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Full Length Research Paper

Influence of tannin on sorghum seed germination

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ABSTRACT

The presence of phenolic substances in the seed coat may inhibit the germination of sorghum seeds, however, it is not known whether during the storage the reduction of these compounds occurs or if a break of dormancy occurs at low temperatures. So, with the interest of verifying the relationship between tannin and seed dormancy, it was intended in this study to evaluate the biochemical, physiological and physical alterations occurred during the development of sorghum seeds with different concentrations of tannin. Seeds of cultivars BR 305 and BR 310, collected at seven development stages (100, 103, 107, 113, 119, 121, 127 days after sowing) according to water content were used. The seeds collected at each stage were divided into two lots, one subjected to drying at 35 ° C, up to the moisture of 12% and the other lot without drying. Seed quality was evaluated by the tests of germination, electric conductivity and enzyme activity. Further, the concentration of tannins in the seeds at each development stage was also determined. Beneficial effect of drying on germination in seeds with higher water content is found. In the seeds evaluated without no drying, the percentage of dormancy is higher when compared to those of seeds subjected to artificial drying. For cultivar BR 305, drying favors the concentration of tannins in seeds collected at different development stages. The same occurred for cultivar BR 310, in seeds collected at 100, 103 and 119 days after sowing. The polyphenoloxidase activity was greter in seeds submitted to the drying of lots with low tannin concentration at both 100 and 103 dasy after sowing.

Keywords: Sorghum bicolor, germination, harvesting, maturation, drying.

INTRODUCTION

The presence of phenolic substances in the seed coat may inhibit sorghum seed germination, Hartmann et al. (1997) defined as chemical dormancy. However, it is not know really if dormancy is due either to the reduction of these compounds or to the storage of seeds under low temperatures (Queiroz, 1979). When the seeds are kept stored at low temperatures, acceptable levels of control of their quality are kept. Even so, loss of viability of these seeds take place, and with aging, the membranes become weak and enzymes lose the catalytic activity. Impaired cellular structure makes the contact of the polyphenoloxidase enzyme with phenolic compounds preferably stored in the vacuoles easy, making inevitable the oxidation of phenols, which converted to quinones, react with proteins, inclusive polyphenoloxidase itself, explaining its lower activity (Amorim et al, 1975).

The development of seeds can be monitored by means of detection of alterations, such as size, water content, dry matter content, germination and vigor. In studies conducted during the maturation of seeds of various species, the dry matter mass has been considered as the best and surest indicative that the seeds have reached physiological maturity (Day, 2001). Vanderlip et al. (1972) reported that after 70 days from flowering, at stage seven, the sorghum grain is considered milky, with a half of the dry matter. In stage eight, three-quarters of the dry matter mass of the seeds have already been accumulated and at stage nine, characterized by the maximum dry matter accumulation, with water content between 25 % to 35 % is considered the point of physiological maturity of this species .

Physiological maturity is not uniform, varying on the same plant. Tillmann et al. (1985) noted a close relationship between water loss and increase of dry matter in seeds, for the three portions of the panicle in sorghum BRS 501 during the maturation process. It was further found that the maximum of germination and dry matter occurred at 47 days after anthesis, on the apical portion and around 54 days after anthesis for the middle and basal portion.

During the drying process, damages to membrane systems can occur, triggering enzymatic mechanisms, with alterations in cellular constituents. The Impairment of the cellular structure facilitates the contact the polyphenoloxidase enzyme, unique to plastids, with phenolic compounds, preferably stored in vacuoles , making inevitable the oxidation of phenols, which converted to quinones can react with proteins, including polyphenol itself (Amorim et al. 1975).

In addition to the enzymatic alaerations, with the reduction of water content, sorghum seeds can be induced to secondary dormancy with the advancement of drying at temperature of 46 ° C - 48 ° C. According to Marcos Filho (2005), inhibition of germination may be caused by substances present in the coat or on the inside part of the seeds, which can block the metabolism preparatory to germination or prevent the free access of oxygen to the embryo or the release of carbonic gas. Various kinds of germination inhibitors, such as tannins, phenolic acids, alkaloids and aldehydes are known.

