

Callose in Soybean Roots: A Biochemical Marker of Seed Physiological Quality under Water Deficit

Natalia Carolina Moraes Ehrhardt-Brocardo, Cileide Maria Medeiros Coelho*,
Clovis Arruda Souza

Santa Catarina State University, Agroveterinary Science Center, Av. Luiz de Camões 2090,
88520-000 Lages, Santa Catarina, Brazil

*Corresponding author. E-mail: souza_clovis@yahoo.com.br

ABSTRACT

Callose is a key component of plant cell walls, synthesized in response to biotic and abiotic stresses. This study aimed to evaluate the accumulation of callose in the roots of soybean seedlings of two cultivars, BS2606IPRO and NA5909 RG, which differed in the physiological quality of the seeds and which were subjected to water deficit during germination. The seed physiological quality was evaluated through germination and vigor tests, with BS2606IPRO showing superior performance (97% germination and 89% vigor) compared to NA5909 RG (91% germination and 40% vigor). Water deficit was simulated using a polyethylene glycol (PEG 6000) solution at -0.2 MPa, -0.3 MPa and -0.4 MPa, while the control used deionized water (0.0 MPa). The seeds were hydrated for 24, 48, 72, and 96 hours post-radicle protrusion, and 13 root tip samples (5 mm) were collected for callose analysis. Callose accumulation in seedlings ranged from 0.018 to 0.356 $\mu\text{g}/\text{root tip}$, with different responses among cultivars under water stress. The cultivar NA5909 RG, 96 h after radicle protrusion, showed an increase of 588% in callose content compared to BS2606IPRO (0.172 and 0.025 $\mu\text{g}\cdot\text{root}^{-1}$, respectively), indicating greater susceptibility to water stress. These findings highlight callose accumulation as a potential biomarker for stress susceptibility in soybean, providing insights for management and cultivar selection.

Keywords: *Glycine max*, β -glucan, germination, vigor, abiotic stress.

INTRODUCTION

Seed quality plays a fundamental role in agricultural productivity, influencing crop establishment, yield potential, and resilience to environmental stresses (Carvalho and Nakagawa, 2012; Marcos-Filho, 2015a). Among the attributes that comprise seed quality, physiological characteristics (germination and vigor) are fundamental for predicting seed performance in the field under different growing conditions (Peske et al., 2003; Marcos-Filho, 2015a; Krzyzanowski et al., 2020). Therefore, ensuring high seed vigor is particularly important in locations prone to abiotic stresses, such as water deficit, where the initial development of seedlings may be compromised (Ehrhardt-Brocardo et al., 2019).

Water availability is a determining factor for the germination process, as it activates metabolic pathways involved in cell growth

and expansion and seedling emergence (Bewley et al., 2013). Thus, a water deficit condition can interrupt these processes, further affecting membrane integrity, enzymatic activity and energy metabolism, also influencing seedling establishment (Lone et al., 2021; Munaweera et al., 2022). Soybean cultivars with contrasting vigor percentages can be classified into high and low vigor groups, which show their ability to overcome abiotic stress during germination (Marcos-Filho, 2015b; Ehrhardt-Brocardo et al., 2019).

Callose is a polysaccharide (β -1,3-glucan) widely recognized for its role in plant defense and stress adaptation. Its synthesis occurs in response to biotic and abiotic stresses, through the formation of physical barriers that restrict pathogen invasion and regulate cell permeability (Chen and Kim, 2009; Vu et al., 2023). Under water deficit conditions, callose deposition has been associated with a

strategy to reduce water loss and, therefore, to increased stress tolerance (Wissemeier et al., 1992; Nedukha, 2015). It was demonstrated in soybean seedlings that callose accumulation varies significantly between different cultivars, with seedlings originating from low vigor seeds showing the greatest deposition under water deficit conditions (Ehrhardt-Brocardo et al., 2019). Some studies have also suggested that callose may function as an indicator of cellular damage, as its excessive accumulation can hinder intercellular communication and nutrient transport (Radford and White, 1998; Wolf and Greiner, 2012).

