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Overcoming dormancy in seeds of B*ixa orellana* L.

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Abstract. Annatto seeds, *Bixa orellana* L., have dormancy mechanisms that make their germination difficult, requiring testing of different methods to enable their reproduction. Thus, the objective was to evaluate the emergence of *Bixa orellana* seeds submitted to solutions and periods of imbibition to overcome dormancy. The experimental design was completely randomized, with a 4×2 factorial arrangement. The evaluated seeds were from the red Piave cultivar, with a solution of distilled water and gibberellic acid (GA_3) at a concentration of 125 mg L^{-1} in the imbibition periods of 0, 4, 10, and 24 hours. Emergence test, shoot height, stem diameter, root length, shoot, and root dry matter, and Dickson's quality index was evaluated. The solutions did not interfere with the development of the plants in the periods. It was concluded that annatto seeds in a GA_3 solution for a period of 24 hours are efficient for overcoming dormancy.
Keywords: Annatto, Emergency, Gibberellic acid.

Introduction

Bixa orellana L., belongs to the *Bixaceae* family, popularly known as annatto, is a tree species whose pigments present in the integument are often used in the cosmetic, pharmaceutical, food and textile industries(Nascimento et al., 2022).

The propagation of annatto plants is preferably done by seeds, however, they have low germination(Picolotto et al., 2013).Studies on the germination of annatto seeds have shown low percentages in natural environments using conventional methods (Castello et al., 2012). Annatto is a species native to the Atlantic Forest and Amazon Forest, standing out for being one of the sources of natural dye and being used in the recovery of degraded areas, where procedures for the production of seedlings are scarce(Neto et al., 2018).

Determining methods to analyze the quality of these seeds is essential for production and commercialization, as they are a potential source of income for small producers, with fast growth and rusticity, in addition to being used for the recovery of degraded areas(Ferreira& Novembre, 2015). The increase in current production is a consequence of the expansion of cultivated areas and not of the improvement in yield, since seed propagation presents problems, due to the dormancy that leads to low germination(Kapoor& Ramamoorthy, 2021).

Several techniques can be used as a treatment to break dormancy, such as pre-germination treatments, which aim to increase the germination rate and seedling production(Lima et al., 2022). So, evaluating the germination potential and methods of overcoming dormancy is essential so that we can further explore this economic activity and ensure greater profitability for producers.

The objective of this research was to evaluate the emergence of *Bixa orellana* L. seeds submitted to solutions and periods of soaking time to overcome dormancy.

Methods

The experiment was conducted under controlled laboratory and greenhouse conditions in the municipality of São João Evangelista, state of Minas Gerais, Brazil.

The experimental design used was completely randomized, with a 4×2 factorial arrangement. The factors were four periods of imbibition time and two solutions, with four replications. The annatto seeds, *Bixa orellana* L., used were from the red Piave grow crops, submitted in the laboratory to soaking in a solution of distilled water (control - H_2O) and a solution of gibberellic acid at a concentration of 125 mg L^{-1} (GA_3), in the periods of 0, 4, 10 and 24 hours, kept in BOD at 25 °C.

In the greenhouse, the seeds were sown in polyethylene bags, 11x22 cm, with substrate in the proportion of 2:1, earth: sand. To analyze the quality of the seeds with the formation of seedlings, the emergence test was conducted, and after the beginning of emergence, daily evaluations were carried out, determining the initial stand on the seven days, and the test was ended after the percentage of emergence stabilized up, evaluating the number of normal seedlings emerged, according to Pereira (1995).

For the emergence speed index (IVE), the number of seedlings emerged from the beginning of emergence was determined daily and the calculation was performed according to Maguire (1962). The average time of emergence and the coefficient of variation of time were evaluated according to(RanaL& Santana, 2006).

The height from the stem to the last leaf of the aerial part and the diameter of the stem were evaluated at 30, 60, 90, and 120 days after sowing. At 120 days after sowing, root length, and green and dry matter of shoots and roots were evaluated. The aerial part and root were placed separately on duly identified Kraft paper and then weighed on an analytical scale, later the plant material was placed in an oven with forced air circulation at a temperature of 65 ºC until reaching a constant mass to determine the dry matter. The results of green and dry matter of plant materials were expressed in grams (g).