The tannin content in sorghum seeds depend on genotype and it may be present or not (Magalhães et al. 2003). Queiroz (1979) noted that the tannin content was highly correlated with seed germination and that, in general, secondary dormancy does not last for more than three months. And as sorghum seeds tend to overcome dormancy in storage, is not really known if this is due to stratification at low temperature or reduction of phenolic compounds such as tannins in the seed coats.

In the absence of accurate information, the alternatives available for determination of the time or the stage most favorable for collection consist in the evaluation of moisture content and use of morphological characteristics of plant parts. Thus, it was aimed with this research work to evaluate biochemical, physical and physiological alterations occurred during the development of sorghum seeds of different concentrations of tannin.

MATERIALS AND METHODS

The analyzes were conducted at the Central Laboratory of Seed Analysis and Food Science Laboratory of the Federal University of Lavras (Universidade Federal de Lavras).

The sowing was conducted in the experimental field of the Department of Agriculture. The soil was prepared in a conventional manner in accordance with the recommendations for the culture. Each treatment consisted of four plots, composed of six rows of 5 m, with a spacing of 0.5 m interrows and 12 plants per linear meter, amounting to30 m^2. The useful area of each plot was made of the four central rows. For planting fertilization, 400 kg / ha of 8-20-10 formulation were used and for topdressing, ammonium sulfate at the dosage of 350 kg / ha, at two applications at 30 and 45 days after planting was employed.

The early harvest was characterized by the milky grain, as described by Varderlip et al. (1972). Harvests were carried out when the seeds reached approximately 48 % , 43 % , 40 % , 35 % , 30 % , 25 % and 20% moisture content, which corresponded to 100 , 103 , 107 , 113 , 119 , 121 and 127 days after sowing, respectively. The panicles were harvested and threshed manually. A part of the seeds was artificially dried in an air circulation oven at 35 ° C, until these reached 12 % moisture content and the other part was not subjected to drying. The experimental design was in completely randomized blocks in a factorial scheme 7x2, seven development stages, with and without drying. Four replications of 50 seeds were used in each treatment and the quality assessed by the tests described next.

Moisture content

Performed by the oven method at 105 º C for 24 hours, according to the Rules for Seed Analysis (Brazil, 2009).

Germination test

the substrate for sowing was paper, moistened with distilled water in amount of 2.5 times as heavy as the dry weight of the paper. The seeds were placed in a germination chamber at 25 °C, and evaluated at four and 10 days after sowing at the end of the test; the seeds which did not germinate were submitted to the tetrazolium test (Brazil, 2009).

Electrical conductivity

The seeds were weighed, placed in plastic glasses with 75 mL of deionized water and maintained at constant temperature of 25 ° C. After 24 hours' soaking, the reading of the electrical conductivity of the solution was carried out with a mass conductivity meter, Digimed mark Model CD -21 and the results were expressed in µS/cm/g according to the methodology described by Krzyzanowski et al. (1999).

Tannin quantification

5g of ground seeds were placed into a test tube and added of 30 ml of 80% methanol. The mixture was boiled for 20 minutes under reflux on a hot plate for tube at 150 ° C. Quantification was performed by the chemical colorimetric method of Folin - Dennis and the readings performed in a spectrophotometer at 760 nm.

