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Original Article

An insight into seed priming response of *Crotalaria ochroleuca* and *Crotalaria spectabilis* during storage

Uma visão sobre a resposta ao priming de sementes de *Crotalaria ochroleuca* e *Crotalaria spectabilis* durante o armazenamento

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Abstract

The proper establishment of plants is essential for the efficient use of resources such as water and light. Besides, even after seed storage and sowing the uniform establishment of plants is essential for their success. *Crotalaria ochroleuca* and *Crotalaria spectabilis* are important medicinal plants with poor seed germination rate, occasionally. The effects of seed priming in both *C. ochroleuca* and *C. spectabilis* were evaluated in seed performance even after seeds storage for up 90-days. Experimental assays were performed in a randomized design with gibberellic acid (GA_3, 100 ppm), polyethylene glycol (PEG 6000, -0.2 MPa) and PEG (-0.2 MPa) + GA_3 (100 ppm) solutions during seed priming in four replicates. Seeds not submitted to priming treatments constituted control. Seeds physiological performance were evaluated immediately and even after 30, 60 and 90-days seed dry-storage. The data obtained in each experiment were submitted to variance analysis (ANOVA) adopting a confidence level of 95%. The effects of seed priming with PEG and GA_3 during seed ageing were significant for germination variables of *C. ochroleuca* and *C. spectabilis*. During dry storage, seed viability of both species gradually decreased and the first symptoms were delayed seed germination, especially more evident for *C. ochroleuca*, even in primed or non-primed seeds. Afterwards, *C. ochroleuca* seeds previously GA_3 primed had higher results of root protrusion (86%), hypocotyls elongation (76%) and complete seedlings (75%) than non-primed seeds (control). These findings shown a good potential of hormopriming to attenuate damage during the seed aging of *C. ochroleuca*.

Keywords: gibberellic acid, polyethylene glycol, seed aging, seedlings development.

Resumo

O estabelecimento adequado das plantas é essencial para o uso eficiente de recursos como água e luz. Além disso, mesmo após o armazenamento e semeadura das sementes, o estabelecimento uniforme das plantas é essencial para o seu sucesso. *Crotalaria ochroleuca* e *Crotalaria spectabilis* são plantas medicinais importantes com baixa taxa de germinação de sementes, ocasionalmente. Os efeitos do condicionamento fisiológico de sementes em *C. ochroleuca* e *C. spectabilis* foram avaliados no desempenho das sementes mesmo após o armazenamento por até 90 dias. Os ensaios experimentais foram realizados em delineamento casualizado com soluções de ácido giberélico (GA_3, 100 ppm), polietilenoglicol (PEG 6000, -0,2 MPa) e PEG (-0,2 MPa) + GA_3 (100 ppm) durante o condicionamento físiológico das sementes, em quatro repetições. As sementes que não foram submetidas aos tratamentos de condicionamento constituíram o controle. O desempenho fisiológico das sementes foi avaliado imediatamente e após 30, 60 e 90 dias de armazenamento. Os dados obtidos em cada experimento foram submetidos à análise de variância (ANOVA) adotando-se nível de confiança de 95%. Os efeitos do condicionamento de sementes com PEG e GA_3 durante o envelhecimento das sementes foram significativos para as medidas de germinação de *C. ochroleuca* e *C. spectabilis*. Durante o armazenamento, a viabilidade das sementes de ambas as espécies diminuiu gradualmente e os primeiros sintomas foram o atraso na germinação das sementes, especialmente para *C. ochroleuca*. Posteriormente, sementes de *C. ochroleuca* previamente condicionadas com GA_3 apresentaram maiores resultados de protrusão radicular (86%), alongamento de hipocótilos (76%) e plântulas normais (75%) do que sementes não condicionadas (controle). Esses achados mostraram um bom potencial do condicionamento fisiológico com fitohormônio para atenuar os danos durante o envelhecimento das sementes de *C. ochroleuca*.

Palavras-chave: ácido giberélico, polietilenoglicol, envelhecimento de sementes, desenvolvimento de plântulas.

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1. Introduction

The genus *Crotalaria* L. species (Family Fabaceae) is distributed throughout the tropics and subtropic regions of the world and has been cultivated in sustainable crop production systems to reduce the application of pesticides against nematodes (Flores and Tozzi, 2008; Muli et al., 2021).

