm³.

Table 1 - Characterization of the cultivars used.

Cultivar	Company	Maturity Group	Cycle[1]	Growth Habit	Stature[2]	Fertility Requirement
BRS1003 IPRO	Embrapa	7.0	Early	Indeterminate	Short/ Medium	Medium/ High

TMG7060 IPRO	Tropical Melhoramento & Genética	6.0	Early	Semi-determinate	Medium	Medium/ High
TMG7063 IPRO	Tropical Melhoramento & Genética	7.0	Super Early	Indeterminate	Medium	High
M6210 IPRO	Monsoy	6.2	Early	Indeterminate	Medium	High
M7739 IPRO	Monsoy	7.7	Super Early	Semi-determinate	Short	Medium/ High
AS3680 IPRO	Agroeste	6.8	Super Early	Indeterminate	Medium	Medium/ High
BMX DESAFIO RR	Brasmax	7.4	Medium	Indeterminate	Medium	Medium/ High

[1] Cycle classification: super early +- 110 days; early +- 125 days; medium +- 140 days; late +- 155 days.

[2] Stature classification: short +- 70cm; medium +- 85cm; high +- 100 cm

2.2 Experimental design and statistical analysis

The experiment was conducted in randomized blocks with five replications in a split-plot design, where the plots were the P nutrition conditions and the seven cultivars were the subplots. The treatment in the plots that received adequate P nutrition or without P fertilization was named as high phosphorus (HP) and low phosphorus (LP), respectivelly. The subplots were the seven cultivars AS3680, BRS1003, DESAFIO, M6210, M7739, TMG7060, TMG7063.

Each replication of the cultivars occupied a subplot of 4 planting rows with 6 plants in each row, spaced 30cm apart, totaling 24 plants/subplot. The plants analyzed were the central ones, to ensure it was not suffering competition between different cultivars. The experimental unit consisted of 1 plant/replication/stage.

After classifying the cultivars in terms of PUE and RE (see section 2.3.3), the response of the groups of cultivars to P availability were analyzed using analysis of variance, using the ExpDes.pt data package from the RStudio statistical program (3.6.3). The means were compared using the Tukey test with a significance level of 5%. Fluorescence and chlorophyll indices analyze were carried out over time, comparing cultivar groups and P availability within each stage.

2.3 Evaluated variables

2.3.1 Gas Exchange

Leaf gas exchange assessments were conducted at stage R5, in the morning, with the first fully expanded leaf from the apex, on the central leaflet. Measurements were taken using an infrared gas analyzer, LI-6400xt model (Li-Cor Inc., Lincoln, Nebraska, USA), with a photosynthetically active photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1}, CO_2 concentration of 400 μmol mol^{-1}, temperature of 28 °C, and relative humidity of 44%. The following variables were recorded: net photosynthetic rate (A_n), stomatal conductance (g_s), transpiration (E). Dark respiration (R_D) was measured during the night period, after 3 hours in the dark.

Using the same apparatus, CO_2 saturation curves for photosynthesis were performed. Measurements were taken at levels of 400, 300, 200, 100, 50, 400, 500, 600, 700, 800, 1000, 1200, and 1300 μmol CO_2 mol^{-1}. The minimum time for stabilization of readings at each concentration was 60 seconds, and the maximum was 120 seconds. Data obtained from the A_n/Ci curve were used to calculate the Rubisco carboxylation rate (V_{cmax}), electron transport rate (J), and triose phosphate utilization (TPU) (Sharkey et al., 2007)).

2.3.2 Leaf phosphorus fractions and total P concentration in the leaf

The P fractions were determined using the methodology described by Yan et al., (2019), adapted from Hidaka & Kitayama, (2013). The leaves used for photosynthetic measurements at the R5 stage were collected and stored in N_2 until the beginning of the analysis. The material was lyophilized and then 50 mg was weighed for use. Extraction was performed three times with 1 mL of cold chloroform: methanol: formic acid (CMF; 12: 6: 1 v/v/v) and three times with 1.26 mL of cold chloroform: methanol: water (CMW; 1: 2: 0.8 v/v/v). The supernatant was collected, and 1.9 mL of water-washed chloroform was added. It was then centrifuged to separate the extract into two layers. The upper aqueous layer (fraction 1) and the lower green layer (lipid P fraction) were transferred to different digestion flasks. The remaining sediment in the tube was extracted with 1 mL of 85% (v/v) cold methanol. The methanol extract was added to the flask containing fraction 1. The Eppendorf with the pellet was left to dry for approximately 15 minutes to remove methanol and chloroform from the sediment. The dry pellet was extracted twice with 1 mL of 5% (p/v) cold trichloroacetic acid (TCA) solution for one hour with constant stirring in a cold environment (4°C). The 5% TCA extract was added to the flask containing fraction 1 (metabolic P). The remaining sediment in the Eppendorf was extracted three times with 1 mL of 2.5% (p/v) TCA at 95°C in a water bath for one hour, with

stirring at 20-minute intervals. The 2.5% TCA extract was added to a digestion flask for subsequent determination of nucleic acid P. The residue remaining from the hot TCA extraction was the residual P fraction. All solutions in the flasks were evaporated in a forced air circulation oven at 55°C for subsequent acid digestion.

Another subsample (50 mg) of the ground dry leaf material was used to determine the total P concentration. This subsample and each of the P fractions were acid-digested under heating. The phosphorus concentrations in the digested samples were determined colorimetrically using the molybdenum blue-based method (Ames, 1966). The sum of P concentrations in the fractions was used to assess the recovery, which was greater than 85% of the total P concentration determined from the leaf.

2.3.3 Responsiveness efficiency and P-use efficiency

The method used to identify and classify the soybean genotypes in terms of efficiency and responsiveness to phosphorus use was proposed initially by (Fageria & Kluthcouski, 1980). This method aims to discriminate soybean cultivars in terms of efficiency in the use of P and the responsiveness to the application of phosphate fertilizer. For this purpose, a graphical representation in the Cartesian plane was used. The x-axis is equivalent to the absorption efficiency in the use of P, regarding the photosynthetic or yield responses (PUEa_{An} or PUEa_{Yield}, respectively), that is, it refers to the average net photosynthesis or yield in an environment with low P availability. In turn, the y-axis is equivalent to the relative changes in photosynthesis or yield referring to P availability (A_{n}RE or $_{\text{Yield}}$RE, respectively), that is, the difference between the photosynthesis or yield at the two levels of phosphate fertilizer divided by the difference between the P content in the soil.

The expressions used to classify cultivars in terms of phosphorus use efficiency were:

$$\text{PUE}a_{An} = A_{\text{nLP}} / [\text{P}]\text{in soil LP} \quad \text{Eqn.1}$$

$$\text{PUE}a_{\text{Yield}} = \text{Grain Yield}_{\text{LP}} / [\text{P}]\text{in soil LP} \quad \text{Eqn.2}$$

$$A_{\text{n}}\text{RE} = (A_{\text{HP}} - A_{\text{LP}}) / ([\text{P}]\text{in soil HP} - [\text{P}]\text{in soil LP}) \quad \text{Eqn.3}$$

$$_{\text{Yield}}\text{RE} = (\text{Grain Yield}_{\text{HP}} - \text{Grain Yield}_{\text{LP}}) / ([\text{P}]\text{in soil HP} - [\text{P}]\text{in soil LP}) \quad \text{Eqn.4}$$

The method recommends that a straight line originating from the average value of each Cartesian axis be drawn, causing the Cartesian plane to be divided into quadrants. The first quadrant represents efficient and responsive cultivars (ER), that is, those that have values above

the average for both Cartesian axes. The second quadrant represents non-efficient and responsive cultivars (NER), that is, those which have values below the average for the abscissa axis and above for the ordinate axis. The third quadrant represents non-efficient and non-responsive cultivars (NENR), that is, those that have values below the averages of the two Cartesian axes. Finally, the fourth quadrant represents efficient and non-responsive soybean cultivars (ENR), that is, those cultivars with values above the average for the x-axis and below for the y-axis.