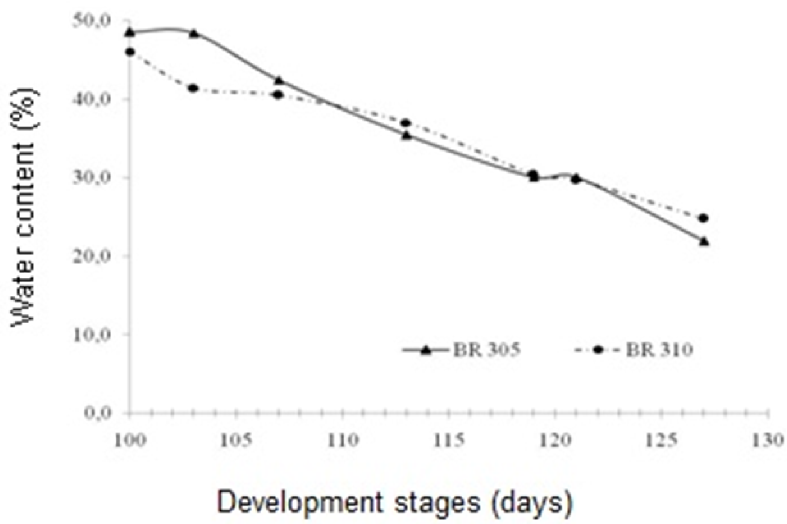

Figure 1. Water contents at the moment of harvest of sorghum seeds, cultivar BR 305 and BR 310 at 100, 103, 107, 113, 119, 121 and 127 days after sowing.

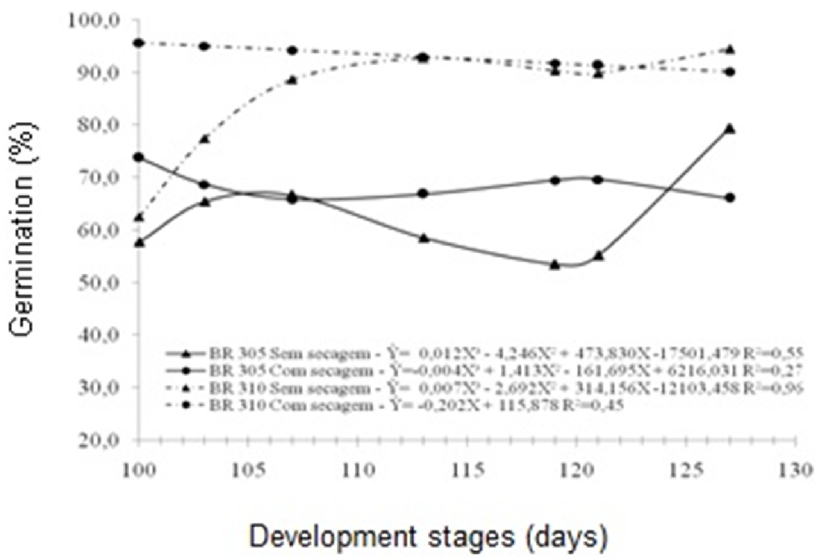

Figure 2. Germination (%) of sorghum seeds BR 305 and BR 310, harvested at different development stages and whether or not subjected to artificial drying.

Quantification of the Polyphenoloxidase

The activity of the enzyme polyphenoloxidase (PPO) was determined according to the method described by Draetta *et al*. (1976). The sorghum seeds were ground in a cooled mill to 4 ° C by adding liquid nitrogen. To each 5g of sample were added 40 ml of buffer solution of 0.1 M potassium phosphate pH 6.0; homogenized in vortex for 5 minutes and filtered through Whatman filter paper number 1 under vacuum. Sample extract was used without DOPA (L- 3 ,4 - dihydroxyphenyalanin) as the blank and the standard curve performed in Schumaz spectrophotometer and the results expressed in U / min / g .

RESULTS

The water contents of the seeds at the moment of collection in the different development stages are shown in Figure 1 and ranged from 48.5 % to 21.9% for lot with a high tannin content (BR 305) and 46.0 % to 24.7 % for lot with a low tannin content (BR 310).