Crotalaria L. species have been reported to contain alkaloids, flavonoids and saponins, having antispasmodic, cardiodepressent and hypotensive properties. *Crotalaria* seeds contain Pyrrolizidine alkaloids and non-proteic amino acids (Kumari and Kumar, 2022). Roots and leaves of *C. spectabilis* are used externally and internally for cure of scabies and impetigo in developing tropical contries (Pandey et al., 2010). Raw leaves of *C. ochroleuca* are used in the treatment of malaria and is the one vegetable most often cited for traditional medicinal purposes (Nakaziba et al., 2021; Kumari and Kumar, 2022).

Crotalaria spectabilis and *Crotalaria ochroleuca* have an indeterminate flowering pattern, which consequently leads to a non-uniform maturity pattern and seed development. Besides, the climatic conditions during seed production may affect the interval from anthesis to harvest time, aggravating non-uniform maturity levels in seed lots. In spite of the advantages of using *Crotalaria* species have increased the demand for high quality seeds in recent years, researchers still must develop efficient procedures for the production of high-quality *Crotalaria* seed lots (Silva et al., 2022a), since its direct-seeded and the seed quality greatly affects field emergence uniformity and crop yield.

Seed quality is a key aspect for obtaining high plant establishment and crop yields. Seed germination (viability) and vigor are factors influencing seed physiological potential which govern the theoretical capacity of seeds to express their vital functions under both favorable and unfavorable environmental conditions. Rapid germination is an important component of the seed vigor concept since it usually corresponds to more rapid seedling emergence in the field (Marcos Filho, 2015; Finch-Savage and Bassel, 2016). However, this attribute can differ greatly among seed lots, and is fundamental for the proper establishment of plants, especially in a scenario with increasingly unpredictable environmental climatic conditions for plant production.

In order to increase seed performance in the field, the seed priming is a technique that allows to improve the performance of seeds physiologically by manipulating imbibition. Priming allows controlled seed hydration to trigger the metabolic processes normally activated during the early phase of germination ('pre-germinative metabolism'), but preventing the seed transition towards full germination (Paparella et al., 2015).

Water can be limited by placing seeds on absorbent paper soaked in an osmoticum of appropriate strength (osmotic priming, such as polyethylene glycol) (Finch-Savage and Bassel, 2016); alternatively, the use of phytohormone gibberellin (GA) for hormopriming has a past of benefits in seeds of several species (Paparella et al., 2015).

Seeds after all these treatments are metabolically advanced, leading to a shortened lag phase on re-imbibition and thus more rapid and uniform germination (Finch-Savage and Bassel, 2016). Its positive impacts including rapid and uniform plant establishment, early vigor, and yield gains, have been observed in various medicinal species including seeds of *Foeniculum vulgare* Mill (Tahaei et al., 2016), *Lepidium sativum* L., *Ocimum basilicum* L. (Noorhosseini et al., 2018) and *Aspilia africana* (Pers.) C. D. Adams (Okello et al., 2022).

In this way, seed priming not only has the potential to enhance the seed vigor and germinability of normal seeds but also has the excellent ability to revive the partially aged seeds (Khan et al., 2016). These include short-term imbibition allowing repair as a result of ageing during seed storage (Farooq et al., 2019; Fabrissin et al., 2021), as previously observed in stored seeds of the medicinal *Citrus limonia* Osbeck (Dantas et al., 2010). Considering the long time between the harvest of *Crotalaria* sp seeds and the beginning of their next use in the season, seed priming could be an useful technology to ensure the fast and proper establishment of the plants.

It has been hypothesized that the biosynthesis of GA during seed priming is an important step in the germination. To our knowledge, the impact of seed priming using polyethylene glycol and gibberellin in seeds of *Crotalaria ochroleuca* and *C. spectabilis* has not been studied. Additionally, the majority of the previous seed priming studies were conducted in other species of *Crotalaria* with focus on hydropriming.

Therefore, research experiments were conducted with the objective to evaluate the effect of seed priming with polyethylene glycol and gibberellin on physiological attributes of freshly harvested and stored seeds of *Crotalaria ochroleuca* and *Crotalaria spectabilis*.

2. Material and Methods

2.1. Seeds production

Experiments were conducted with seeds of *Crotalaria ochroleuca* and *Crotalaria spectabilis* developed on mother plants cultivated in optimum experimental conditions at the Research Farm of Grande Dourados University (22º13'16" S, 54º48'02" W and altitude of 430 m), Mato Grosso do Sul state, Brazil, between March and August 2019. The climate of the experimental site is characterized as Cwa, according to the Köppen classification. The soil of the experimental site is classified as clayey Rhodic Ferralsol (FAO, 2006). During both species seeds production the climatic data were gathered at the agro–meteorological observatory; the daily average of rainfall was 1.84 mm, the maximum and minimum averages temperatures were of 28.7 °C and 16.8 °C, respectively.