The Dickson quality index (IQD) was determined, obtained as a function of shoot height (H), stem diameter (D), shoot dry matter (MSPA) and root dry matter (MSR). and total dry matter (MST) (Dickson et al., 2011), following the formula:

$$IQD = [MST/(H/D) + (MSPA/MSR)]$$

Statistical analysis

The data obtained were subjected to analysis of variance, mean test using Tukey and polynomial regression ($p<0.05$), tested for normality and homogeneity and, when necessary, transformed into arcsen$\sqrt{x}/100$. Statistical analyzes were performed using the *software* R 4.1.3.

Results and discussion

The annatto seeds submitted to soaking for less than 24 hours presented low emergence and the GA_3 solution for 24 hours had higher emergence, overcoming seed dormancy, as shown in Table 1. By using the H_2O solution, it was possible to observe the low percentage of emergence (Table 1), thus presenting the need to use methods to overcome dormancy as the use of GA_3 solution to break seed dormancy and achieve better results in emergence. Despite reports of studies on the low germination of annatto seeds(Lopes et al., 2008; Neto et al., 2018),many authors attribute its low germination to the impermeability of the tegument(Castello et al., 2012; Fernandes et al., 2021; Pereira et al., 2012),to which methods of overcoming dormancy can be assigned to improve seed viability.

Table 1. Percentages of the averages of the initial stand (EI), emergence (E), emergence velocity index (IVE), average time (TM) and coefficient of variation of time (CVT) submitted to H_2O and GA_3 solutions in different periods of time to overcome dormancy in annatto seeds.

Solutions	EI (%)	E (%)	IVE	TM	CVT (%)
H_20	38 c	36 b	0,36 c	15,56^{ns}	5.62 b
GA_3/0h	31 c	38 b	0,35 c	15,52^{ns}	5,04 c
GA_3/4h	44 b	44 b	0,40 c	16,45^{ns}	6,38 b
GA_3/10h	50 ab	50 b	0,49 b	17,13^{ns}	6,02 bc
GA_3/24h	56 a	81 a	0,61 a	16,44^{ns}	8.06 a
CV (%)	15,42	18,92	9,18	13,16	8,33

Means followed by the same letter in the column do not differ according to Tukey's test ($p<0.05$). ns: not significant. CV (%): coefficient of variation.

In this work, the treatment with GA_3 solution for a period of 24 hours was efficient to overcome the dormancy imposed by the tegument of the annatto seed (Table 1), being possible in the initial stand test and the emergence velocity index to separate the solutions in levels of distinction with the GA_3/0h and H_20 solution lower and the GA_3/24h higher and presenting a higher percentage of emergence compared with the other solutions, therefore as mentioned by authors due to the tegument constitute a physical barrier hindering the entry of water with the need for methods to overcome dormancy(Amaral et al., 2000; Custódio et al., 2002; Picolotto et al., 2013).Annatto seeds, when submitted to mechanical and sulfuric acid scarification, absorb water satisfactorily, overcoming the dormancy imposed by the tegument, and the use of GA_3 also increases the speed of germination(Bocatto & Forti, 2020),thus evidencing the need for methods to overcome the dormancy of annatto seeds. Studies with the use of acids promote the overcoming of dormancy in annatto, soursop and murici seeds(Lopes et al., 2008; Murakami et al., 2011; Picolotto et al., 2013; Rego et al., 2018).

The reduced capacity for emergence observed in seeds in periods shorter than 24 hours in Table 1 was similar to that observed by Lopes et al. (2008) when KNO3 solution was used. According to Amaral et al. (2000), low germination can be explained by the impermeability of the tegument and its rigidity, preventing the entry of water, because in the absence of water the germination process is not initiated.