2.3.4 Chlorophyll a fluorescence

The measurements of chlorophyll a fluorescence variables were performed at each stage of development, using the Mini-PAM pulse-amplitude-modulated fluorometer (Heinz Walz, Effeltrich, Germany). Evaluation took place at the end of the water stress recovery period in the morning, on the first fully expanded leaf from the apex, on the central leaflet. Minimum fluorescence (F_0) and maximum fluorescence (F_m) were measured in the early morning after leaf acclimation in the dark for at least 30 minutes. The obtained values were used to determine the maximum quantum efficiency of photosystem II (*PSII*; F_v/F_m) according to Eqn. 5:

$$F_v/F_m = (Fm - F_0)/Fm) \quad \text{Eqn.5}$$

After determining F_v/F_m, the plant tissue was exposed for 30 seconds to a photosynthetic photon flux density (PPFD) of 1000 μmol m^{-2} s^{-1}, and then a saturating light pulse was applied. From this process, the following variables were determined: F, the fluorescence in *steady state* before the saturating light pulse; and Fm', the maximum fluorescence of the illuminated plant tissue. The effective quantum yield variables of PSII in illuminated plant tissue (ϕ_{PSII}); non-photochemical quenching of fluorescence (NPQ) (Bilger & Björkman, 1990); electron transport rate (ETR), were calculated according to the equations:

$$\phi_{PSII} = (Fm' - F)/Fm' \quad \text{Eqn.6}$$

$$NPQ = Fm - Fm'/Fm' \quad \text{Eqn.7}$$

$$ETR = \phi PSII \times PPFD \times \alpha, \quad \text{Eqn.8}$$

Where α, defined as 0.47 for soybean (Gallé et al., 2013), is the product of the proportions of photons destined for the two photosystems (MELIS et al., 1987).

At stage R5, a steady-state light curve (SSLC) was performed. The leaflet was dark-adapted for approximately 30 minutes, and then readings were taken at intensities of 0, 8, 80, 160, 260, 385, 530, 780, 1060, 1610 µmol m^{-2} s^{-1}. The following light response curves were obtained ETR_{max} and $NPQ_{max.}$

2.3.5 Chlorophyll índices

The measurements chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b* ratio and total chlorophyll índices were performed at each stage of development, simultaneously with the fluorescence measurements, using a ClorofiLOG portable chlorophyll meter (Falker, Brazil). Three measurements were made at each central leaflet from among the same leaves used for fluorescence measurements of chlorophyll *a*.

2.3.6 Morphological and growth assessments

In the V2, R2, R7, and R8 stages, leaf area, and dry mass of leaves, stem, and root were analyzed. Leaf area (LA) was obtained using the ImageJ program. Leaves, stems, and roots were dried in a forced air circulation oven at 65°C to obtain the total dry mass (Total DM), leaf dry mass (Leaf DM), shoot dry mass (Shoot DM), root dry mass(Root DM). With these data, the following were calculated leaf mass per area (LMA) to according to the equation:

LMA = Leaf DM (g)/ LA (cm^2) Eqn.9

3- Results

The graphical dispersion of the genotypes in the quadrants revealed that the productive responsiveness ($_{Yield}$RE) is related to the photosynthetic responsiveness (A_nRE) to P fertilization. Cultivars that are not photosynthetically responsive to P fertilization are also not productively responsive, they are M7739 (ENR, Fig. 2A, 2B), DESAFIO and M6210 (NENR; Fig. 2A, 2B). Cultivars photosynthetically responsive to P fertilization are also productively responsive, they are AS3680 (NER; Fig. 2A, 2B), TMG7060 (NER; Fig. 2A, 2B), BRS1003, TMG7063. However, PUEa_{An} does not correlate with PUEa_{Yield}. The cultivars BRS1003 and TMG7063 are classified as photosynthetically efficient and responsive (ER, Fig. 2A), but are classified as non-efficient and productively responsive (NER, Fig 2B).

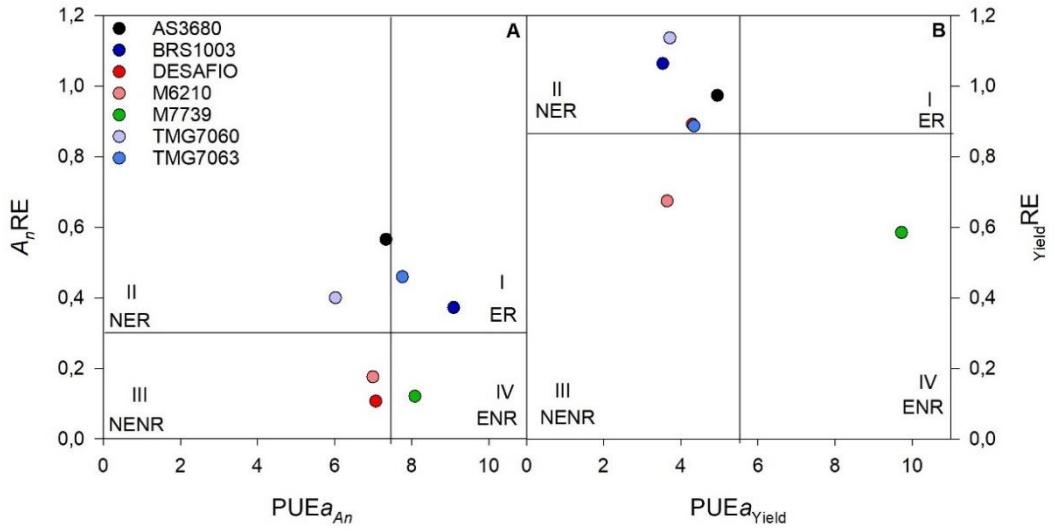

Fig. 2 Classification of soybean cultivars (AS3680, BRS1003, DESAFIO, M6210, M7739, TMG7060, TMG7063) for P efficiency and responsiveness (RE) based on photosynthesis (A_nRE; A) and based on grain yield ($_{Yield}$RE; B)

P-responsive cultivars (ER and NER) showed higher values of *A*, V_{cmax}, *J*, *TPU*, ETR_{max}, when subjected to the HP condition (Fig. 3A, 3B, 3D, 3F, 3G). The group of non-responsive cultivars (ENR, NENR) presented the lowest *A* values in HP. The results of this group were similar between the nutritional conditions of HP and LP in the photosynthetic parameters: *A*, V_{cmax}, *J*, *TPU*, ETR_{max} (Fig. 3A, 3B, 3D, 3F, 3G).

Stomatal conductance was lower due to P deficiency only in NER cultivars (Fig. 3C), but did not impact plant transpiration (Fig. 3E). The *E* was lower in LP only in ER cultivars. Cultivars efficient in the use of P (ER, ENR) showed greater non-photochemical extinction when subjected to high irradiance and LP (Fig. 3H).

The cultivars showed lower respiratory rates in LP, with the exception of NENR, which showed the same respiratory rate in HP and LP (Fig. 3I). Under P deficiency, all cultivars showed reduced P concentration in leaves at the R5 stage (Fig. 3J).

