SUBSTRATES AND WATER AVAILABILITY ON THE EMERGENCE AND INITIAL GROWTH OF *Alibertia sessilis* Schum. SEEDLINGS

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Abstract

The cultivation of native species in the Cerrado has encountered various problems, for instance, the slow growth and the lack of knowledge about the cultivation practices. In order to better use the available water resources and to produce quality seedlings, this study aimed to evaluate different substrates and water levels on the emergence and initial growth of *Alibertia sessilis* Schum. seedlings. The effects of the following substrates were evaluated: dystroferric Red Latosol (Oxisol), Oxisol +sand (1:1 v/v), Oxisol + sand + semi-decomposed poultry litter (1:1:0.5 v/v) and Oxisol + commercial-substrate-CS (1:1 v/v) associated with the levels of irrigation of 25, 50, 75 and 100% of the water holding capacity in the substrate (WHC). The best results for emergence percentage, initial growth and quality of seedlings were observed in the substrates Oxisol + sand 2 + poultry litter (1:2:0,5) at 100% of WHC. There was no emergence at 25% of WHC.

Keywords: Rubiaceae; marmelinho; fruit of the Cerrado

Resumo

Substratos e disponibilidade hídrica na emergência e crescimento inicial de mudas de Alibertia sessilis Schum. O cultivo de espécies nativas no Cerrado tem esbarrado em vários problemas, entre eles, o crescimento lento e a carência de conhecimentos sobre as práticas culturais. A fim de melhor utilizar os recursos hídricos disponíveis e produzir mudas de qualidade, objetivou-se no presente trabalho avaliar diferentes substratos e níveis de água na emergência e no crescimento inicial das mudas Alibertia sessilis Schum. Foram avaliados os efeitos dos substratos Latossolo Vermelho distroférrico (LVd), LVd +areia (1:1 v/v), LVd + areia + cama de frango semidecomposta (1:1:0,5 v/v), LVd + areia 2 + cama de frango semidecomposta (1:2:0,5 v/v) e LVd + substrato comercial -SC (1:1 v/v) associados aos níveis de irrigação de 25, 50, 75 e 100% da capacidade de retenção de água no substrato (CRA). Os melhores resultados para porcentagem de emergência, crescimento inicial e qualidade das mudas foram observados nos substratos LVd + areia 2 + cama de frango (1:2:0,5) a 100% da CRA. Não houve emergência a 25% da CRA. Palavras-chave: Rubiaceae; marmelinho; frutífera do Cerrado.

INTRODUCTION

The cultivation and production of seedlings of native species in the Cerrado have encountered several problems, such as slow growth, basic information about their biology, forest utilization, management, cultivation treatments, and lack of awareness of their economic potential (SCALON; JEROMINE, 2013; NUNES *et al.*, 2014, SCALON *et al.*, 2015, GORDIN *et al.*, 2016).

Some studies have already been conducted addressing the effect of substrates and water levels on the emergence and production of seedlings of some native species in the Cerrado, with varying results observed. In the species *Hancornia speciosa*, the substrates dystroferric Red Latosol (Oxisol), Oxisol + sand (1:1) or Oxisol + Bioplant[®] (1:1) can be recommended for the emergence and initial growth of plants in water availability from 75% to 100% of WHC (GORDIN et al., 2016). For the production of *Campomanesia adamantium seedlings*, the best substrate was the mixture of dystroferric Red Latosol (Oxisol) + sand + Bioplant[®] (1:1:1 v/v) at 75% and 100% of the water holding capacity in the substrate (DRESCH *et al.*, 2016). However, for the species *Eugenia pyriformis*, the highest percentages of emergence and better plant growth were observed in the substrate of dystroferric Red Latosol (Oxisol)+ sand + poultry litter (1:2:0.5 v:v:v) at 50% of WHC (SCALON; JEROMINE, 2013).