As the seeds were immersed in GA_3 solution for a period of time of 24 hours, the tegument may have undergone a degradation process, which caused the entry of water into the seeds favoring the germination process. Results found by Neto et al. (2018), recommend immersion in water for four and eight hours with vegetal biostimulant to break the dormancy of annatto seeds to promote better germination, so without the use of vegetal biostimulant, treatments less than 24 hours were inefficient to break seed dormancy, also observed in this work that treatments in soaking in solution less than this period showed low emergence (Table 1), caused by the impermeability of the tegument to water, as reported by Mendes et al. (2006) .

Among the variables analyzed, only the mean time of emergence was not significantly influenced by the solutions with different time periods (Table 1). According to Ranal and Santana (2006), the coefficient of variation of time measures the degree of dispersion of germination around the mean time. Thus, the results show that germination was concentrated near the mean time, with values up to 8.06% (Table 1), revealing that the germination period of the seed in general is not long. Therefore, Pereira et al. (2009) reports that relatively low coefficients of variation of time, show the low relative dispersion of the emergence process in relation to time.

When evaluating the increase in diameter and height of the plants during the periods, both were not significant, but the plants developed satisfactorily during the periods, reaching heights of up to 210 mm and diameter of 5 mm in Figures 1 and 2. Results of the survey of the diameter of the stem and height of the cotton plant in periods of up to 110 days(Jácome et al., 2003)and in coffee seeds for 150 days(Marana et al., 2008)with the use of GA_3 solution, found no difference in the production and vegetative development of the plant in these periods. Vendruscolo et al. (2016) in the application of GA_3 solution in the culture of *Eucalyptus sp.* obtained vigorous vegetative growth enabling greater establishment in the field with high potential for accumulation of biomass of economic interest. In this sense, the diameter of the stalk and plant height are parameters that can be evaluated in the process of development and growth of seedlings.

No statistical differences were observed in the grouping of the averages of the parameters evaluated in Figure 3, not affecting the vegetative development of the plants over the periods. In general, the present work presented the lowest mean for the ratio corresponding to the seedlings produced in the H_20 solution that obtained the lowest growth in height and diameter in Figures 1 and 2. The action of gibberellin can stimulate the growth of the stem and roots in plant development (Taiz et al., 2017).

The use of gibberellin solution can provide morphophysiological adjustment of the plants by allocating more photo-assimilates to the plant(Vendruscolo et al., 2016),Thus the use of GA_3 solution provided an increase in biomass in a way adequate for investment in the leaves, stem and roots for greater absorption of soil solution to support growth, so the use of gibberellic acid is a promising practice. Therefore, the evaluation of plant quality through parameters such as height, diameter, green and dry matter along with Dickson's quality index are important tools to identify if the work is being conducted properly, so Fonseca et al. (2002) and Marana et al. (2008) report the importance of these parameters as good indicators of seedling quality being considered robust in the results.

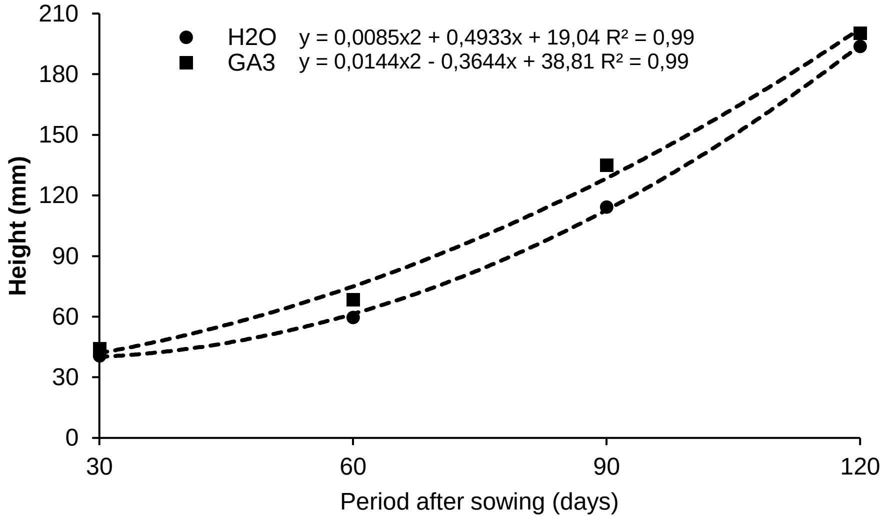

Figure 1. Height of annatto plants as a function of periods after sowing of seeds subjected to H_2O and GA_3 solutions.