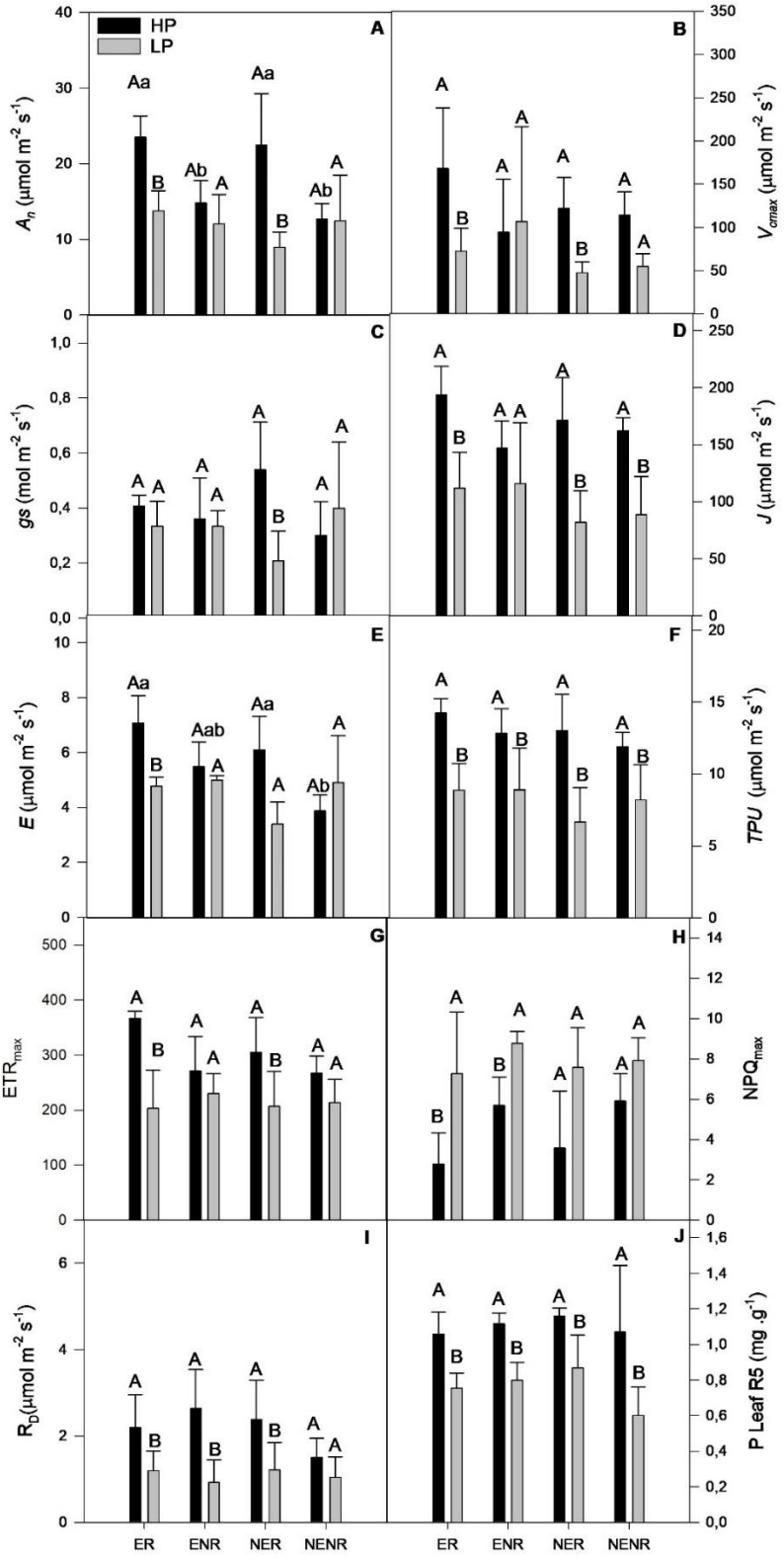

Fig. 3 Net photosynthetic rate (A_n; A), Maximum carboxylation rate (V_{cmax}; B), stomatal conductances (g_s; C), electron transport capacity (J; D), transpiration (E; E), triose phosphate utilization (TPU; F), maximum electron transport rate (ETR_{max}; G) and maximum nonphotochemical fluorescence quenching (NPQ_{max}; H), respiration dark (R_D; I), P concentration in leaves in R5 (PLeafR5; J) in groups of efficient and responsive (ER), efficient and non-responsive (ENR), non-efficient and responsive (NER), non-efficient and non-responsive soybean cultivars. Capital letters compare high phosphorus (HP) and low

phosphorus (LP) conditions. Lowercase letters compare cultivar groups (ER, ENR, NER, NENR), using the Tukey test at 5%.

The ENR cultivar presented the lowest percentage and concentration of P-nucleic acid (Fig. 4A, 4B) and the highest percentage of P-metabolic (Fig. 4C), regardless of the P condition. It also presented the lowest percentage and concentration of P-lipid when subjected to LP (Fig. 4E, 4F). The NENR group presented the lowest concentration of P-metabolic when subjected to LP.

The percentage of P-residual was similar in HP and LP for all cultivars (Fig. 4G), however, the concentration of P-residual was lower in HP for ENR and higher for NENR (Fig. 4H).

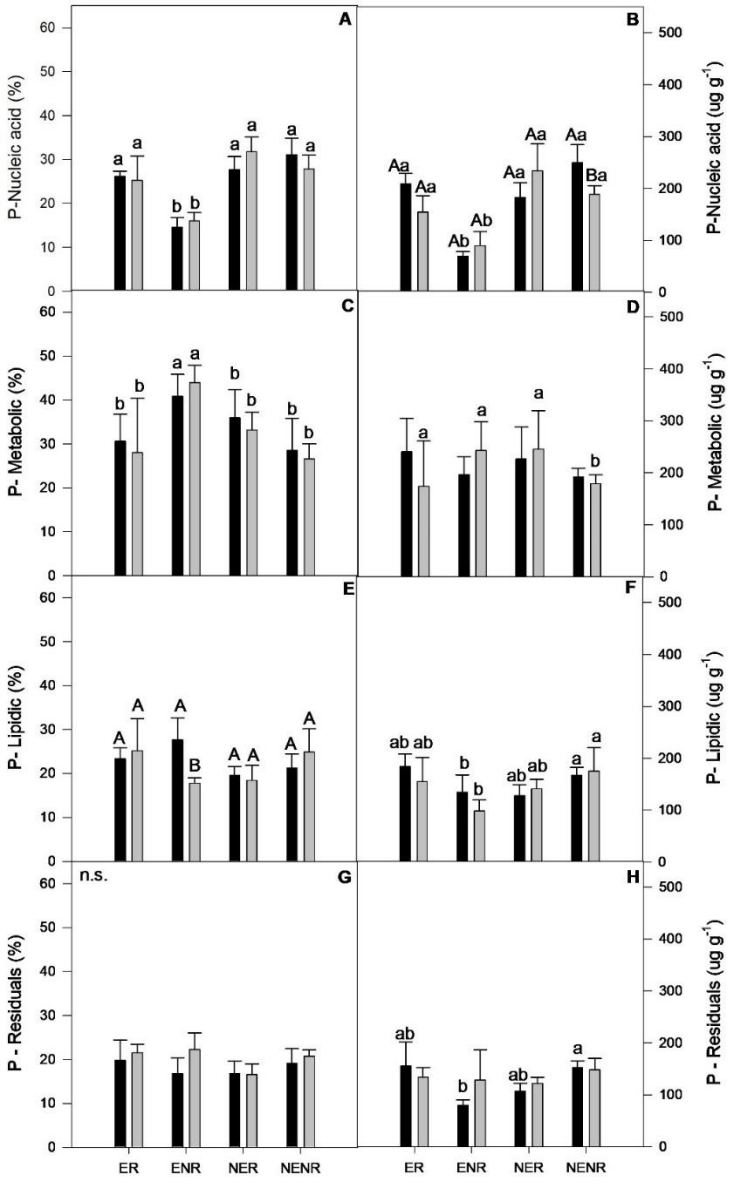

Fig.4 Percentage of phosphorus fraction allocation (A, C, E, G) and P fraction concentrations (B, D, F, H) in nucleic acids (A, B), metabolic (C, D), lipid (E, F), residual (G, H) in groups of efficient and

responsive (ER), efficient and non-responsive (ENR), non-efficient and responsive (NER), non-efficient and non-responsive soybean cultivars at R5 stage. Capital letters compare high phosphorus (HP) and low phosphorus (LP) conditions. Lowercase letters compare cultivar groups (ER, ENR, NER, NENR) using the Tukey test at 5%.

At the beginning of development (V2), the cultivars reduced maximum PSII quantum efficiency under P deficiency, with the exception of ENR (Fig. 5A, 5C, 5D). The F_v/F_m of the ENR cultivar was superior to all other cultivars, regardless of P fertilization (Fig. 5B). At flowering (R1), there was a reduction in F_v/F_m, but it was quickly recovered until R2. While the cultivars NER and NENR showed a reduction in F_v/F_m under P deficiency in R2 and R3 (Fig. 5C, 5D), the cultivars ER and ENR (Fig. 5A, 5B) reduced the maximum efficiency of PSII only in R5. At the end of the life cycle, non-efficient cultivars showed a greater decline in F_v/F_m in LP.

Only the ENR cultivar did not reduce the effective quantum yield of PSII in P deficiency, in V2 (Fig. 5E, 5F, 5G, 5H). The ER cultivars showed lower ϕ_{PSII} (Fig. 5E) and ETR (Fig. 5I) in LP in the reproductive stages, while the non-efficient cultivars showed a reduction ϕ_{PSII} (Fig. 5G, 5H) and ETR (Fig. 5K, 5L) only in R7.

Cultivars efficient in the use of phosphorus showed greater non-photochemical quenching (Fig. 5M, 5N) in phosphorus deficiency during the longer life cycle than non-efficient cultivars (Fig. 5O, 5P). Furthermore, these values were higher than those observed in non-efficient cultivars.