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Studies on the relationship water-plant can feed into greater knowledge of the processes of absorption, transport and water loss, and provide strategies of management of plants grown in environments subject to deficit or excess of water in the soil. The amount of water stored and available to these plants varies, among other factors, with the amount of organic matter and texture of the substrate. The plants will respond differently to each condition for cultivation, especially concerning growth, quality, and metabolism (SCALON; JEROMINE, 2013; ROCHA *et al.*, 2016; DRESCH *et al.*, 2016).

The substrates usually used to produce seedlings may consist of a mixture of mineral and organic components. Different substrate formulations have been used to produce seedlings, such as mixtures containing humus, carbonized rice hull, chicken manure, coffee husk, charcoal, and Plantmax[®] (DRESCH *et al.*, 2016; SCALON; JEROMINE, 2013; GORDIN *et al.*, 2016). The poultry litter has been utilized in many regions where the broiler farming is well developed, which generates large availability of organic waste. The addition of organic matter to the soil provides greater moisture to the soil in cultivation under irrigation suspension (ROCHA *et al.*, 2016), as well as contributes to the physical-chemical properties of the soil, such as cation exchange capacity, the formation of complexes and chelates with numerous ions, improving structure, aeration and retention of moisture (FORNASIERI FILHO, 1992).

The *Alibertia sessilis* (Vell.) K. Schum. - Rubiaceae, known as "marmelinho" or "marmelo-do-cerrado", is a sub-bush species and demonstrates great food and medicinal importance (RODRIGUES; CARVALHO, 2001). Its fruits are consumed *in natura* or used as jellies and pies by the regional population, its leaves are eaten by cattle. Additionally, along with its branches, its leaves are used in medicinal preparations, as compress, bath, and poultice and are employed to heal skin affections (ALMEIDA *et al.*, 1998). Thus, as for most species of the Cerrado, there is little information on the process of seedling formation of this species and its potential for use in projects to recover degraded areas.

Considering the hardiness of this species, we hypothesized that it is a hydrically little demanding and that its cultivation is easily carried out in substrates poor in organic matter. Thus, we aimed to assess if different substrates and water levels affect the emergence and initial growth of seedlings of *Alibertia sessilis*.

MATERIAL AND METHODS

The exsiccata of the botanical material is deposited in the Herbarium of the Federal University of Grande Dourados (UFGD) under the Number 5410. The fruits of *A. sessilis* were collected when they were mature with a coat of brown coloring. The collection was held at Fazenda Santa Madalena, located in Dourados-MS, at 23°8'17''S and 55°8'15''W, featuring an elevation of 436m with typical vegetation of the Cerrado. After collecting the fruits, they were processed on a sieve with running water to remove the pulp and to select the whole seeds without external signs of injury or deformation. The selected seeds were dried on a sieve in the shade in a laboratory environment and the sowing occurred on the following day.

The sowing was performed on the substrates: 1) dystroferric Red Latosol (Oxisol); 2) Oxisol + sand (1:1 v/v); 3) Oxisol + sand + semi-decomposed poultry litter (1:1:0.5 v/v); 4) Oxisol + sand 2 + semi-decomposed poultry litter (1:2:0.5 v/v); and 5) Oxisol + commercial substrate - CS (1:1 v/v), without any additional chemical fertilization on any substrate. The commercial substrate was composed of pine bark, coconut fiber, vermiculite, and macro-and micronutrients, which were not disclosed by the manufacturer, with a pH between 5.2 and 6.5 and the electrical conductivity between 0.6 and 1.4 μ s cm⁻¹. The semi-decomposed poultry litter was in fermentation for around 60 days, humification phase of the organic material.

The levels of 25%, 50%, 75% and 100% of the water holding capacity were used in the irrigation, which was performed on alternate days. The water holding capacity (WHC) was determined by adopting the content of water retained by the substrate after drainage of the excess (SOUZA *et al.*, 2000).

The seeds with no pre-germination treatment were sown in 50×190 mm tubes at a depth of 2 cm and kept in greenhouse under plastic screen to avoid the incidence of rainfall, and the brightness was reduced in 30%.