During the seed production treatments necessary for crop development were applied. The seeds were mechanized harvest in August 2019. A triage was performed in the laboratory to remove potentially immature or damaged seeds. After harvest, the seeds water content of *C. ochroleuca* was 11% and *C. spectabilis* presented 9.7%, both assessed gravimetrically after an oven drying at 105 ± 3°C. The results were expressed at wet basis (Brasil, 2009).

2.2. Experimental details and laboratory study

To know the water content levels attained by *C. ochroleuca* and *C. spectabilis* seeds in accordance with their triphasic pattern of water uptake, we analyzed samples of 40 seeds, replicated in four subsamples of 10 seeds. We maintained the samples in a germination chamber (Biochemical Oxygen Demand) at alternating temperatures of 20° C:30 °C and a photoperiod of 8 h:16 h (light: dark; fluorescent white light). The mass over time was recorded for both species seeds with a scale balance (precision: 0.001 g) and plotted as a mass increment curve. The fresh mass of the seed was recorded over time to plot fresh mass curves (Figure 1). After this, seed priming was undertaken for 18 h.

Prior to the osmotic and GA_3 treatments, a preliminary experiment was conducted to determine the effect of different osmopriming solutions on the germination and vigor of both *Crotalaria* species. Five seed osmopriming treatments were used, which were replicated four times in a randomized casualized design using the *C. ochroleuca* and *C. spectabilis* seeds. The seeds osmopriming treatments undertaken were polyethylene glycol (PEG 6000) solutions at -0.2, -0.4, -0.6 and -0.8 MPa (data not shown), in a germination chamber (B.O.D.) at alternating temperatures of 20 °C:30 °C and a photoperiod of 8 h:16 h (light:dark; fluorescent white light). The PEG solutions were prepared according to Michel and Kaufmann (1973). Before initiating priming studies, seeds were surface sterilized using sodium hypochlorite solution for 1 min followed by through washing with distilled water to prevent any fungal infection.

2.3. Seed priming and storage

According to the early pre-treatments the PEG solution at -0.2 MPa was more suitable to the osmopriming of the *C. ochroleuca* and *C. spectabilis* seeds for efficiently preventing root protrusion and corresponding to the osmotic solution that uses less product in relation to the other tested solutions. Seeds of *C. ochroleuca* and *C. spectabilis* were disposed over germitest paper moistened with either distilled water, PEG (-2.0 MPa) and/or GA_3 (100 ppm). In both cases, the volume used to moisten the paper was equal to 2.5 times the germitest paper mass (in g). The solution of 100 ppm GA_3 was prepared by dissolving 100 mg of GA_3 in ethyl alcohol (2 mL) followed by dilution to 1.0 L using distilled water.

Seed priming was performed for 18 h per treatment in a germination chamber at alternating temperatures of 20 °C:30 °C followed by drying of seed (25 °C, 24 h) to its original moisture content. Four replicates were used, each with 50 seeds.

To study whether seed priming enhance the seed performance even after storage, seeds were disposed inside paper bags in climatized chamber (20 °C, 55% relative humidity). We used a split-plot design to analyze these processes for each *Crotalaria* species: priming solutions (distilled water; 0 MPa and 0 ppm), PEG (-2.0 MPa), GA_3 (100 ppm) and PEG (-2.0 MPa) with GA_3 (100 ppm) x four storage periods (0, 30, 60 and 90 days). The main plot was composed of samples from each seed priming treatment while the sub-plot was storage periods. Four replicates were used, each with 50 seeds.

2.4. Data collection

2.4.1. Germination characters

Seeds were neatly arranged "between paper" moistened with distilled water. The germination test was performed in a germination chamber (Biochemical Oxygen Demand) at alternating temperatures of 20 °C:30 °C and a photoperiod of 8 h:16 h (light: dark; fluorescent white light).

The germination assessments were performed daily at the same time. The embryo protrusion and the seeds with hypocotyl elongation were expressed in percentage. The young plants observed at 21 DAS were analyzed in relation to their development potential, expressed as "normal seedlings" in terms of survival in optimum field conditions (Brasil, 2009). The root protrusion and germination index were calculated according to Xia et al. (2023) (Equations 1, 2):

$$\Sigma \frac{RPt}{Dt} \tag{1}$$

where RPt refers to the root protrusion at t and Dt refers to root protrusion days;

$$\Sigma \frac{Gt}{Dt} \tag{2}$$

where Gt refers to the germination at t and Dt refers to germination days.