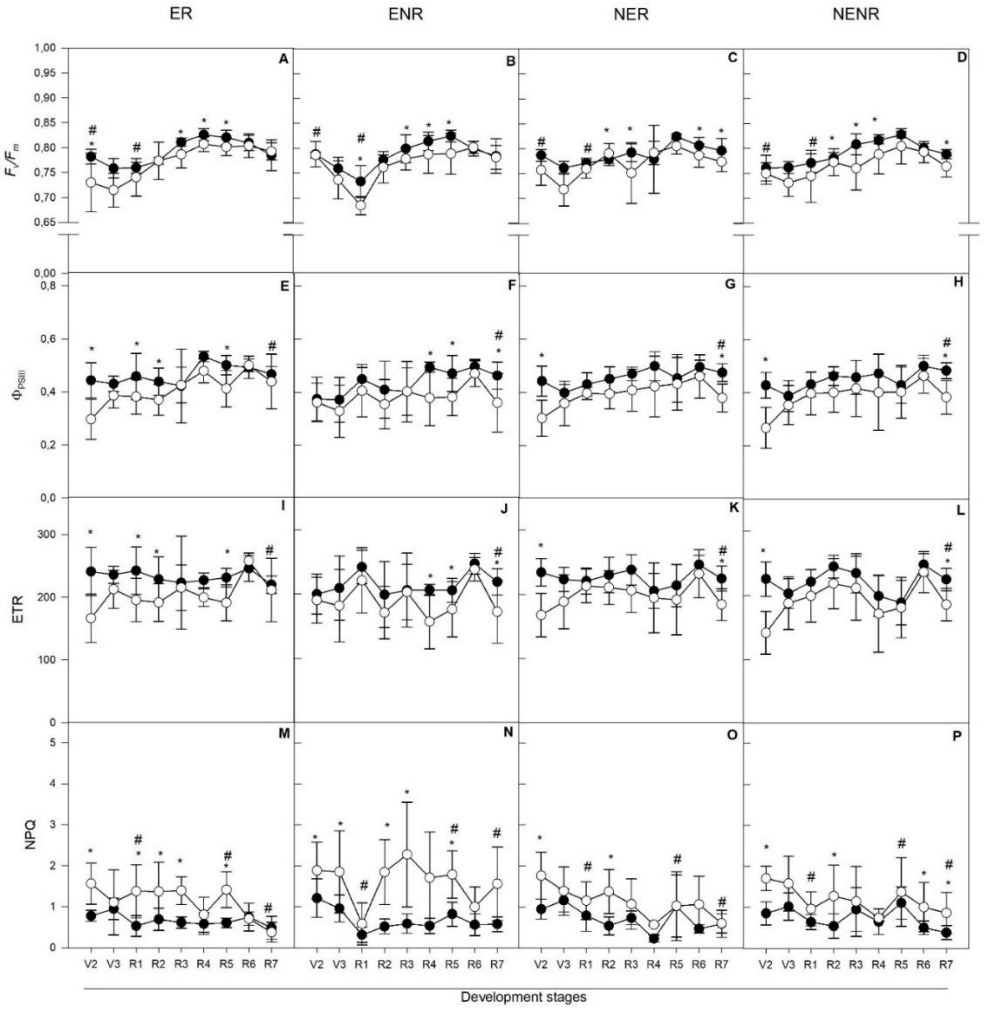

Fig. 5 Maximum quantum efficiency of photosystem II (F_v/F_m; A, B, C, D), effective quantum yield of PSII (ϕ_{PSII}; E, F, G, H), electron transport rate (ETR; I, J, K, L), nonphotochemical fluorescence quenching (NPQ, M, N, O, P) during the development stages of soybean cultivars efficient and responsive (ER; A, E, I, M), efficient and non-responsive (ENR; B, F, J, N), non-efficient and responsive (NER; C, G, K, O), non-efficient and non-responsive (NENR; D, H, L, P) soybean cultivars.The asterisks represent the difference between the nutritional conditions of
high phosphorus (HP) and low phosphorus (LP). The hash corresponds to the statistical difference between the groups of cultivars, using the Tukey test at 5%.

In V2, ER cultivars showed the highest levels of total chlorophyll (Fig. 6A), chlorophyll *a* (Fig. 6E) and chlorophyll *b* (Fig. 6I) under conditions of high P availability (HP), while NENR cultivars showed the lowest values (Fig. 6D, 6H, 6L, 6P). From R1 onwards, ER (Fig. 6A, 6E, 6I, 6M) and NER (Fig. 6C, 6G, 6K, 6O) cultivars increased total chlorophyll, chlorophyll *a* and chlorophyll *b* indices under P deficiency (LP), while NENR (Fig. 6D, 6H, 6L,6P) cultivars showed an increase in these indices only in LP, in R2.

The ENR cultivar did not show a significant difference in chlorophyll indices between HP and LP conditions throughout the development cycle, but showed the highest indices in HP in the R2 and R3 stages (Fig. 6B, 6F, 6J, 6N). The cultivars did not show changes in pigments between HP and LP at the grain filling stage (R5).

Under phosphorus deficiency, NENR cultivars reduced total chlorophyll and chlorophyll *b* indices at R6, while ENR showed the highest total chlorophyll, chlorophyll *a* and chlorophyll *b* values at this stage (Fig. 6C, 6G, 6H, 6O). At the end of the cycle, in R7, the ER cultivars maintained the highest levels of total chlorophyll, chlorophyll *a* and chlorophyll *b* in LP, and the NENR and NER cultivars showed higher chlorophyll *a/b* ratio values (Fig. 6O, 6P).

