

RESEARCH NOTE

Pre-germination treatments and storage of cassava seeds and their correlation with emergence of seedlings¹

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ABSTRACT - Despite propagation of the cassava crop be done by cuttings for commercial purposes, the majority of accesses keeps active the sexual propagation system; what is essential to genetic breeding programs. Cassava seeds, however, have low and uneven germination rates. Considering such event, the objective of this study was to evaluate the efficiency of the storage, and different pre-germination treatments, on cassava seedling emergence. For this, an experiment was carried out with seeds obtained by open pollination between plants of four sweet cassava cultivars, with 10 pre-germination treatments; in addition to a control treatment. The viability of seeds subjected to different treatments was assessed by tetrazolium test; and the assessment of seedling emergence was performed by daily counts of the number of emerged seedlings. Data obtained were expressed in: emergence percentage; emergence speed index; and mean time of seedling emergence. It was concluded that storage, at 4 °C, during one year, increases seed emergence percentage and favors cassava seedling emergence speed; and that the dry heat (60 °C), during seven and 14 days, also favors their emergence speed .

Index terms: *Manihot esculenta*, seed technology, seed dormancy, seed germination.

Tratamentos pré-germinativos e armazenamento de sementes de mandioca e sua correlação com a emergência de plântulas

RESUMO - Apesar da propagação de mandioca ser efetuada vegetativamente para fins comerciais, a maioria dos acessos mantém ativo o sistema de propagação sexual, o que é fundamental para programas de melhoramento genético. Entretanto, as sementes de mandioca apresentam germinação baixa e desuniforme. Considerando este fato, o objetivo deste trabalho foi avaliar a eficiência do armazenamento, e de diferentes tratamentos pré-germinativos, na emergência de plântulas de mandioca. Para isso, foi conduzido um experimento com sementes originadas da polinização aberta entre plantas de quatro variedades de mandioca de mesa, nas quais foram testados 10 tratamentos pré-germinativos, mais a testemunha. A viabilidade das sementes submetidas aos tratamentos foi avaliada pelo teste do tetrazólio e a avaliação da emergência das plântulas foi realizada através da contagem diária do número de plântulas emersas. Os dados observados foram expressos em porcentagem de emergência, índice de velocidade de emergência e tempo médio de emergência das plântulas. Pelos resultados obtidos, pode-se concluir que o armazenamento, a 4 °C, durante um ano, aumenta a porcentagem de emergência das sementes e favorece a velocidade de emergência de plântulas; e que o calor seco (60 °C), durante sete e 14 dias, também favorece a velocidade de emergência das plântulas.

Termos para indexação: *Manihot esculenta*, tecnologia de sementes, dormência, germinação.

Introduction

Cassava (*Manihot esculenta* Crantz) is a bushy perennial, whose probable center of origin and diversity is Brazil

(Olsen, 2004). The crop is one of the most important products of Brazilian agriculture, as much from the economical view point as sociocultural. The species is cultivated chiefly for the use of its tuberous roots, rich in starch, which are consumed

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in the processed forms of meal and starch (among others), cooked and fried “in natura” (among other modes) (El-Sharkawy, 2003) or, in lower scale, in chemical industry and animal feeding (Vilpoux, 2008).

Although, for commercial purposes or livelihood, the cassava cultivation is exclusively performed by means of vegetative propagation by using “seed-cuttings”, majority of accesses maintains active the sexual propagation system (Vieira et al., 2008). From evolutionary view point, the maintenance of sexual propagation system active through crossbreeding, allows constant gene recombination and gene combination of different origins in a single genotype; what confers higher adaptation capacity to environmental changes to the species. Nevertheless, the vegetative reproduction allows immediate adaptation to environmental conditions, by means of higher genetic characteristics fixation, selected consciously or unconsciously within the segregating population (Elias et al., 2001; Duputié et al., 2009).

The fact that the cassava plant keeps a system of sexual propagation activated is essential for the genetic breeding programs for the species, once it allows for the direct crossbreeding between previously defined parental varieties, and subsequent selection of individuals within the segregating population originating from such crossings (Fukuda et al., 2002). However, one of the limiting factors to genetic breeding of the species is the low and uneven germination of true seeds of cassava; what may be attributed to environmental factors such as luminosity and temperature or to intrinsic causes as seed dormancy, or a combination between both factors (Fukuda and Cerqueira, 1986).