In the design of experiments, completely randomized designs in schema of subdivided plot were used. The plot consisted of each of the five substrates, and the subplot consisted of each of the four water levels. Four repetitions of twenty seeds were used for each treatment.

The percentage and emergence speed index (ESI) (NAKAGAWA, 1999) were assessed and the assessments lasted 60 days after sowing. 175 days after emergence, the seedlings were assessed for growth and quality, being determined: stem height (cm) and root length (cm) using graduated ruler; root collar diameter (mm) with digital caliper; chlorophyll content (SPAD), measured with the instrument chlorophyll meter SPAD 502; leaf area (cm²), using Leaf Area Meter LCO 3000; dry mass of root and aerial part (g), using Forced-Air Oven at 60°C; height: diameter ratios of the root collar (HDR); ratio of the shoot dry mass, and the root dry

mass (RPAR); f Dickson quality index (DQI), according to the formula [total dry matter / (HDR + RPAR)] (DICKSON *et al.*, 1960).

After a normality test (Lilliefors Test) and a homogeneity test (Bartlett's Test) to analyze the variance of the characteristics studied, emergence percentage data were transformed into $\sqrt{(x+1)}$. The results were submitted to variance analysis and, when the effect showed significant, the means obtained were compared by the test of Tukey at 5% of probability. The analyses were performed using the computer program SISVAR (Ferreira, 2011).

RESULTS

There was no emergence in the lowest water holding capacity (25%), therefore, this treatment was not considered in the statistical analysis of any characteristic assessed. The seeds of *Alibertia sessilis* took about 50 days to cease the emergence (data not shown), presenting low percentage and ESI by the end of this period (Figure 1a and 1b).

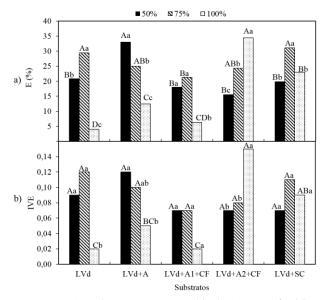


Figure 1. Percentage of emergence (E) and emergence speed index (IVE) of *Alibertia sessilis* Schum. seeds due to different substrates and water holding capacity (CRA). Uppercase letters compare the same CRA in different substrates, and lowercase letters compare the different CRA in the same substrate. LVd = distroferric Red Latosol; A = sand; CF = semi-decomposed poultry litter; SC = commercial substrate.

Figura 1. Porcentagem de emergência (E) e índice de velocidade de emergência (IVE) das sementes de *Alibertia sessilis* Schum. em função de diferentes substratos e capacidade de retenção de água (CRA). Letras maiúsculas comparam a mesma CRA nos diferentes substratos e letras minúsculas comparam as diferentes CRA no mesmo substrato. LVd = latossolo Vermelho distroférrico; A = areia; CF = cama de frango semidecomposta; SC = substrato comercial.

The highest values of percentage of emergence and ESI were observed in the cultivation in Oxisol + S2+ PL (1:2:0.5) and 100% of the WHC, with values that did not vary significantly from the cultivation in Oxisol + sand at 50% WHC. The results varied in the different substrates and water levels assessed, showing lower values in the substrate Oxisol and 100% WHC.

It can be observed that 175 days after emergence, the height of seedlings was higher when cultivated at 100% WHC in the substrate Oxisol + CS (Figure 2a). Similar results were observed for the diameter of the root collar (Figure 2b) that did not change when compared to that of seedlings grown in Oxisol + S2 + PL.

Root length was higher when grown with less water availability (50% WHC) and in the substrate Oxisol + S (Figure 2c).