2.5. Statistical analyses

Data were analyzed separately for each species. When significant effects of priming methods were detected during seed storage/aging ($P < 0.05$), germination characters data were subjected to polynomial regression analysis for which the significance of regression was assessed by the F test with Sisvar® software.

3. Results

The water content increased over time in both species' seeds (Figure 1), indicating that there is no inhibition of metabolism that can block the germination process. Interestingly, although with very similar shapes between the seeds, the water absorption pattern of the two *Crotalaria* species was different. This relationship is unique with temperature and with sample composition, and so isotherms were constructed for both species seed.

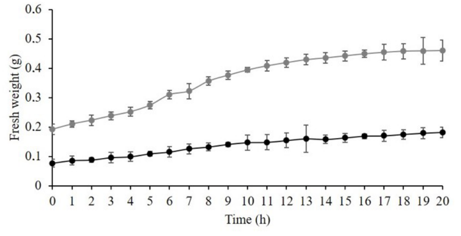

Figure 1. Mass increment over time in *Crotalaria ochroleuca* and *Crotalaria spectabilis* measured at 20-30 °C and a photoperiod of 8 h:16 h (light: dark). Bars represent standard deviation.

In spite of the mass increment over time, water uptake of *C. ochroleuca* seeds was slightly but continuous without no obvious triphasic pattern (Figure1). After 10 hours of water exposure, the seeds reached twice the initial fresh mass (0.148 g); in the following 10 hours, water absorption occurred even more gradually and the seeds had a fresh mass of 0.182 g (Figure 1).

Through the shape of water sorption isotherms of *C. spectabilis* exhibited fast increase in water uptake (Figure 1). It is often critical whether a sorption–desorption experiment has reached equilibrium and whether water contents remain constant over an extended period of time is one of the criterions. Seeds reached twice the initial fresh mass (0.38 g) after 9 hours of water exposure and came to a constant water content after 16 hours with fresh mass of 0.449 g (Figure 1).

The effects of seed priming with PEG and GA_3 during ageing were significant for germination measurements of *C. ochroleuca* and *C. spectabilis* and had adjusted polynomial equations and high regression coefficient. However, the effects of seed priming and seed ageing were distinct for each *Crotalaria* species seeds.

3.1. Crotalaria ochroleuca

Priming treatments also significantly influenced radicle protrusion. Compared to the control (dry seed), all priming treatments had similar results of radicle protrusion for up to 30-days storage (Figure 2A). After 60-days storage, all priming treatments with GA_3 content had a positive impact on radicle protrusion (in average, 86%). This became evident at 90-days storage; seed priming with GA_3 with or without PEG (-2.0 MPa) showed, respectively 76% and 77% radicle protrusion. However, control and osmotic priming showed 65% and 61% radicle protrusion (Figure 2A).

Similarly, there was also improvement in shoot parameters at the same storage periods. Other priming treatments showed the same pattern for up to 60-days storage and did not differ from the control (Fig 2B). Afterwards, seed priming with GA_3 associated or not with PEG (-2.0 MPa) provided higher results of hypocotyl elongation (76% and 75%, respectively) when compared to the control (64%) or only with osmotic treatment (60%) (Figure 2B).

The final normal complete seedlings showed similar behavior and did not differ by priming treatment compared to the control treatment for up to 30-days storage (Figure 2C). All priming treatments showed higher results (in average, 78%) compared to the control (70%) at 60-days storage. As increase of the storage period, the seedlings formation reduced, and was more pronounced in seeds that were not submitted to the priming treatments. Particularly, at the end of 90 days of storage, the seeds previously conditioned with GA_3 showed, on average, 75% of complete seedlings and the unconditioned seeds showed a result of 60% of seedlings (Figure 2C). In general, at 90 days storage, the normal seedlings decrease even in seeds treated with PEG, GA_3 or not.

The lowest results of radicle protrusion, hypocotyl elongation and normal seedlings was observed in control and isolated PEG treatment at the end of storage period (Figure 2).

Both priming techniques and the control showed similar seed vigor without evident differences by the velocity parameters and showed decrease in the radicle protrusion speed index throughout storage periods (Figure 3A). Similarly, germination speed index reduced across the time and, especially after 60-days storage (Figure 3B).