Despite advances in studies on seed vigor and forms of adaptation to stress, the relationship between callose deposition in seedlings originating from seeds with differences in physiological quality remains little explored. In Ehrhardt-Brocardo et al. (2019), when investigating seedlings of different soybean cultivars, observed a significant increase in callose content (approximately 35.5%) in seedlings subjected to water deficit induced by polyethylene glycol solutions. Based on this, a better understanding of these dynamics can help in the development of screening tools to select stress-tolerant genotypes and improve seed production practices (Corbineau, 2012; Hampton et al., 2016). Thus, this study aims to investigate callose deposition in roots of soybean seedlings under simulated water deficit conditions, comparing two cultivars with differences in seed physiological quality, in order to elucidate the role of this parameter as a biochemical marker of susceptibility to water deficit.

MATERIAL AND METHODS

This study was carried out using seeds from two soybean cultivars (BS2606IPRO and NA5909 RG) that presented seeds with contrasting physiological qualities. The samples were homogenized and reduced to obtain average samples. Using these average samples, working samples were obtained for different tests, as described in the Brazilian Standards for Seed Analysis (Brasil, 2009). The seeds were stored in a dry chamber with

a relative humidity of $50 \pm 5\%$ and a temperature of $10 \pm 2^\circ\text{C}$ until analysis.

Seed Quality Assessment: the germination test was conducted with 400 seeds. Previously, seed samples of both cultivars were subjected to preconditioning in polystyrene crystal boxes (*Gerbox*[®]) with aluminum screens suspended using 40 mL of deionized water and placed in germination chambers (model *Mangelsdorf*) for 16 h at 25°C . Afterwards, the seeds were distributed equidistantly on Germitest paper towels moistened with deionized water at a proportion of 2.5 mL g^{-1} of dry paper, where they remained in the germination chamber at $25 \pm 2^\circ\text{C}$. The count of normal, abnormal, and dead seedlings was performed on the fifth (first evaluation) and eighth (second evaluation) days after sowing. The results were expressed as a percentage of normal seedlings (Brasil, 2009). The accelerated aging test was performed with 400 seeds, divided into two replicates and distributed in crystal polystyrene boxes (*Gerbox*[®]) with aluminum screens suspended inside. After obtaining the samples, 40 mL of deionized water was added to the bottom of the boxes and these were placed in an aging chamber for 48 h at 41°C (Marcos-Filho et al., 2009). Subsequently, germination was performed, and the methodology and results evaluation methods were conducted as previously described (Brasil, 2009).

Water Deficit Simulation: the water deficit simulation was performed using 200 seeds with polyethylene glycol solution prepared at -0.2 MPa , -0.3 MPa and -0.4 MPa (Villela et al., 1991). A control treatment (0.0 MPa) was conducted using deionized water. The seeds were hydrated for 24, 48, 72 and 96 h after radicle protrusion. At each interval, 13 root tip samples (5 mm in length) for each treatment were excised for callose quantification, following the method described by Zhang et al. (1994).

Callose Quantification: root samples were pretreated with ethanol and extracted with NaOH at 80°C , followed by centrifugation. The supernatant reacted with aniline blue, HCl, and glycine-NaOH buffer (pH 9.5) and incubated before fluorescence measurement.

Callose content was determined using an RF-6000 Fluorescence Spectrometer (Shimadzu, Japan) coupled to LabSolutions RF software with excitation at 398 nm and emission at 495 nm. Curdlan reagent was used as a standard, and callose content was expressed in micrograms per root tip ($\mu\text{g root tip}^{-1}$).

Statistical Analysis: the experimental design was a completely randomized design and analysis of variance (F Test) was performed and the means were compared using Tukey's test ($P < 0.05$) in Assistat software (Silva and Azevedo, 2002).