In this sense, the treatments of pre-germination have been successfully tested for the improvement of cassava seeds germination rate (Pujol et al., 2002). However, in choosing pre-germination treatments, aiming at improving this parameter, it is important to consider the easiness of its completion and its applicability to a large number of seeds. Another important aspect is that the treatment of seed pre-germination is able to standardize the emergence and the seedling development, in addition to improving the germination rate, in a way to facilitate assessments of hybrids within genetic breeding programs.

This manner, the objective of this study was to assess efficiency of seed storage and of seed different pre-germination treatments, on emergence of cassava seedlings.

Material and Methods

The seeds of cassava used in the experiment were obtained in an area of Embrapa Cerrados, originating from crossings by open pollination among plants of sweet cassava varieties IAC 576-70 (Japonesinha), IAPAR 19 (Pioneira), BGNC 751

(Japonesa), and BGMC 1289 (Moura); which are the most cultivated in the region of Federal District and surroundings. Because of natural dehiscence of the fruits, they were packed still immatures into voile bags at equal phenological stage and the seeds were systematically collected after their dispersal. The seed harvest was performed in two steps: the first in the year 2009 and the second in the year 2010. The seeds harvested in 2009 were then packaged into Kraft paper bags and stored under refrigeration, at 4 °C, during one year; and the seeds harvested in 2010 were stored into Kraft paper bags, under natural environmental conditions, during one month, until experiment installation date.

Before treatments, all seed have been sorted as true-seeds or pseudo-seeds, by immersion in water at room temperature. The floating seeds were sorted as pseudo-seeds and eliminated; and true-seed (submerged seeds) were removed from water and dried under natural environment, following the methodology described by Ogburia and Adachi (1995).

The following treatments were assessed: I - storage of seeds at 4 °C, during one year; II – dry heat (60 °C), during seven days; III - dry heat (60 °C), during 14 days; IV – mechanical scarification, followed by seed immersion into a 100 ppm of gibberellic acid solution, during 24 h; V – mechanical scarification, followed by seed immersion into 200 ppm gibberellic acid, during 24 h; VI – mechanical scarification of seeds; VII - acid scarification of seeds, by immersion into concentrated sulfuric acid, during 5 min.; VIII - acid scarification of seeds, by immersion into concentrated sulfuric acid, during 10 min.; IX - immersion of seeds into water, at 80 °C, during 2 min.; X - immersion of seeds into water, at 90 °C, during 2 min.; and XI - control (storage of seeds, at room temperature, during 30 days, without any previous treatment).

Mechanical scarification was achieved by manual friction of seeds on water sandpaper until removal of a small area of seed coat, in the opposite side of caruncle. For dry heat treatment, the seeds were kept in dry forced air circulation oven at 60 °C, during seven and 14 days.

After treatment, the tetrazolium test was applied to a subsample of 10 seeds each, for each treatment, aiming at verifying their viability. For this, the seeds were pre-conditioned by direct immersion in distilled water at 30 °C, for a 24 h period. Subsequently, the seeds were longitudinally sectioned, for exposing embryo and cotyledons and immersed in a tetrazolium solution at 0.5%, at 35 °C, during 5 h, and then assessed for their coloration, according methodology proposed by Wetzel et al. (2006).

For assessing emergence of seedlings, polypropylene tubes (volume of 175 cm³) were filled with substrate composed by: one third of vermiculite; one third of soil; and one third of the commercial brand substrate Germinar Forte®. The seeds

were sown at 2 cm deep inside these tubes, which were then kept under greenhouse conditions with daily irrigation. A randomized block experimental design was used with four replications, composed by 40 seeds each, totaling 40 tubes.

Daily counts of number emerged seedlings were performed during 65 days, starting from the sowing. In such count, were considered only plants showing potential to keep developing and generate normal plants (Brasil, 2009). The computed data were expressed in percentage emergence, emergence speed index, and mean time of seedling emergence; according to the equations (1), (2), and (3) as follow:

$$EP = (E/Et) \times 100 \quad (1)$$

Where: EP = emergence percentage; E = total of seedlings that have emerged within each replication; and Et = total number of seeds within each replication.

$$ESI = E1/N1 + E2/N2 + \dots + Ei/Ni \quad (2)$$

Where: ESI = emergence speed index; E1, E2... Ei = number of seedlings emerged at the first, second... and last count; N1, N2... Nn = number of days after the sowing until the first, second... and last count (Maguire, 1962).

$$MTE = (E1 \times N1) + (E2 \times N2) + \dots + (En \times Nn) / Etot \quad (3)$$

Where: MTE = mean time (days) for emergence of seedlings; E1, E2... En = number of seedlings that have

emerged at the first, second... and last day; Nn = number of days taken to emergence; Etot = total of seedling that have emerged per treatment (Rodolfo-Junior et al., 2009).