3.2. Crotalaria spectabilis

Unconditioned freshly seeds had radicle protrusion of 66% and osmo or hormopriming seeds ranged from 57 to 64% (Figure 2D). However, significant increases in the radicle protrusion were observed in response to PEG and GA_3 treatment at 30-days storage. For instance, in the absence of conditioning, radicle protrusion was 56%; after conditioning with PEG (-2.0MPa) or GA_3 (100 ppm) the radicle protrusion of these seeds increased to 70% and 62%, respectively (Figure 2D). However, the combination of the two conditioning agents did not differ from the control (Figure 2D). Results observed in the following storage periods were similar between the control and the conditioning treatments, reaching radicle protrusion of 61 to 64% at the end of 90 days of storage (Figure 2D).

A similar pattern to the radicle protrusion was observed to the hypocotyl elongation. After 30-days storage, PEG-treated seeds had 67% hypocotyl elongation. At the same time, seeds treated with GA_3 had, on average, 59% and the control had 55% of hypocotyl elongation (Figure 2E). However, control and conditioned seeds along the subsequent storage periods ranged from 61 to 66% of hypocotyl elongation (Figure 2E). At the end of storage, the control result was 64% and the conditioned seeds showed 63% of hypocotyl elongation, with no obvious differences between them (Figure 2E).

There was also a significant interaction between priming treatments and storage periods for normal seedlings. However, no apparent increase in seedling formation was caused by the application or by the absence of seed conditioning (Figure 2F). Fresh seeds showed results ranging from 55% (with PEG conditioning) to 63% (control and priming with GA_3). However, despite the increase in results between 30 to 60 days of storage, variations in complete seedling formation results did not exceed 6 percentage points between control and priming treatments (Figure 2F). At the end of storage, PEG priming results had 57%, control had 63% and GA_3 priming had 62% normal seedlings (Figure 2F).

The root protrusion index results showed the same behavior as the root protrusion percentage, since these results are related and infers about the speed of radicle emission. Through this parameter it was possible to verify that the conditioned seeds increased the speed of root protrusion at 30 days of storage (Figure 3C). For instance, seeds treated with PEG (-2.0 MPa) had index of 32, and the control and other conditioned seeds ranged from 27 to 29 of root protrusion speed index (Figure 3C). Nevertheless, in the other storage periods, the results of root protrusion speed were similar between conditioned and non-conditioned seeds, ranged from

12 to 15 at 60-days storage (Figure 3C). Seeds 90-days stored had radicle protrusion index ranged from 10 to 14 (Figure 3C).

Initially, the germination speed index ranged from 8 to 10 between the control and priming treatments (Figure 3D). Similar to the root protrusion index results, at 30-days storage PEG (-2.0 MPa) treatment had the highest result (16); and the results ranged from 13 to 15 (Figure 3D). After that, it also decreased and became almost constant after 60-days until 90-days storage.