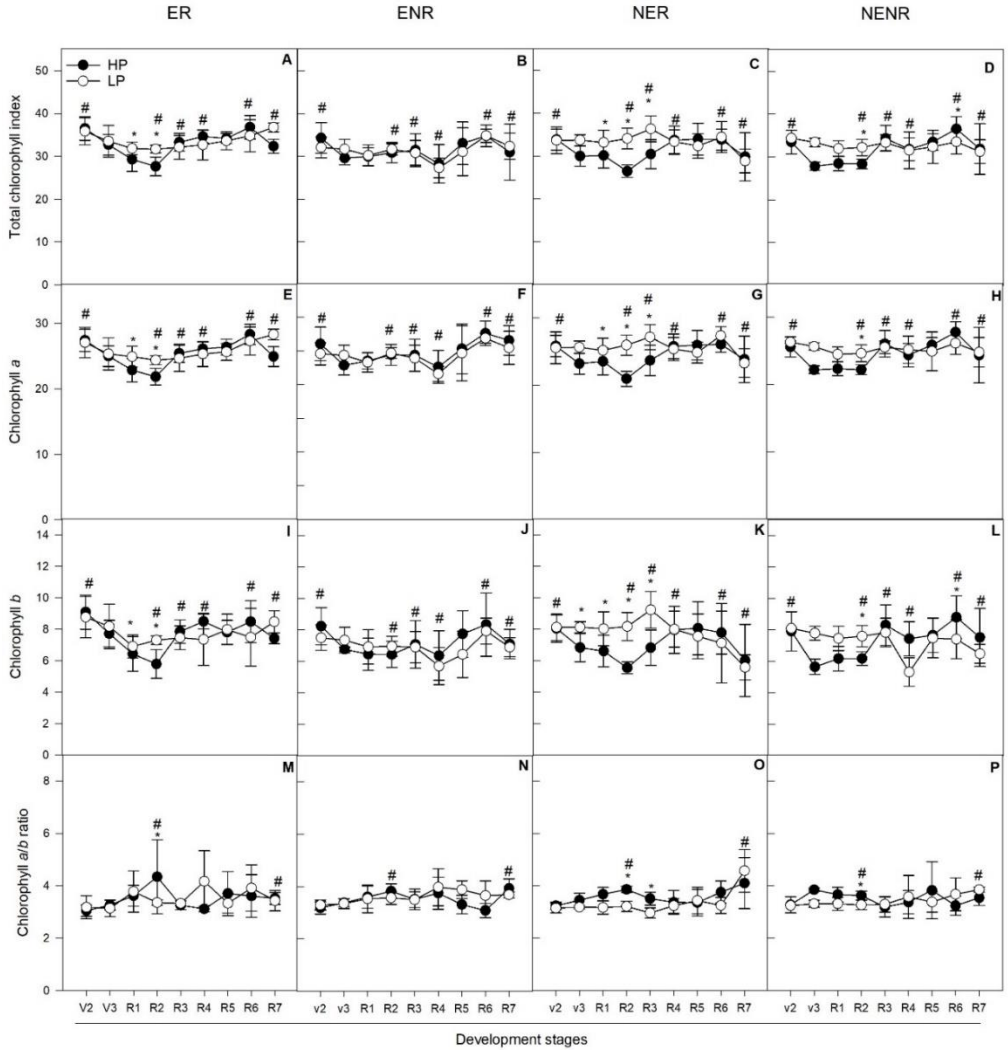

Fig. 6 Total Chlorophyll index (A, B, C, D), Chlorophyll a (E, F, G, H), Chlorophyll b (I, J, K, L), Chlorophyll a/b ratio (M, N, O, P) during the development stages of soybean cultivars efficient and responsive (ER; A, E, I, M), efficient and non-responsive (ENR; B, F, J, N), non-efficient and responsive (NER; C, G, K, O), non-efficient and non-responsive (NENR; D, H, L, P) soybean cultivars. The asterisks represent the difference between the nutritional conditions of high phosphorus (HP) and low phosphorus (LP). The hash corresponds to the statistical difference between the groups of cultivars, using the Tukey test at 5%.

In general, plant growth (leaf, stem, root and total dry matter production) was greater in the treatment with high phosphorus availability (HP) than with low phosphorus availability (LP) at all stages of development (Table 2). From R2 to R7, there was differentiation between

the cultivars regarding the dry mass of the stem, leaves and total, with the cultivar ENR presenting the highest mass and the cultivars NER and NENR presenting the lowest (Table 2).

All cultivars had similar leaf areas in V2, regardless of phosphorus nutrition (Table 2). The ENR cultivar was the only one to reduce leaf area by R2 under phosphorus deficiency. The other cultivars reduced leaf area under phosphorus deficiency only in R5 and maintained this difference in R7. The cultivars ER, NER and NENR lost many leaves in R7, resulting in a reduction in leaf area. However, the ENR cultivar prolonged leaf lifespan in LP, equalizing the leaf area between HP and LP conditions.

The cultivars showed similar LMA in V2, regardless of P availability (Table 2). In R2, only ENR showed LMA in HP equal to LP. This value was higher than other cultivars. In R5, ER cultivars showed a reduction in LMA in LP, while NER increased. At the end of the cycle, all cultivars reduced LMA under P deficiency, except ENR.

All cultivars showed higher yields in productive traits under the HP condition (Table3)

Table 2 - Leaf Area, leaf dry mass (Leaf DM), shoot dry mass (Shoot DM), root dry mass (Root DM), total dry mass (Total DM) and leaf mass per area (LMA) during the development stages of soybean cultivars efficient and responsive (ER), efficient and non-responsive (ENR), non-efficient and responsive (NER), non-efficient and non-responsive soybean cultivars. Capital letters compare high phosphorus (HP) and low phosphorus (LP) conditions. Lowercase letters compare cultivar groups (ER, ENR, NER, NENR) using the Tukey test at 5%.

Cultivar	Stages	Leaf Area (cm^2 $plant^{-1}$)		Leaf DM (g $plant^{-1}$)		Shoot DM (g $plant^{-1}$)		Root DM (g $plant^{-1}$)		Total DM (g $plant^{-1}$)		LMA (g m^2)	
		HP	LP	HP	LP	HP	LP	HP	LP	HP	LP	HP	LP
ER	V2	71,250	76,791	0,297	0,208	0,127	0,098	0,515	0,315	0,940	0,632	0,0041	0,0034
		Aa	**Aa**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**		
	R2	139,40	119,896	1,008	0,379	0,389	0,155	0,912	0,444	2,369	1,003	0,0072	0,0032
		A	**Aa**	**Aab**	**Bab**	**Aab**	**Bab**	**A**	**B**	**Aab**	**Bab**	**Aa**	**Bb**
	R5	161,28	87,223	1,477	0,537	0,951	0,327	1,313	0,628	4,767	1,826	0,0142	0,0099
		A	**Bb**	**Aab**	**Bab**	**Aab**	**Bab**	**A**	**B**	**Aab**	**Bab**	**A**	**B**
	R7	92,658	40,319	0,826	0,321	1,183	0,671	1,173	0,549	7,550	2,686	0,0132	0,0074
		A	**B**	**Aab**	**Bab**	**Aab**	**Bab**	**A**	**B**	**Aab**	**Bab**	**A**	**B**
ENR	V2	74,509	81,486	0,302	0,249	0,136	0,130	0,446	0,312	0,886	0,692	0,0044	0,0031
		Aa	**Aa**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**		
	R2	140,50	80,670	1,343	0,580	0,536	0,434	0,871	0,498	2,833	1,592	0,0095	0,0071
		A	**Bb**	**Aa**	**Ba**	**Aa**	**Ba**	**A**	**B**	**Aa**	**Ba**	**Aa**	**Aa**
	R5	246,87	142,458	1,966	0,910	1,385	0,645	1,810	0,745	6,058	2,621	0,0080	0,0064

		A	**Ba**	**Aa**	**Ba**	**Aa**	**Ba**	**A**	**B**	**Aa**	**Ba**	**A**	**A**
	R7	94,736	62,489	1,549	0,630	2,004	0,869	1,586	0,815	9,969	4,028	0,0144	0,0095
		A	**A**	**Aa**	**Ba**	**Aa**	**Ba**	**A**	**B**	**Aa**	**Ba**	**A**	**A**
NER	V2	71,709	70,405	0,293	0,237	0,126	0,107	0,562	0,313	0,982	0,659	0,0041	0,0033
		Aa	**Aa**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**		
	R2	152,08	125,792	1,132	0,388	0,380	0,145	0,955	0,445	2,632	1,005	0,0074	0,0031
		A	**Aa**	**Aab**	**Bab**	**Aab**	**Bab**	**A**	**B**	**Aab**	**Bab**	**Aa**	**Bb**
	R5	245,85	61,385	1,413	0,421	0,823	0,230	1,306	0,475	4,385	1,32	0,0091	0,0126
		A	**Bb**	**Ab**	**Bb**	**Ab**	**Bb**	**A**	**B**	**Ab**	**Bb**	**B**	**A**
	R7	88,802	24,195	1,018	0,101	1,102	0,224	1,150	0,337	6,782	1,315	0,0120	0,0044
		A	**B**	**Ab**	**Bb**	**Ab**	**Bb**	**A**	**B**	**Ab**	**Bb**	**A**	**B**
NENR	V2	77,526	68,788	0,318	0,243	0,140	0,107	0,559	0,373	1,011	0,724	0,0041	0,0043
		Aa	**Aa**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**		
	R2	130,54	120,626	0,940	0,352	0,362	0,153	0,979	0,446	2,343	0,975	0,0071	0,0030
		5**A**	**Aa**	**Ab**	**Bb**	**Ab**	**Bb**	**A**	**B**	**Ab**	**Bb**	**Aa**	**Bb**
	R5	158,57	79,790	1,350	0,514	0,870	0,290	1,284	0,550	4,177	1,538	0,0148	0,0140
		1**A**	**Bb**	**Aab**	**Bab**	**Aab**	**Bab**	**A**	**B**	**Aab**	**Bab**	**A**	**A**
	R7	80,136	30,645	1,175	0,246	1,284	0,316	1,300	0,459	7,215	1,839	0,0228	0,0079
		A	**B**	**Aab**	**Bab**	**Aab**	**Bab**	**A**	**B**	**Aab**	**Bab**	**A**	**B**

Table 3- Flower dry mass (Flower DM), pod dry mass (Pod DM), grain dry mass (Grain DM) of soybean cultivars efficient and responsive (ER), efficient and non-responsive (ENR), non-efficient and responsive (NER), non-efficient and non-responsive soybean cultivars. Capital letters compare high phosphorus (HP) and low phosphorus (LP) conditions. Lowercase letters compare cultivar groups (ER, ENR, NER, NENR) using the Tukey test at 5%.