By the analysis of germination (Figure 2), in seeds not subjected to drying in lot BR 305, reduction in the

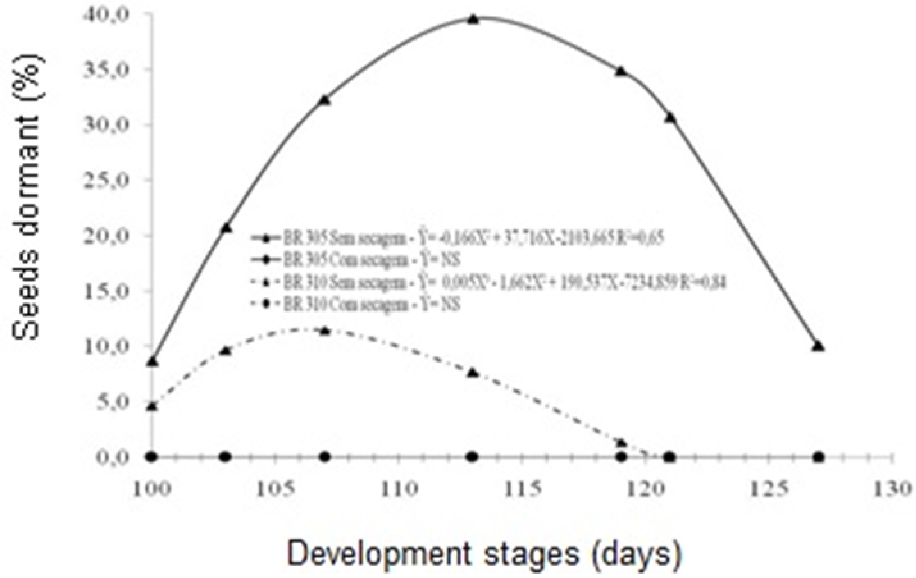

Figure 3. Percentage of dormant sorghum seeds BR 305 and BR 310, harvested at different development stages and whether or not subjected to artificial drying

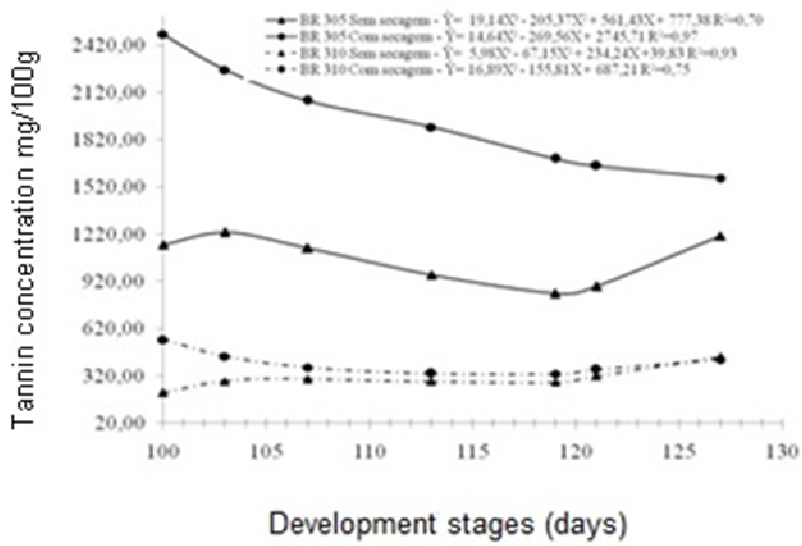

Figure 4. Tannin concentration in sorghum seeds BR 305 and BR 310, collected at different development stages and whether or not subjected to artificial drying

germination values from 107 and up to 119 days with increase at 121 days.

The non-germinated seeds were submitted to the tetrazolium test to confirm the condition of dormancy. The highest percentage of dormant seeds, of cultivar BR 305, occurred when these were with 35% moisture, 113 days (Figure 3). However, for seeds of this same lot, subjected to artificial drying, reduction in the percentage of germination at 107 days was found, its being kept until the last development stage. Therefore, with drying, the percentage of dormant seeds was null.

By analyzing the results of the tannin concentration (Figure 4), it is found that, after drying, tannin concentration was reduced up to a minimum of 327.8 mg/100, 119 days after the sowing of the seed lot, for seeds BR 310, with later increase. However, without artificial drying, increase in the concentration of tannin with advancing development stages was observed, reaching the highest concentration at 127 days.