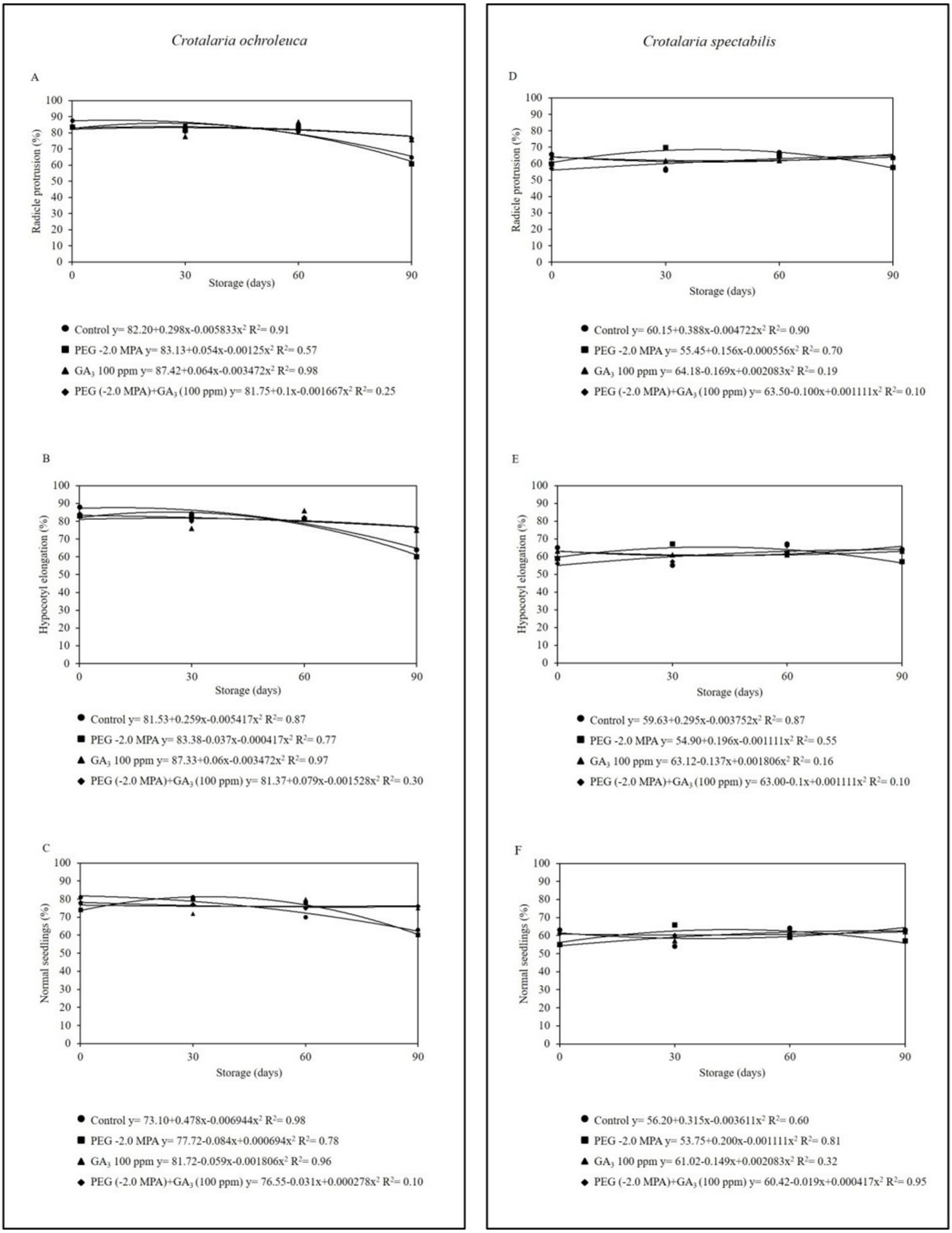

Figure 2. Effect of priming treatments on germination and initial growth of *Crotalaria*. A - Radicle protrusion; B - Hypocotyl elongation; C - Normal seedlings of *Crotalaria ochroleuca*; D - Radicle protrusion; E - Hypocotyl elongation; F - Normal seedlings of *Crotalaria spectabilis*.