RESULTS AND DISCUSSION

From the analysis of seed physiological quality, a significant difference was observed between the two cultivars (Table 1). The tests indicated that, overall, the BS2606IPRO cultivar demonstrated superior performance compared to the NA5909 RG cultivar in

terms of seed physiological quality. The BS2606IPRO cultivar obtained a higher germination percentage and vigor, suggesting that its seeds are more efficient in initial establishment and seedling development when compared to the NA5909 RG cultivar. On the other hand, the NA5909 RG cultivar seeds performed worse in both tests, evidencing lower physiological quality, which may reflect lower adaptability and resistance to environmental stresses during the early stages of plant development. In the study by Ehrhardt-Brocardo et al. (2019), soybean cultivar selection included seeds with similar germination rates but significant differences in vigor, demonstrating the influence of this physiological attribute on seedling performance. In this study, a different design was adopted, selecting cultivars that differ in both germination and vigor, enabling a more comprehensive analysis.

Table 1. Physiological seed quality of two soybean cultivars

Cultivars	Germination	Accelerated Aging
	%	
BS2606IPRO	97 a	89 a
NA5909 RG	91 b	40 b
CV (%)	2.16	6.81

Mean values followed by the same letter do not differ according to Tukey's test ($P < 0.05$).

Water deficit simulation demonstrated that PEG 6000 treatment at -0.4 MPa effectively induced stress, significantly reducing germination rates compared to the control (Table 2). This finding is corroborated by

previous studies demonstrating that increasing PEG concentrations potentiate the effects of water limitation on seed metabolism (Hampton et al., 2016).

Table 2. Germination test with polyethylene glycol (PEG 6000) solution at potentials of -0.2 MPa, -0.3 MPa, -0.4 MPa and the control (0.0 MPa)

Cultivars	Osmotic Potencial (MPa)				CV (%)
	Control	-0.2	-0.3	-0.4	
BS2606IPRO	97 aA	95 aA	88 aA	85 aA	5.13
NA5909 RG	91 bA	86 aA	76 aA	23 bB	11.43
CV (%)	2.64	4.24	6.68	20.06	

The mean values followed by the same letter do not differ according to the Tukey test ($P < 0.05$), lowercase in the column and uppercase in the row.

Based on the results obtained, it was found that callose accumulation levels were similar in both cultivars under control conditions, indicating a minimal physiological

response to stress and suggesting similar behavior in a favorable germination environment (Table 3). However, when the seeds were subjected to water deficit conditions,

the NA5909 RG cultivar, with lower physiological quality, showed significantly higher callose accumulation compared to the

BS2606IPRO cultivar, demonstrating a more intense response to stress (Table 3).

Table 3. Callose content ($\mu\text{g}/\text{root tip}$) in two soybean cultivars under induced water deficit

Cultivars	Treatment	
	water	PEG
	$\mu\text{g}/\text{root tip}$	
BS2606IPRO	0.09329 aA	0.02549 bB
NA5909 RG	0.08557 aB	0.17535 aA

The mean values followed by the same letter do not differ according to the Tukey test ($P < 0.05$), lowercase in the column and uppercase in the row.

This increase in callose content can be interpreted as an adaptive mechanism, in which the deposition of this polysaccharide acts to protect cells against damage caused by water limitation, a characteristic often associated with stress resistance processes (Wolf and Greiner, 2012). Thus, the cultivar NA5909 RG demonstrates a greater dependence on this accumulation of callose to mitigate the effects of water deficit, while BS2606IPRO showed lower deposition even under adverse conditions, possibly due to genetic or physiological characteristics that confer greater resistance to water loss or greater efficiency in stress tolerance (Ehrhardt-Brocardo et al., 2019).

In all hydration periods evaluated, the BS2606IPRO cultivar presented higher

callose content when germinated in water compared to induced water deficit, suggesting a more efficient response to water availability during the germination process (Figure 1).

In contrast, the NA5909 RG cultivar did not present a consistent pattern of callose accumulation, either in germination conditions in water or under water deficit, except at 96 h after radicle protrusion, when, under stress, it accumulated significantly more callose. This behaviour indicates that BS2606IPRO maintains stable callose levels throughout the hydration periods, while NA5909 RG presents a delayed and intensified response, peaking under water deficit conditions (Figure 1).