Data obtained were subjected to ANOVA, at 5% error probability by T test, through Genes program (Cruz, 2001). The emergence percentage data were transformed by $\sqrt{x} \times 0.50$, before analysis; however, the original data are shown in the Table. The means of treatments were compared by the Scott-Knott test, at 5% probability. Variability data, resulting from seed assessment through tetrazolium test and after being subjected to different treatments, have not been statistically analyzed.

Results and Discussion

Results of ANOVA have revealed statistically significant differences among treatments for emergence percentage, emergence speed index, and emergence mean time of cassava seedlings.

The seeds without treatment and stored at room temperature, during 30 days (control), have presented 100% viability by tetrazolium test, and all seeds assessed have shown cotyledons and embryo stained; indicating that were alive and suitable for germination. The same result was obtained for the seeds subjected to the treatments: storage at 4 °C, during 1 year, under dry heat (60 °C) during seven and 14 days, after the mechanical scarification followed by immersion in gibberellic acid solution (100 and 200 ppm) during 24 h; and mechanical scarification followed by immersion in concentrated sulfuric acid, during 5 and 10 min.; what confirms that such pre-germination treatments do not cause any embryonic damage that could endanger germination of the seeds (Table 1).

Table 1. Means of emergence percentage (EP), mean time of emergence (MTE), and emergence speed index (ESI) of cassava seedlings from seeds subjected to different pre-germination treatments; and percentage of viability (VIAB), assessed by tetrazolium test, after each treatment.

Pre-germination treatments	EP (%)	MTE (days)	ESI	VIAB (%)
Storage at 4 °C during one year	88 A*	30.24 E*	0.0200 A*	100
Control	80 B	49.47 B	0.0104 C	100
Dry heat (60 °C) during 14 days	79 B	36.62 D	0.0154 B	100
Dry heat (60 °C) during seven days	76 B	35.17 D	0.0153 B	100
Mechanical scarification + 200 ppm GA3, during 24 h	37 C	43.29 C	0.0062 D	100
Mechanical scarification + 100 ppm GA3, during 24 h	30 D	45.87 C	0.0044 E	100
Mechanical scarification	29 D	44.91 C	0.0043 E	100
Acid scarification (5 min.)	16 E	55.76 A	0.0017 F	100
Acid scarification (10 min.)	4 F	55.0 A	0.0005 G	100
Water immersion (80 °C) during 2 min.	0 F	-	-	0
Water immersion (90 °C) during 2 min.	0 F	-	-	0

*Means followed by the same letters in the columns do not statistically differ between each other by Scott-Knott test, at 5% probability.

The treatments of immersion in water at 80 °C and 90 °C, for 2 min., have provoked damages to seeds, turning them unviable; what was verified by lack of staining in the cotyledons and embryos of all seeds subjected to the tetrazolium test and confirmed by the non-emergence of seedling (Table 1). Likewise, the treatment of immersion in water, at 80 °C, 90 °C, and 100 °C, for 2 min., in seeds of cassava tree, commonly known in Brazil as “Maniçoba” (*Manihot glaziovii* Muell. Arg., Euphorbiaceae), was not efficient to overcome seed dormancy (Rodolfo-Junior et al., 2009).

Seeds subjected to storage, at 4 °C, during 1 year, have presented emergence percentage of 88%, showing significant increase in relation to control, which had only 80% emergence. Martins et al. (2009) found that seeds of “Maniçoba”, stored into paper bags, for a period of until 150 days and submitted to scarification before the sowing, have presented trend of emergence percentage increment along storage period. Similar results were obtained by Canuto and Canuto (1981) for seed on another species of “Maniçoba” (*Manihot pseudoglaziovii* Pax & Hoffm., Euphorbiaceae); by Santarém and Aquila (1995) for seeds of Senna [*Senna macranthera* (Collard.) Irwin et Barn., Leguminosae], and by Lopes et al. (2009), for seeds of “Imbu” (*Spondias tuberosa* Arruda, Anacardiaceae)