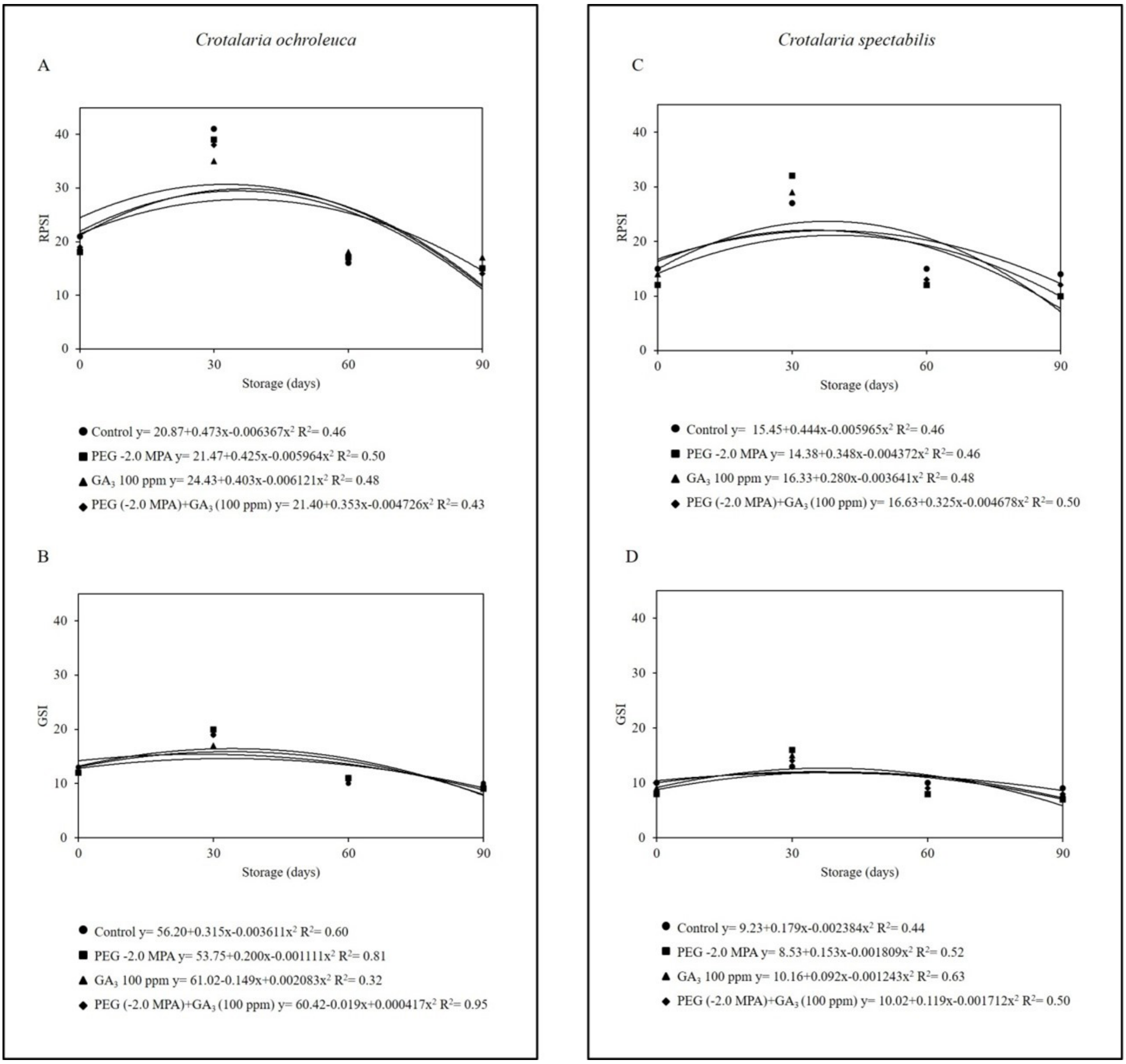

Figure 3. Effect of priming treatments on speed germination and initial growth of *Crotalaria*. A - Radicle protrusion speed index; B - Germination speed index of *Crotalaria ochroleuca*; C - Radicle protrusion speed index; D - Germination speed index of *Crotalaria spectabilis*.

4. Discussion

Seed germination and seedling establishment are among highly critical developmental phases of plants. Nonetheless, the establishment of plants is essential for the efficient use of resources such as water and light. Thus, uniform, and proper establishment of plants is a pre-requisite for their success. Delayed emergence and inappropriate establishment can reduce the future growth rate of the plants (Abbasi Khalaki et al., 2021). Particularly for *Crotalaria* sp, seed germination in the laboratory is low, as well as emergence in the field. Thus, seed priming might increase seed germination.

Unconditioned seeds of *C. ochroleuca* stored for up to 60-days showed higher results of root protrusion and hypocotyl elongation, which are characteristics of early seedling growth. However, after that, primed seeds with GA_3 (with or without PEG) showed higher results for seedling growth and normal seedling formation, reaching increases of more than 10 percentage points in the seedlings characteristics. This indicates that seed exposure to gibberellin could attenuate the negative impacts of seed deterioration/aging.

Therefore, herein our results agree with previous reports concluding that seed priming with GA_3 increased seed germination and seedlings growth parameters due to effect on cell division and elongation in soybean (Manoharlal and Saiprasad, 2018). Likewise, priming involves pre-germinative, controlled hydration of seeds to eliciting factors (natural or synthetic or a combination of both) at early developmental stages (Paparella et al., 2015) and boosts stress tolerance potential as seen for *C. ochroleuca* during seed aging. Besides, improvements due to GA_3 priming imprint epigenetic memory that increases the ability of the seed to deal with future stresses. Stress memory is the result of epigenetic changes as well as the accumulation of signaling proteins or transcription factors.

Epigenetic changes are inherited through mitosis and meiosis and hold important roles in stress tolerance and acclimation processes (Sen and Puthur, 2020).

However, for *C. spectabilis* no expressive result for the germination measurements differentiated primed and non-primed seeds, even after storage though the most expressive losses were in the germination speed of the seeds. It should be highlighted that, in a previous study *C. spectabilis* seeds showed a high physiological performance even after stored for up to approximately 150-days in uncontrolled environmental conditions, deteriorating afterwards, although without losing their germination capacity up to 270 days of storage (Silva et al., 2022b). In parallel, it has already been hypothesized that the ability to respond to priming treatment might be genetically controlled (Noorhosseini et al., 2018). In view of this, it is suggested that seeds that survive longer may be less responsive to priming effects, or that priming effects are actually determined according to genetic traits.