Cultivar	Flower DM (g plant^{-1})		Pod DM (g plant^{-1})		Grain DM (g plant^{-1})	
	HP	LP	HP	LP	HP	LP
ER	0.059**A**	0.02**3B**	2.785**A**	0.739**B**	1.582**A**	0.405**B**
ENR	0.081**A**	0.078**A**	3.153**A**	1.158**B**	1.675**A**	0.554**B**
NER	0.164**A**	0.026**B**	2.324**A**	0.441**B**	1.186**A**	0.210**B**
NENR	0.060**A**	0.022**B**	2.443**A**	0.567**B**	1.125**A**	0.249**B**

4- Discussion

Soybean cultivars grown in soil with low and sufficient P presented different strategies in the use of P, indicating genetic variability in their responses to P limitation. Characteristics related to growth, P allocation and photosynthetic capacity combined with response time,

highlighted the plasticity of cultivars in response to P availability. Contrary to our hypothesis, the efficient and non-responsive cultivar (ENR), and not the efficient and responsive (ER) one, showed similar photosynthetic rates under nutritional conditions, the result of a differential allocation in P fractions.

The cultivar ENR responded to phosphorus deficiency at the beginning of reproductive growth with a reduction in growth, but presented higher levels of total chlorophyll, F_v/F_m, ϕ_{PSII} and NPQ in R2 compared to the other cultivars. The superior performance of the photosynthetic apparatus may be related to the positive effect of high nitrogen availability in the tissues of these plants. In other words, nitrogen was not fully used by metabolism due to phosphorus limitation, but it favored investment in components of the photosynthetic machinery, such as chlorophyll molecules. The authors Bulgarelli et al., (2019) and Thomas et al., (2006) observed a high N:P in studies with *Eucalyptus spp.* and there was no reduction in chlorophyll content under P deficiency.

Furthermore, at the stage of high energy demand for grain filling, the ENR cultivar was able to allocate a greater proportion of P in metabolic forms. The increase in this organic P fraction may have favored photosynthetic performance in conditions of low P availability (LP), making it similar to that of high P availability (HP). The reduction in the proportion of P in nucleic acids may be a result of the greater allocation of metabolic P and, additionally, the formation of proteins to support growth. Studies demonstrate that a large amount of P in nucleic acids is related to P-rich ribosomal RNA (Hayes et al., 2022; Mo et al., 2019). Furthermore, higher plant growth rates require greater investment in ribosomal RNA to produce the proteins needed for growth. Therefore, the higher yield in the growth parameters of this cultivar when compared to the others under P deficiency conditions is justified.

ENR cultivars adopted the strategy of reducing P-lipid under P deficiency, replacing membrane phospholipids with galactolipids and sulfolipids during leaf development. This lipid remodeling released more P for other functions and parts of the plant, improving tolerance in P-depleted soils (Soumya et al., 2022). Most lipid membranes in chloroplasts do not contain phosphorus and those that do can be partially replaced without affecting electron transport in photosystem II and chloroplast development. The results showed that photosynthetic rates and ETRmax were not affected by the reduction of phospholipids in soybean leaves, indicating that photosynthetic capacity was maintained even with less lipidic P.

The lower P responsiveness of the ENR group can be attributed to the fact that the maximum growth potential was already reached with lower P availability. Thus, while P efficiency is related to the ability to grow under low P inputs, responsiveness is associated with increased

growth potential with additional P inputs. Therefore, the non-responsiveness to P addition may be indicative of high PUE under low P, which is a desired characteristic as long as yields are maintained (Hammond et al., 2009).

The soybean cultivars with high responsiveness to P, ER and NER showed an increase in V_{cmax}, J, TPU, ETR when fertilized with P. Medina et al., (2022) evaluated one of the cultivars belonging to the ER group of this work, the cultivar TMG7063, which was also efficient in P-deficient conditions, due to high carboxylation capacity, RuBP regeneration and triose-P export. This demonstrates the productive stability of the cultivar in the field and in nutrient solution. The reductions in V_{cmax} and J values at the lowest doses of P may have occurred as a consequence of decreases in the activation state and the amount of rubisco (Singh et al., 2019), due to TPU limitation over time (Zhu et al., 2016). Activation of rubisco by the enzyme rubisco activase (RCA) requires the hydrolysis of ATP (Waheeda & Chiu, 2022); therefore, with lower energy production under P deficit, rubisco activation may be lower. Furthermore, P deficiency reduces the expression of RCA genes (such as GmRCAβ) that are associated with P efficiency in soybean (Chu et al., 2018). Another potential limitation of RuBP regeneration is the lower activity of enzymes in the Calvin-Benson cycle that also depend on ATP, such as 3-phosphoglycerate kinase, NADP-glyceraldehyde-3-phosphate dehydrogenase, sedoheptulose-1,7-bisphosphate phosphatase and ribulose -5-phosphate kinase (Tantray et al., 2020).

The big difference between ER and NER was the photoprotection ability, related to non-photochemical excess energy dissipation in a regulated manner, presented by ER cultivars since V2. An increased NPQ value is important for the performance of soybean cultivars under phosphorus deficit. Reduction in ATP synthase activity at low P stress limits proton export to the chloroplast stroma, acidifying the lumen. With this process, there is a reduction in ETR under P deficiency (Carstensen et al., 2018), as also observed in this study, and the reduction in lumen pH activates NPQ components. The dissipation of excess energy may be related to the xanthophyll cycle, in which zeaxanthin, converted from violaxanthin, can dissipate excess energy and protect photosystems from overexcitation and possible damage.

Despite having one of the highest average concentrations of P in the leaves, the NENR cultivars had the lowest photosynthetic rates. The low photosynthetic efficiency may be related to the increase in LMA in R5. The increase in LMA causes a decline in photosynthetic rates through greater resistance to CO_2 diffusion through mesophyll (Hidaka & Kitayama, 2011), which may have contributed to lower V_{cmax} values in this group of cultivars. In previous work (unpublished data), Medina et al. observed a reduction in gm under P deficiency. Furthermore, NENR cultivars showed an increase in the concentration of lipid P in P-deficient plants,

indicating a possible increase in the thickness or hardness of the membranes, causing an increase in the resistance to CO_2 entry and a reduction in V_{cmax} values in HP (Hayes et al., 2022).

Phosphorus limitation mainly affects leaf expansion, which directly impacts biomass production, while reduced photosynthesis may or may not occur (Fredeen et al., 1990; Gutiérrez-Boem & Thomas, 2001). At the beginning of development (V2), the leaf area of cultivars under low P availability was not affected, as initial growth largely depends on P reserves in the seed (Veneklaas et al., 2012). The duration of dependence on seed P reserves varies depending on seed mass, seed P concentration, soil P availability, and seedling P need. In maize seedlings, significant uptake of P from the soil begins about 5 days after sowing, but use of seed P reserves continues for about 2 weeks (Nadeem et al., 2011). Due to the depletion of the P reserve and/or efficiency in the use of P, the ENR cultivar reduced leaf expansion at full flowering, while the other cultivars reduced it only at grain filling. The reduction in LA caused an increase in LMA, but it did not resulted necessarily in low photosynthetic efficiency.

Interestingly, photosynthetic efficiency in the use of P (PPUE) varies by an order of magnitude in any LMA and part of this variation can be attributed to some factors such as N and P concentration in leaves and leaf lifespan. According to Hidaka & Kitayama, (2011) high PPUE in nature is achieved mainly by leaves with low P concentrations and high P remobilization after senescence. Such characteristics are expected in high LMA leaves with long shelf life and low photosynthetic capacity. In soybeans, the maintenance of the leaves attached to the stem for a longer time at the end of the cycle also demonstrates an efficient strategy carried out by ENR.

Despite the reduction in leaf area under P deficiency at full flowering, ENR maintained biomass gain. Carbon gain at this stage favored the development and setting of flowers under P deficiency. Greater biomass accumulation is associated with the formation of more flowers in the reproductive stage. Phosphorus application reduces flower abortion (L.-W. Xu et al., 2024). The formation of pods in soybeans is directly related to the height of the stem and branches. Although the photosynthetic rate did not differ between HP and LP for the ENR and NENR cultivars, there was greater grain production in HP due to stem height and leaf area.

5- Conclusion

Soybean genotypes showed clear differences in photosynthetic response efficiency under low P availability. Cultivars that are not photosynthetically responsive to P fertilization are also not productively responsive.

The most efficient cultivar under P deficiency was also less responsive. The photosynthetic efficiency of this cultivar was supported by greater allocation of metabolic P and reduction of lipid P, in addition to the more robust photosynthetic apparatus from the initial stages of development.