Seeds stored at 4 °C, during 1 year, have presented emergence mean time of 24 to 30 days; what represents a difference of almost 20 days in relation to control, which has presented emergence mean time of 47 to 49 days. The seeds subjected to mechanical scarification, as well as to mechanical scarification followed by immersion into solutions of gibberellic acid at 100 and 200 ppm, during 24 h, have shown lower emergence mean time than the seeds of control treatment; although statistically similar between each other. In seeds of *Manihot glaziovii* and White Leadtree [*Leucaena leucocephala* (Lam.) de Wit, Fabaceae], the mechanical scarification was also efficient in promoting increase in seedling emergence speed (Teles et al., 2000; Rodolfo-Junior et al., 2009). Likewise, seedling emergence speed was larger after mechanical scarification followed by immersion into gibberellic acid solution in seeds of Cherimoya (*Annona squamosa* L., Anonaceae), the cross between two different Cherimoya species (*Annona cherimola* Mill. X *Annona squamosa* L.) (Stenzel et al., 2003), and two species within the family Caesalpinoideae: Orchid-tree (*Bauhinia monandra* Britt.) and Mountain ebony (*Bauhinia unguolata* L.,) (Alves et al., 2000).

In the case of cassava seeds, it is possible that the action of heat and mechanical scarification have provoked ruptures on seed coat, facilitating expansion of embryo and consequently seedlings emergence; thus reducing the mean time for that such process to occur.

For the emergence speed index (ESI), the treatment that presented the best result was the storage at 4 °C during

one year, which ESI was of 0.002, followed by treatments of dry heat (60 °C) for seven and 14 days; which ESI were of 0.0153 and 0.0154, respectively, and by control, with ESI of 0.0104. These results demonstrate that the seed storage, under such same conditions, favored the cassava seedlings emergence speed, although the last treatments have not altered the seedling final emergence percentage. That means that these treatments may increase cassava seedling emergence uniformity in the field; thus accelerating establishment of plants and the progress of activities within genetic breeding programs for the crop.

Similarly, seeds of *Manihot glaziovii* and *Manihot pseudoglaziovii* stored into paper bags, during a period of until 150 days, and subjected to scarification before sowing, have shown a trend of linear increase of emergence speed along storage period (Martins et al., 2009). For many plant species, it is known that seed storage at room temperature is sufficient to attenuate intensity of dormancy, accelerating germination and emergence of seedlings. Such fact was already observed in seeds of many species within the genus *Brachiararia* (Poaceae) (Gonzalez et al., 1993, Martins and Silva, 2006) and other species of Poaceae family such as: Bluestem grass (*Andropogon gayanus* Kunth.) (Eira, 1983) and millet (*Panicum maximum* Jacq.) (Conde and Garcia, 1985). For seeds of the palm tree “Tucumã” (*Astrocaryum aculeatum* G. Mey., Arecaceae), has occurred improvement on the emergence time mean and emergence speed index, when occurred combination of the factors: higher moisture content in seeds x treatment of pre-germination with dry heat (Nazário and Ferreira, 2010).

The good performance of seeds stored at 4 °C, during 1 year, in the emergence and emergence speed of seedling, may be explained by the fact that freshly-harvested cassava seeds present dormancy and need from 3 to 6 months of storage, to be suitable to germination process (Jennings and Iglesias, 2002). Species that have evolved within humid tropical regions have developed mechanisms of water absorption impediment to avoid germination soon after dispersion, this way assuring survival (Dias, 2005).

According to Pujol et al. (2002), the ecology of seed germination of domesticated species in the genus *Manihot* displays characteristic features of adaptation to unstable environments; and these seeds may remain viable and dormant during long periods, when the edaphoclimatic conditions are unfavorable to germination and development of seedlings.

In this sense, the storage of cassava seeds, during until one year and the treatment of dry heat (60 °C, during seven and 14 days) were promising for the increment on emergence speed of seedlings. The dry heat treatment has been particularly convenient for large scale uses, once it is fairly simple to be conducted and does not demand seed storage during 1 year;

what facilitates conduction of genetic breeding programs for crop. Notwithstanding, precautions have to be taken concerning combination between temperature and time of exposure, once prolonged exposures may cause damages to seeds because of protein denaturing and embryonic tissues degradation.

Conclusions

The storage at 4 °C, during one year, increases emergence percentage and favors emergence speed of cassava seedlings; and the dry heat (60 °C) treatment, during seven to 14 days, also favors emergence speed of seedlings.

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