In Figure 5, it is observed for the values of electrical conductivity, which from the 107 days, the seeds from lot BR 310 not subjected to artificial drying presented the

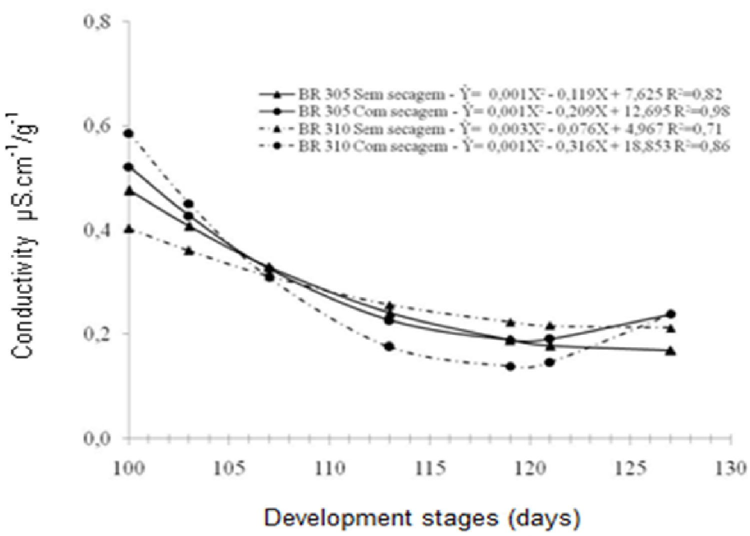

Figure 5. Electric conductivity of sorghum seeds BR 305 and BR 310 , harvested at different stages of development and whether or not subject to artificial drying

highest values of leaching. Opposite behavior was shown by the seeds harvested and subjected to drying. But for lot BR 305, there were no great variations in the values of electrical conductivity among seeds which were given or not drying. In general, reduction in the conductivity values along the development is verified.

The activity of the enzyme polyphenoloxidase (Figure 6) was higher in seeds subjected to drying of the lot with a low concentration of tannin, at 100 and at 103 days after sowing. Shortly after the third maturation stage, however, reversal in this trend was noticed, with the highest activity of the enzyme in the seeds which were not subjected to drying.

DISCUSSION

Significant interactions between drying and development stage for both lots were observed for all the tests conducted. The highest values of germination of cultivar BR 305 were observed when the seeds were harvested with 22% of moisture content and not subjected to drying. Other authors have observed better germination when the seeds presented 30% (Andrade et al;1988) or 42% of water content (Borba et al. 1993).

For the seeds with a low tannin content (BR 310) and not subjected to drying (Figure 2), there was a behavior similar to that of lot 305 BR behavior with regard to germination, increase having occurred in that value, till 113 days, with the maximum germination coinciding with the reduced in water content. The germination of the seeds of cultivar BR 310, regardless of drying was higher than that of cultivar BR 305, at all development stages. For Muscolo et al. (2001), phenolic compounds, such as tannin, inhibit the enzymes glucose -6- phosphate - dehydrogenase, glucose phosphate isomerase and aldolase, which are related to the synthesis of sugars, leading to decreased seed germination.

The highest percentage of dormant seeds, of cultivar BR 305, occurred when these seeds were with 35% of moisture, at 113 days (Figure 3). The results of dormancy in the seeds subjected to artificial drying are similar to those observed in seeds **of lots** BR 305 and BR 310. In the seeds without drying of lot BR 310, there was an increase in the percentage of dormant seeds to a maximum of 12 %, to 107 days, when they had moisture content of 40 %. From that stage, there was a decrease in these values , with zero to 121 and 127 days, when harvested with 25 % and 20% water content, respectively. For lot BR 305, the maximum value of dormancy occurred at 113 days, when they had 35 % moisture content, with further decrease in these values , coinciding with the reduction in water content.

By analyzing the results of the tannin concentration (Figure 4), it is found that after drying, tannin concentration was reduced up to a minimum of 327.8 mg/100, 119 days after sowing, for the seeds of lot BR 310, with further increase. However, without artificial drying, an increase in the concentration of tannin with advancing development stages was found, reaching the highest concentration at 127 days.