The biggest difference between efficient and inefficient cultivars, regardless of responsiveness, is the ability to dissipate excess energy in a regulated manner.

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Supplementary material

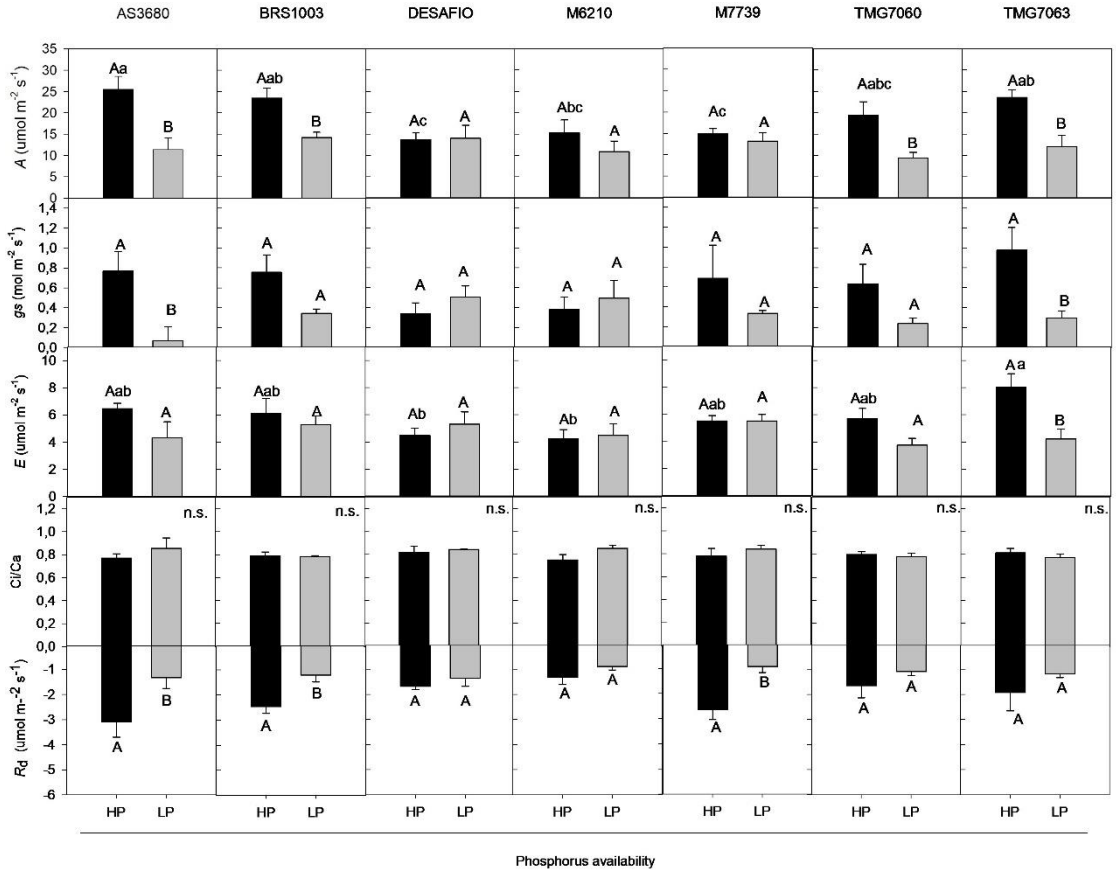

Fig. S1 Net photosynthetic rate (*A*), stomatal conductances (g_s), transpiration (*E*), ratio of intercellular to ambient CO2 concentrations (Ci/Ca), respiration dark (R_D) of cultivars AS3680, BRS1003, DESAFIO, M6210, M7739, TMG7060, TMG7063. Capital letters compare high phosphorus (HP) and low phosphorus (LP) conditions. Lowercase letters compare cultivars, using the Tukey test at 5%.

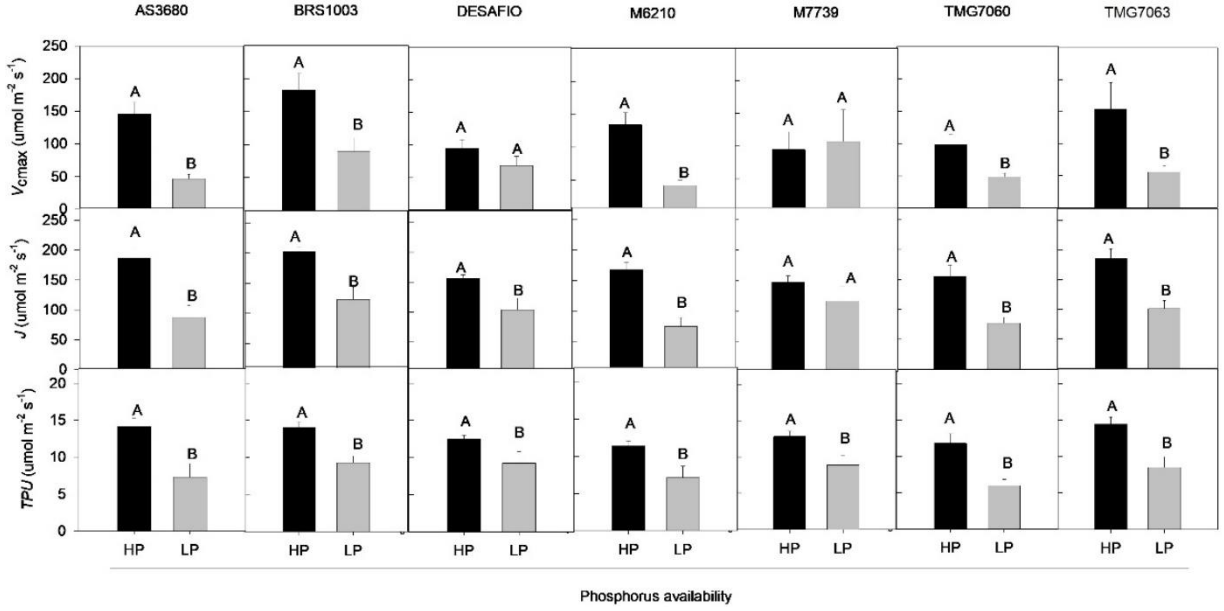

Fig. S2 Maximum carboxylation rate (V_{cmax}), electron transport capacity (*J*), triose phosphate utilization (*TPU*) of cultivars AS3680, BRS1003, DESAFIO, M6210, M7739, TMG7060, TMG7063. Capital letters compare high phosphorus (HP) and low phosphorus (LP)

conditions. Lowercase letters compare cultivars, using the Tukey test at 5%.