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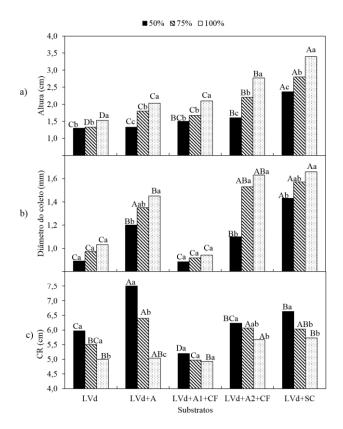


Figure 2. Height (a), diameter of the root collar (b) and root length – CR (c) of *Alibertia sessilis* Schum seedlings. due to different substrates and water holding capacity (CRA). Uppercase letters compare the same CRA in different substrates, and lowercase letters compare the different CRA in the same substrate. LVd = distroferric Red Latosol; A = sand; CF = semi-decomposed poultry litter; SC = commercial substrate.

Figura 2. Altura (a), diâmetro do coleto (b) e comprimento da raiz – CR (c), de mudas de *Alibertia sessilis* Schum. em função de diferentes substratos e capacidade de retenção de água (CRA). Letras maiúsculas comparam a mesma CRA nos diferentes substratos e letras minúsculas comparam as diferentes CRA no mesmo substrato. LVd = latossolo Vermelho distroférrico; A = areia; CF = cama de frango semidecomposta; SC = substrato comercial.

Both the chlorophyll content and the leaf area were lower in the seedlings grown at 50% of WHC on virtually all assessed substrates (Figure 3a and 3b). In the substrate Oxisol + S2 + PL (1:2:0,5), SPAD was higher in all levels of water holding capacity assessed (Figure 3a), showing no variation from the substrate Oxisol + CS at 100% of WHC.

Shoot and root dry mass were higher in substrates containing more sand and poultry litter (Oxisol + S2 + PL) or when commercial substrate was added (Oxisol + CS) at 75 and 100% of WHC (Figure 4a and b). Although the root length was higher under 50% WHC, this behavior was not repeated for the dry mass.

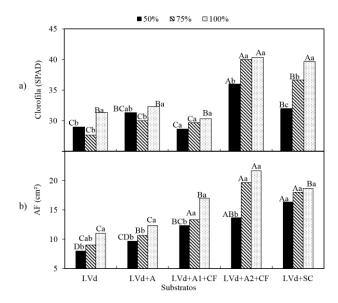


Figure 3. SPAD index (a) and leaf area – AF (b) of *Alibertia sessilis* Schum. seedlings due to different substrates and water holding capacity (CRA). Uppercase letters compare the same CRA in different substrates, and lowercase letters compare the different CRA in the same substrate. LVd = distroferric Red Latosol; A = sand; CF = semi-decomposed poultry litter; SC = commercial substrate.

Figura 3. Índice de clorofila (a) e área foliar – AF (b) de mudas de *Alibertia sessilis* Schum. em função de diferentes substratos e capacidade de retenção de água (CRA). Letras maiúsculas comparam a mesma CRA nos diferentes substratos e letras minúsculas comparam as diferentes CRA no mesmo substrato. LVd = latossolo Vermelho distroférrico; A = areia; CF = cama de frango semidecomposta; SC = substrato comercial.

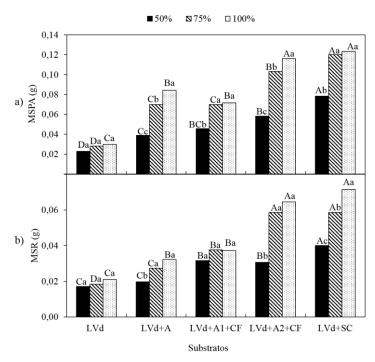


Figure 4. Shoot dry mass (a) and root (b) of *Alibertia sessilis* Schum. seedlings due to different substrates and water holding capacity (CRA). Uppercase letters compare the same CRA in different substrates, and lowercase letters compare the different CRA in the same substrate. LVd = distroferric Red Latosol; A= sand; CF = semi-decomposed poultry litter; SC = commercial substrate.