In the seeds of cultivar BR 305 submitted to artificial drying, there was decrease of the tannin concentration with advancing maturation and higher tannin values when compared to those of non-dried seeds. The tannin concentration did not vary greatly with the development stage of this cultivar, the lowest concentration of tannin for these seeds being recorded at 119 days after sowing. It can be still found that for lot BR 305, drying favored the

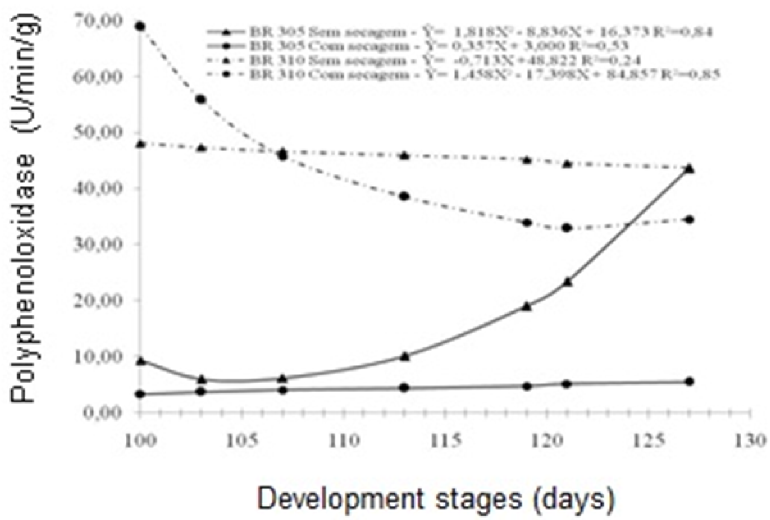

Figure 6. Polyphenoloxidase activity (U / min / g) of sorghum seeds BR 305 and BR 310, harvested at different development stages and whether or not subjected to artificial drying

concentration of this compound at different development stages. However, for lot BR 310, drying did not favor the concentration, greater difference being found for the first season.

In general, reduction in the values of conductivity throughout development (Figure 5) was noticed. With advancing maturity, the structural organization of cell membranes occurs, which explains the decrease in electrical conductivity values. The natural or artificial drying promotes membrane structuration, however, for the lot with a low concentration of tannin (BR 310), artificial drying appears to be less harmful to the membrane system than natural drying as the maturation process proceeds.

The activity of the enzyme polyphenol oxidase (Figure 6) was greater in seeds submitted to drying of the lot with a low concentration of tannin at 100 and 103 days after sowing. Shortly after the third maturation stage, however, reversal in this trend was found, with highest activity of the enzyme in the seeds which were not subjected to drying.

However, for the lot with the highest concentration of tannin, less activity was observed in seeds subjected to artificial drying, regardless of the development stage. For the seeds without drying, there was an increase in enzyme activity throughout development, its being more pronounced from 107 days on, with the maximum activity value at 127 days.

The reduction of the activity of polyphenoloxidase enzyme in dry seeds of lot BR 310 coincides with the reduction of the tannin concentration and lower electrical conductivity values. This demonstrates that with the maintenance of seeds' membrane structure, the presence of this enzyme is reduced, which remains compartmentalized in plastids.

There are in the literature, a great deal of controversies regarding the activity of polyphenoloxidase enzyme. Murata et al. (1995) observed a decrease in the specific activity of polyphenoloxidase enzyme during ripening of 'Fuji' apple fruits. In other fruits, increases have been recorded which according Mowlah et al. (1982), may be associated to the fall in polyphenol content. Another issue for the difference in the activity of this enzyme may be excess product formed, which can reduce the activity. These differences may, still, be related to the extraction and detection of the activity of this enzyme and even to the process of determination of phenolic compounds .

CONCLUSIONS

The drying of sorghum seeds harvested at early development stages promotes germination. The incidence of dormancy is higher in seeds not subjected to drying. There was an increase in the polyphenoloxidase activity during the development of the seeds without drying. There is no relationship between the concentration of tannin and dormancy in sorghum seed.

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