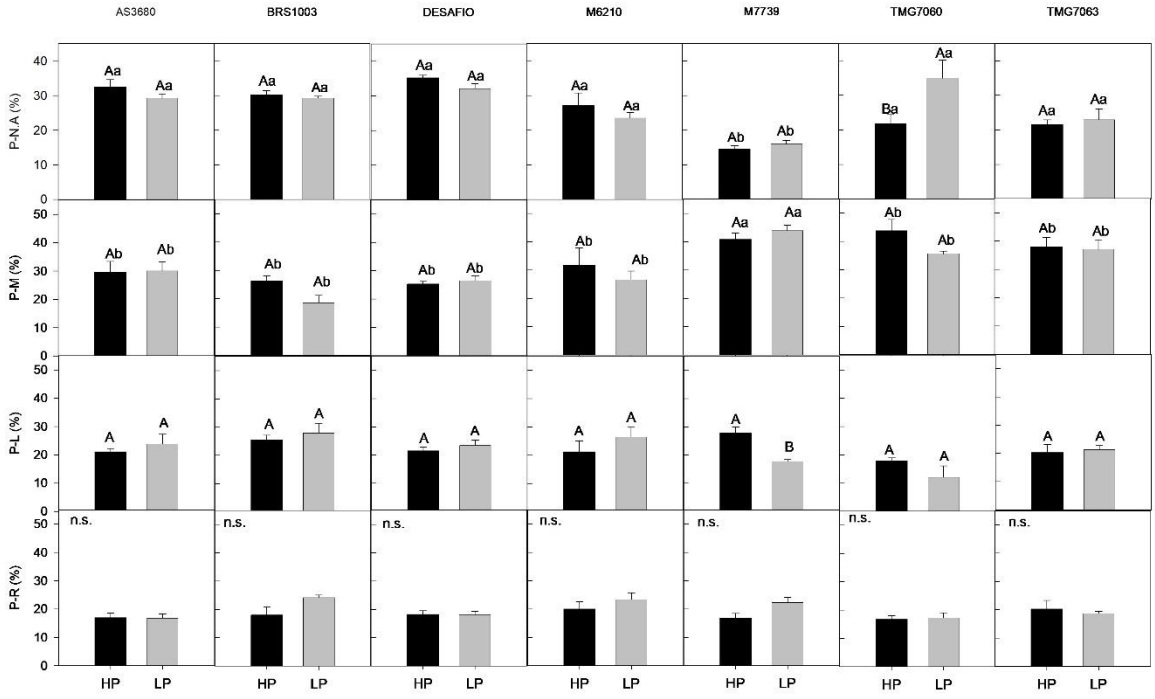

Fig. S3 Allocation of P fractions in percentage (%) and concentration of organic P fractions of cultivars AS3680, BRS1003, DESAFIO, M6210, M7739, TMG7060, TMG7063. Capital letters compare high phosphorus (HP) and low phosphorus (LP) conditions. Lowercase letters compare cultivars, using the Tukey test at 5%.

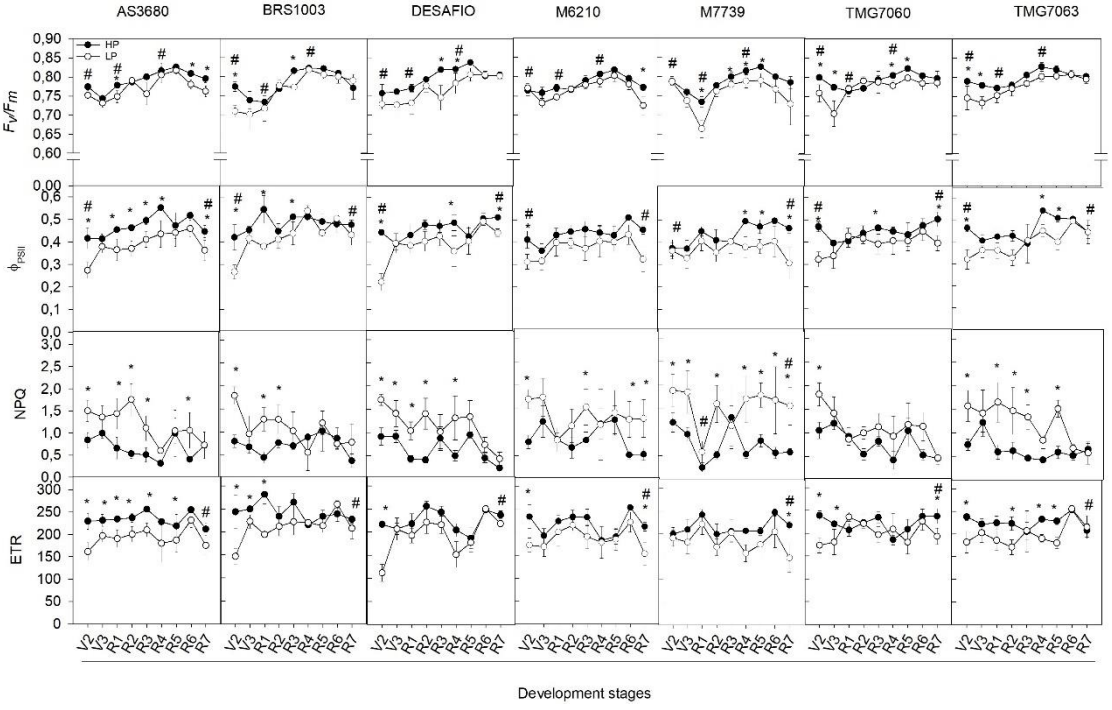

Fig. S4 Maximum quantum efficiency of photosystem II (F_v/F_m), effective quantum yield of PSII (ϕ_{PSII}), electron transport rate (ETR), nonphotochemical fluorescence quenching (NPQ) during the development stages of cultivars AS3680, BRS1003, DESAFIO, M6210, M7739, TMG7060, TMG7063 under high

phosphorus (HP) and low phosphorus (LP). The hash corresponds to the statistical difference between the groups of cultivars, using the Tukey test at 5%.

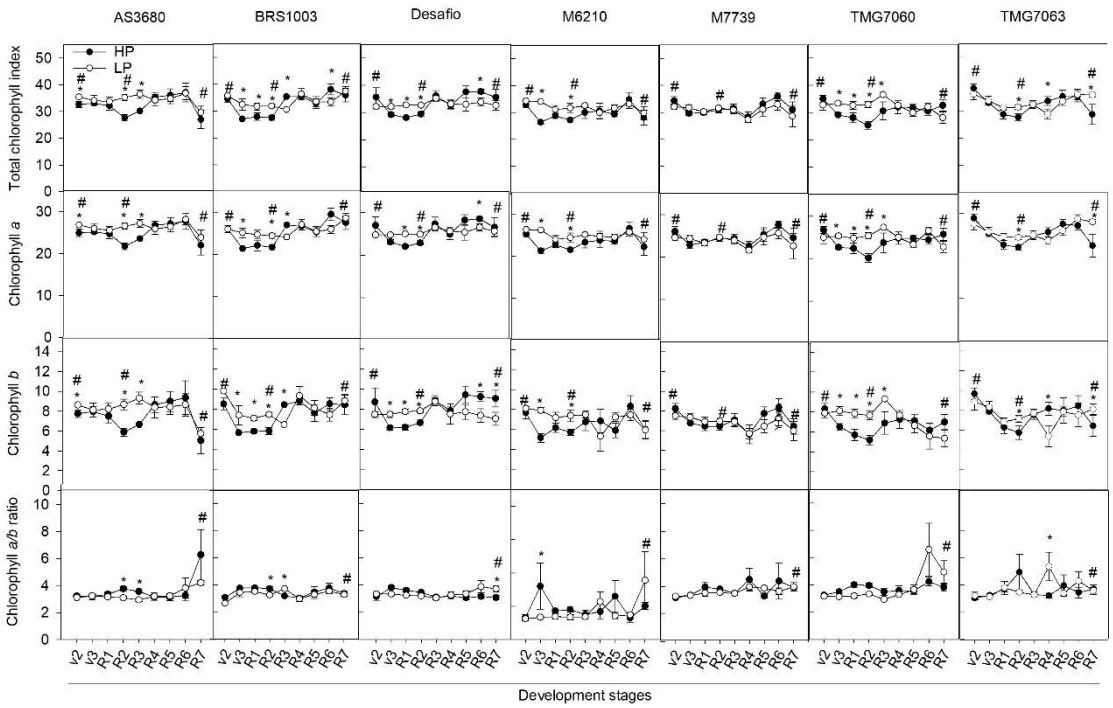

Fig. S5 Total Chlorophyll index, Chlorophyll *a*, Chlorophyll *b*, Chlorophyll *a/b* ratio during the development stages of AS3680, BRS1003, DESAFIO, M6210, M7739, TMG7060, TMG7063 under high phosphorus (HP) and low phosphorus (LP). The hash corresponds to the statistical difference between the groups of cultivars, using the Tukey test at 5%.

Final Considerations

Phosphorus (P) deficiency significantly affects photosynthesis and grain production of different soybean genotypes. Combined with water stress, there is a reduction in the quantity and quality of grains.

P deficiency influences the recovery capacity of soybean cultivars after recurrent water deficits, affecting physiological parameters such as stomatal resistance and mesophilic conductance. Under conditions of P deficiency, the recovery of photosynthesis and stomatal conductance is faster, especially in cultivars sensitive to water stress that have gone through multiple deficit cycles. However, this deficiency also results in lower protein content and higher unsaturated fatty acid content in grains.

Our studies have shown that the efficiency and responsiveness of soybean cultivars to P addition vary, indicating genetic variability in their responses to P limitation. P-efficient cultivars demonstrate greater metabolic P allocation and effective mechanisms for dissipating excess energy, while P-responsive cultivars have better photosynthetic and grain yield.

Therefore, strategies to increase P use efficiency and recovery capacity under drought stress are crucial to improving soybean sustainability and productivity in phosphorus-deficient soils.