Anyway, both agriculture and plant conservation require the maintenance of seed germination vigor and viability during storage (Waterworth et al., 2019). However, seed ageing has been well-recognized as the major cause of reduced vigor and decline in germination; a range of deteriorative changes during seed ageing, such as enhanced leakage of solutes indicative of early membrane deterioration, reduced enzyme activity (e.g., enzymes for germination, free-radical scavenging enzymes), and reduced respiration, protein and DNA synthesis, amongst others. This led to the hypothesis that free radicals produced during ageing cause profound cellular damage (Powell, 2022). Nevertheless, more research is needed to discover how priming methods contribute to modulate seed longevity.

Though there was a reduction in the emission of the radicle, hypocotyl and seedling development of *C. ochroleuca* over time seed storage, it was more drastic in non-primed GA_3 seeds. Similar to our results, many other researchers also observed a positive impact of hormopriming using GA_3 on germination and emergence attributes of seeds of medicinal plants. Priming with GA_3 (250 ppm) in aged seeds of *Anthemis tinctoria* (Falahhosseini et al., 2019) and *Achillea millefolium* (Rasoolzadeh et al., 2020) had positive effect and efficiency on increasing seed vigor and seedling length. Improvements were attributed to the profile of the enzymatic metabolism such as catalase enzyme, key for seed repair against ageing ROS-induced damage during priming treatment.

In summary, hormopriming with GA_3 have good potential to improve crop establishment and growth of *C. ochroleuca*. In addition to the novelty in relation to the species for which there are no data in the literature, the study sheds some light on the circumstances of evaluating the efficiency of the priming technique. Our results show the importance of evaluating seeds that were submitted to priming techniques during storage. According to the results with freshly harvested seeds of *C. ochroleuca*, there was no obvious effect of seed priming. However, expressive results were observed after 60 days of storage and corroborated at 90 days of storage. Although all germination and seedling growth characteristics decreased as seed aging, priming with GA_3 had a positive potential in mitigating the effects of seed deterioration of *C. ochroleuca*. This is of great relevance as *C. ochroleuca* seeds are not generally sown immediately after harvesting and remain stored until the summer crop harvest.

It should be noted that the expressive results of percentage and speed of root protrusion observed at 30 days of storage of PEG primed seeds of *C. spectabilis* were not visualized in complete seedling appearance (Figure 2F). Thus, through the subsequent storage periods, the seed priming was not expressive in relation to the control. These results reinforce the need to evaluate the complete formation of the seedlings parts in order to validate the stress-ameliorating potential of the priming technique.

Our results indicate that the methodology for evaluating the efficiency of the seed conditioning technique should consider some peculiarities. First, it is essential to evaluate the effect of treatments over the seed storage period, for at least 30 to 90 days. Second, it is necessary to consider the complete formation of the seedling's parts and not only the root protrusion as a criterion for germination of seeds previously submitted to priming.

Therefore, the primary root emission does not necessarily mean that the seedling will develop completely. This information is fundamental, considering that the priming technique is aimed at the full and uniform establishment of plant stands. According to Louis et al. (2023) the knowledge about the duration of stress memory in seed priming is only beginning to emerge and ocurrent understanding of epigenetic memory and the stability of the epigenome in successive generations is very limited.

Overall, data of this study provide useful information concerning improvement of *Crotalaria ochroleuca* seed germination after priming with GA_3 and submitted to storage. Considering that *Crotalaria* seeds face multiple environmental stresses throughout their lifespan that not only reduce their performance, but may also negatively affect crop productivity, further research needs to be considered if *Crotalaria* plants raised from primed seeds also exhibit enhanced tolerance toward abiotic stresses. This would be of great value, as the *Crotalaria* crop is aimed also to improve the soil attributes for the succeeding crop.

5. Conclusions

For *Crotalaria spectabilis* no expressive result for the germination measurements differentiated primed and non-primed seeds.

Crotalaria ochroleuca reduced the emission of the radicle, hypocotyl and seedling development after 60-days storage. However, seed priming with GA_3 increased seed germination of *C. ochroleuca.* Priming with GA_3 had a positive potential in mitigating the effects of seed deterioration of *C. ochroleuca.*

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