Figura 4. Massa seca da parte aérea (a) e raiz (b) de mudas de *Alibertia sessilis* Schum. em função de diferentes substratos e capacidade de retenção de água (CRA). Letras maiúsculas comparam a mesma CRA nos diferentes substratos e letras minúsculas comparam as diferentes CRA no mesmo substrato. LVd = latossolo Vermelho distroférrico; A = areia; CF = cama de frango semidecomposta; SC = substrato comercial.

The relation aerial parts/root (RAPR) did not vary significantly between the different levels of WHC (Figure 5a). We observed that the higher the root dry mass the lower was the relation aerial parts/root.

However, in the relation height/diameter (RHD), it was higher under larger water availability, especially in the substrates Oxisol + S2 + PL and Oxisol + CS (Figure 5b). The highest values of Dickson quality index were obtained in seedlings grown in substrates Oxisol + S2 + PL and Oxisol + CS and under 75% and 100% of WHC (Figure 5c).

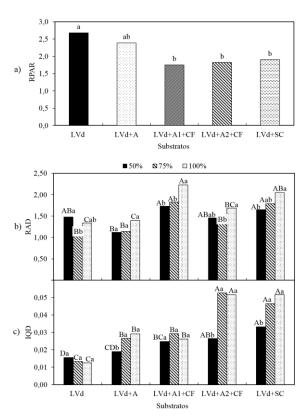


Figure 5. Relation aerial parts/root - RPAR (a), relation height /diameter (b), and Dickson quality index –IQD (c) in *Alibertia sessilis* Schum seedlings. due to different substrates and water holding capacity - CRA (b, c). Uppercase letters compare the same CRA in different substrates, and lowercase letters compare the different CRA in the same substrate. LVd = distroferric Red Latosol; A = sand; CF = semi-decomposed poultry litter; SC = commercial substrate.

Figura 5. Relação massa seca da parte aérea/ raiz – RPAR (a), relação altura /diâmetro (b) e índice de qualidade de Dickson – IQD (c) em mudas de *Alibertia sessilis* Schum. em função de diferentes substratos e capacidade de retenção de água – CRA (b, c). Letras maiúsculas comparam a mesma CRA nos diferentes substratos e letras minúsculas comparam as diferentes CRA no mesmo substrato. LVd = latossolo Vermelho distroférrico; A = areia; CF = cama de frango semidecomposta; SC = substrato comercial.

DISCUSSION

Seeds of *Alibertia sessilis* showed low germination potential independent of substrates and tested water holding capacities. It is observed in the literature that the germination potential of the species of the genus *Alibertia* is quite variable; for example the seeds of *A. edulis* under controlled temperature conditions of 20°, 25° and 30° C in BOD presented from 91 to 81% of germination and from 0,52 to 0,48 of GSI (NUNES et al.,

2014).It has been observed in the literature, that the potential germination of the species of the genus Alibertia is quite variable; for example the seeds of A. edulis under controlled temperature conditions of 20, 25 and 30 °C in BOD, have 91 to 81% germination and 0,52 to 0,48 for the IVG (NUNES *et al.*, 2014). However, they may present, on average, 20% to 80% of germination and reduction in the germination potential, which was attributed to the temperature and storage time associated with seed moisture content (BENEDICT *et al.*, 2016). Thus, these differences between both species of the same genus and of the same native environment can be attributed to the seed water level at the time of sowing or as according to the time in which the seeds were stored after harvesting and processing before sowing.

The water restriction in the cultivation at 25% WHC impaired imbibition and metabolism of the *A. sessilis* embryonic axis since this condition affects the speed and percentage of germination of the seeds by reducing cell expansion. It is noteworthy that the increased water supply caused by 100% of WHC also impaired germination and growth processes, which may be due to the reduction of soil oxygenation. The limitations to the diffusion of oxygen may cause the germination to stop by favoring the ethanol production in the cells of the embryonic axis, which is toxic to the normal metabolism due to acidification and death of the seeds (KOLB; JOLY, 2010).