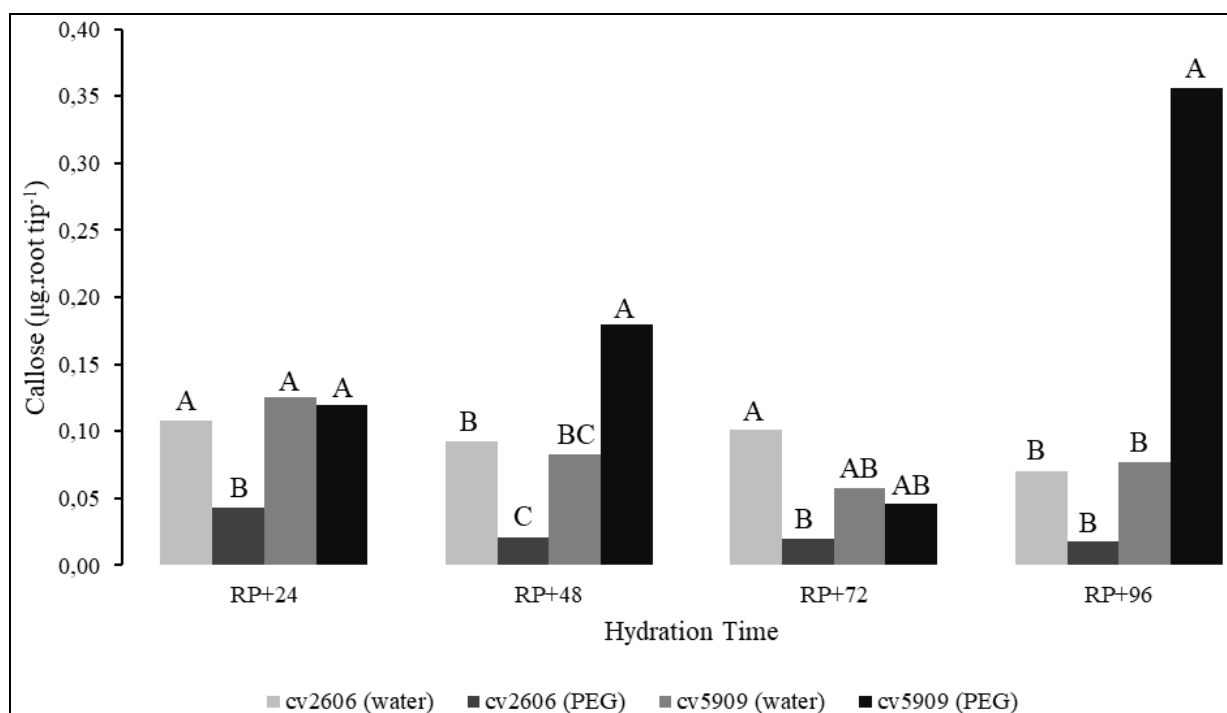


Figure 1. Callose content in roots of soybean seedlings under water deficit induced at different hydration times

These patterns corroborate that seed vigor is inversely related to callose accumulation, since low vigor cultivars exhibit greater deposition of this polysaccharide under water stress as a compensatory mechanism (Ehrhardt-Brocardo et al., 2019). Callose, accumulated primarily in plasma membranes and plasmodesmata, blocks cellular communication and water transport, protecting cells under stress (Nedukha, 2015; Sivaguru et al., 2000). Furthermore, during water deficit, its deposition in the phloem criviform tubes reduces the transport of aqueous solutions (Nedukha, 2015).

Thus, while the low physiological quality cultivar NA5909 RG relies on late and accentuated callose deposition to mitigate cellular damage, the cultivar BS2606IPRO maintains greater membrane integrity and does not rely on this defense mechanism, suggesting a more efficient process to respond to water stress (Kavan et al., 2019).

CONCLUSIONS

The callose accumulation in the roots of soybean seedlings is closely associated with seed physiological quality and response to water stress. Seedlings from seeds of low physiological quality exhibited higher callose accumulation, suggesting a protective, yet potentially limiting, mechanism under stress conditions. This response highlights the role of callose as a biochemical marker that can aid in identifying soybean cultivars with greater susceptibility or tolerance to water stress. Understanding these biochemical and physiological responses contributes to the advancement of seed quality assessment methodologies and the selection of resilient genotypes for seed production.

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