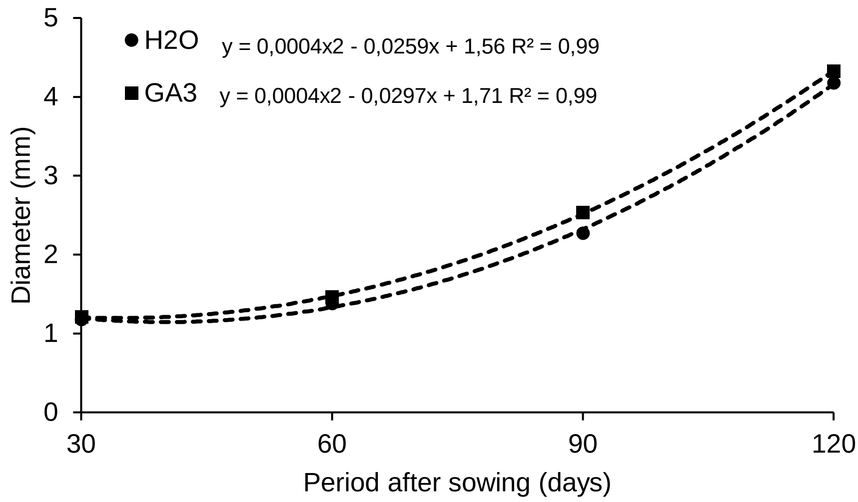

Figure 2. Annatto plant collection diameter as a function of periods after sowing of seeds subjected to H_2O and GA_3 solutions.

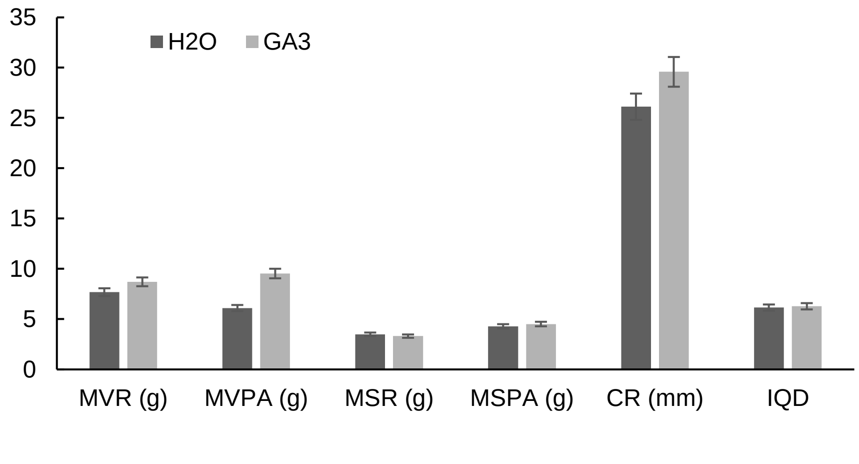

Figure 3. Root Green Matter (MVR), Shoot Green Matter (MVPA), Root Dry Matter (MSR), Shoot Dry Matter (MSPA), Root Length (CR) and Dickson Quality Index (IQD) after 120 days of sowing annatto seeds subjected to H_2O and GA_3 solutions.

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Conclusion

Annatto seeds have greater emergence with a solution of gibberellic acid at a concentration of 125 mg L^{-1} for a period of 24 hours, overcoming seed dormancy. The solutions of distilled water and gibberellic acid did not interfere in the development of the plants until the period of 120 days. Research related to gibberellic acid in the growth and development of annatto plants is necessary and relevant.

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