The lowest values of emergence in the substrate containing only distroferric Red Latosol and at 100% of WHC may be ascribed to the clayey characteristic of this type of soil, triggering the lowest water and aeration availability for the seeds when the substrate was maintained at 100% of WHC. Similarly, this water holding capacity also reduced the emergence percentage and the ESI of *Eugenia pyriformis* (SCALON; JEROMINE, 2013), which was also attributed to the reduction of aeration of the substrate.

The best germination results observed in substrates containing addition of poultry litter and sand, especially in greater proportion, are attributed to the beneficial effect of the addition of organic matter and sand in the substrate. This result has been reported in the literature for other species, as this mixture presents materials that enable good porosity and drainage in their composition, which allow the movement of water and air in the substrate and favor the germination and emergence (FORNASIERI FILHO, 1992; SILVA *et al.*, 2009; SCALON; JEROMINE, 2013; GORDIN *et al.*, 2016).

In this sense, when assessing the effect of different substrates on the production of seedlings of *Hancornia speciosa*, Silva *et al.* (2009) observed better germination in the substrate with carbonized rice hull, which provided better drainage and, consequently, better aeration. In order to produce seedlings of *Eugenia pyriformis*, the substrate should be composed of distroferric Red Latosol + sand + poultry litter (1 : 2 : 0,5) (SCALON; JEROMINE, 2013).

In addition to affecting the germination processes, the water restriction due to WHC of 50% reduced the growth of seedlings of *A. sessilis*. Lowest water availability leads to decreased cell expansion, caused by dehydration of the protoplast, and increased concentration of the solutes (MARTINS *et al.*, 2010). As a consequence of dehydration, morphological, physiological and metabolic modifications are observed through the whole plant, which affect the production and translocation of photoassimilates to the new growth areas (SAPETA *et al.*, 2013).

The highest root growth of seedlings observed under lower water availability in the substrate corroborates the information from literature, which suggests higher synthesis of abscisic acid in the cells of the root tissue of species under this condition. This triggers greater expansion and, consequently, higher root growth, although this higher growth is not accompanied by mass accumulation (TAIZ; ZEIGER, 2013). This response enables deepening of the root system and greater soil exploration, which leads, as a consequence, to the obtention of water and nutrients that the plant needs. However, the lower root growth in substrate with high WHC, depending on the type of soil used in the seedlings cultivation, suggests that the high water availability and the very clayey characteristic of Oxisol may have caused a restriction or barrier to the development of the root system of the seedlings, which is associated with deficient aeration to supply the growth activities.

The plants grown in substrate containing poultry litter and commercial substrate presented higher chlorophyll content, which may be related to the higher nitrogen content found in the mixture. This nutrient is an important constituent of the chlorophyll molecule. However, in the substrate Oxisol + sand + poultry litter (1 : 1 : 0,5), the results were not satisfactory due to the lower proportion of sand, an important component for good aeration of the substrate. The reduction of chlorophyll levels of seedlings submitted to the holding capacity of 50% occurs due to the lower synthesis of pigments since no yellowing or leaf abcission were observed. Plants submitted to water deficit present lower content of photosynthetic pigments, which may be attributed to the degradation of chlorophyll or to the deficiency in the synthesis by the reduction of chlorophyllase (GHOLAMI *et al.*, 2012, ASHARAF; HARRIS, 2013).

The smallest leaf area was observed in cultivated plants under lower water availability (similar to the accumulation of dry mass). Gordin et al. (2016) observed in their review that, as the leaf expansion is a process

governed by the cell turgor under water deficit, a reduction in the leaf expansion and size can occur. It would lead to the decrease in the photosynthetic activity and, consequently, to the accumulation of dry mass and plant growth.

In relation to the accumulation of biomass in the shoot and root part, we observed that the seedlings of *A. sessilis* presented a similar behavior when cultivated in substrates containing poultry litter + sand or commercial substrate; a more balanced distribution of mass (around 1.5 times) between the shoot and root part was observed, as on the relation RAPR. The seedlings with higher root biomass are more likely to survive water stress in the dry season once in their natural environment. The greater the development of the root system the greater the chance of surviving in the field since the seedling presents better absorption of water and support on the soil (LIMA *et al.*, 2008). Thus, we believe that seedlings of *A. sessilis* cultivated under these conditions would have good chances of survival when transplanted.

The highest height of the seedlings observed in the cultivation with higher water availability in the substrates containing poultry litter and commercial substrate provided greater shoot dry mass, which was reflected in the RAPR and HDR calculation. However, it should be noted that a growth twice as high as the aerial part in relation to the diameter is not a favorable feature, and it is characterized as etiolation. In this condition, the plant may not be able to sustain itself and fall on very windy days, or due to any other adverse factor. Camera and Endress (2008) comment that the HDR indicates the quality of seedlings to be carried to the field. The ideal is that a balance between these characteristics exists.

The impaired effect of water deficit on the growth of *A. sessilis* has also been reported for other native species. The seedlings of *Hymeneae. courbaril* L. develop better in irrigation depths from 90 to 100% of potential evapotranspiration, and the water deficit reduces the height, diameter, leaf area and shoot dry mass of the seedlings (SILVA *et al.*, 2016). Seedlings of *Campomanesia adamantium* and *Hancornia speciosa* also grow better when grown in 75% and 100% of WHC; however cultivation under water restrictions (25% and 50% WHC) causes growth reduction (DRESCH *et al.*, 2016; GORDIN *et al.*, 2016).

Cultivation in substrate containing Oxisol + Sand 2 + PL and Oxisol + CS under 75% and 100% of WHCprovided the best conditions for production of seedlings of *A. sessilis*, because, besides the increasing of chlorophyll content and leaf area, characteristics that are not computed in the DQI calculation, it was the condition that provided the highest DQI of the seedlings. However, it is important to emphasize the slow growth because the plants had, on average, only 4 cm in height in the best cultivation condition at the end of 175 days after emergence. The DQI is a good indicator of quality of seedlings since the balance of the biomass distribution, an important parameter to be used in the assessment of the quality, is considered in its calculation; besides it was used to assess the quality of seedlings of several native species of the Cerrado (SCALON; JEROMINI, 2013, DRESCH *et al.*, 2016, GORDIN *et al.*, 2016). However, it should be noted that the DQI was lower than the minimum value of 0.20, as recommended by Hunt, under all conditions of cultivation (1990). Nevertheless, it is considered that the higher the DQI the better the quality of the seedlings (CESAR *et al.*, 2014).

Although native species of the Cerrado have germination potential, they present slow growth, which can lead to a late emergence or even low survival rate. In this work, a great non-uniformity was observed in the stand of the seedlings of *A. sessilis*, which has also been observed for other native fruits species in the Cerrado.

Thus, the results confirm the hypothesis of the work and suggest that *A. sessilis* germinate in substrate that is poor in organic matter and has low water availability (50% of WHC), a condition that represents its natural occurrence environment. However, if cultivated in environment with very sandy soil and availability of organic matter, the species needs higher water availability (100% WHC).

In order to optimize its growth, the cultivation with higher water availability (75 and 100% of WHC) and the addition of organic matter present in the semi-decomposed poultry litter or in the commercial substrate are recommended. These results suggest that, despite being a native species of an environment with low organic matter availability in the soil and subject to water deficit at some seasons of the year (as a characteristic of the Cerrado region where it is found), the species responds positively to higher water availability and organic matter in the seedlings production phase.

CONCLUSIONS

- The substrates Oxisol + sand at 50% of WHC and Oxisol + sand 2 + poultry litter (1:2:0.5 v/v) at 100% of WHC provide the highest percentages of emergence.
- The seedlings present higher quality and shoot growth at 175 days after the emergence on the substrates Oxisol + sand 2 + poultry litter (1:2:0.5 v/v) and Oxisol + CS at 75, and 100% of WHC.

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