Influence of Different Pre-treatments on Enhancing Seed Germination in Cassava

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Abstract

Cassava is mainly propagated vegetatively by stem cuttings. However, cassava seeds are widely used by the breeders with the objective of developing new variety with its adaptability in varying environmental conditions, disease resistance and high yield. But the seeds having limiting factors like hard seed coat, light, temperature and other intrinsic factors lead to poor seed germination. The aim of this study was to enhance seed germination in cassava, which takes 16 to 20 days under normal condition. The efficacy of different treatments like mechanical scarification, water treatment and gibberellic acid treatment were investigated. It was found that seed germination percentage was significantly varied among different treatments (p<0.01) and maximum seed germination at 7th day after treatment was observed for GA3 50 ppm seed treatment (T5), followed by GA3 60ppm for 30 minutes on cotyledons (T14). When comparing the results, seeds soaked overnight in GA3 50ppm (T5) had significant effect on germination (66.67 %) and can be used as a best treatment for getting early germination in cassava.

Key words: Seed germination, mechanical scarification, gibberellic acid treatment, cassava seed treatment

Introduction

Cassava (Manihot esculenta Crantz), popularly known as manioc, tapioca or yuca, belongs to the family Euphorbiaceae, is a major staple food in the developing world, providing a basic diet for around 500 million people. The crop is neo tropical in origin and is now extensively cultivated in tropical and subtropical region for its edible tuberous root. World cassava production was estimated to be 270.2 m t, whereas in India the annual production was 8.1 m t from an area of 0.22 m ha (FAOSTAT, 2015). Nigeria is the world’s largest producer of cassava with a production of 54.8 m t from an area of 7.1 m ha. Cassava root is a good source of carbohydrates, high levels of vitamins, minerals and dietary fiber.

Cassava is propagated vegetatively by stem cuttings, although many accessions maintain its sexual propagation system. In traditional agro ecosystem, farmers allow volunteer seedlings to grow; later promising seedlings were selected and used for clonal selection (Elias et al., 2001).

Pattern of genetic diversity suggests that the incorporation of sexual hybridization and clonal selection of the crop results in the development of new varieties with good agronomic as well as morphological characters, thereby high intra varietal crop diversity. In vegetative reproduction, the genetic material gets fixed that helps in the immediate adaptation of plants to the environmental condition. Manihot species exhibit a wide range of genetic diversity which results from easy cross pollination and high heterozygosity (Fukuda et al., 1996). The products of sexual reproduction are important in the evolutionary dynamics of this clonally propagated crop (Mc Key et al., 2001). Cassava seeds act as a filter for virus diseases and other pathogens that tend to accumulate in vegetative cuttings and the plants originating from seedlings are normally or in most cases are free of such systemic pathogens (Lozano and Nolt, 1989).

Sexual propagation through seeds is very essential in cassava breeding program, that allows the cross breeding between two different parents which are contrasting in
trait of interest. However, the limiting factors like hard seed coat and other intrinsic factors may lower the seed germination rate and that may be overcome through pre-treatments. Seed germination is a complex process that depends on the genetic constituents of the seeds and on several environmental factors such as the growing medium (Asrar, 2009) temperature, light and salinity (AL-Helal, 1998). Propagation through seeds have manifold advantages like enhancing the multiplication rate several times, keeping the dreaded cassava mosaic diseases under check, easy seed storage and transport (Rajendran et al., 2000). Under ambient condition, cassava seeds can be stored up to six months without any loss of viability and the viability declines gradually with storage duration and climatic condition. Seeds for storage should be kept at 5°C and 60% relative humidity (IITA, 1978). There is a sharp decline in germination percentage after 8 months and the normal time period of cassava seed germination occurs between 16 to 20 days after sowing (Rajendran et al., 2000). Cassava seeds exhibit physiological dormancy and to break dormancy, different treatments have been used. Seed germination and seedling growth are known to be regulated by exogenous hormones and treating the seeds with growth regulators helps to improve seed germination. Studies reported that the dormancy maintenance and release depends mainly on intrinsic balance of abscisic acid (ABA) and GA₃ which indicates that GA₃ has pronounced effect in regulating seed germination and dormancy. Growth regulators are used to break seed dormancy and to improve seed germination in many plant species (Pallais et al., 1991; Karam and Al-Salem, 2001; Bahrani et al., 2008; Zeinalabedini et al., 2009; Deng et al., 2010; Zeng et al., 2010). Cassava has very thick seed coat that is impermeable to water that can be overcome through mechanical scarification of the seeds (Pujol et al., 2002). Keeping the above, the present study was done to determine the effect of different seed treatments to enhance germination in cassava.

Materials and Methods

Seed material

The present investigation was undertaken in ICAR- Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala under glasshouse condition (2013). The seeds from CMD resistant cultivar, MNga-1 (Sree Padmanabha) were used in the germination experiments. Due to the natural dehiscence of the fruits, they were packed in white muslin cloth and seeds were collected after their dispersal. Before treatments, non-viable seeds were separated from the viable seeds by immersing in water at room temperature. The floating seeds were non-viable and eliminated; and submerged seeds were separated from water and dried under sunlight.

Experimental procedure

A total of 510 seeds were used in the germination experiment. There were 17 different treatments (Table 1) which included water, GA₃ treatment, mechanical scarification, combinatorial treatments and control (dry seeds). Each treatment was done with three replications and 10 seeds were used for each replication.

Water treatment

The water treatments were conducted at two different time intervals. In these two treatments, the seeds were soaked in water for 24 hours and 48 hours before sowing (Rajendran et al., 2000). Cassava seeds exhibit physiological dormancy and to break dormancy, different treatments have been used. Seed germination and seedling growth are known to be regulated by exogenous hormones and treating the seeds with growth regulators helps to improve seed germination. Studies reported that the dormancy maintenance and release depends mainly on intrinsic balance of abscisic acid (ABA) and GA₃ which indicates that GA₃ has pronounced effect in regulating seed germination and dormancy. Growth regulators are used to break seed dormancy and to improve seed germination in many plant species (Pallais et al., 1991; Karam and Al-Salem, 2001; Bahrani et al., 2008; Zeinalabedini et al., 2009; Deng et al., 2010; Zeng et al., 2010). Cassava has very thick seed coat that is impermeable to water that can be overcome through mechanical scarification of the seeds (Pujol et al., 2002). Keeping the above, the present study was done to determine the effect of different seed treatments to enhance germination in cassava.

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Mechanical scarification

In mechanical scarification, the seeds were initially soaked in water overnight and afterwards the seed coat was removed through physical abrasion using dissection needle.

Table 1. Cassava seed pre-treatments

<table>
<thead>
<tr>
<th>Treatments</th>
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<tbody>
<tr>
<td>T₁ Control (dry seeds)</td>
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<tr>
<td>T₂ 24 hour water treatment</td>
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<tr>
<td>T₃ 48 hour water treatment</td>
</tr>
<tr>
<td>T₄ Mechanical scarification</td>
</tr>
<tr>
<td>T₅ GA₃ treatment 50ppm</td>
</tr>
<tr>
<td>T₆ Seed coat removal and GA₃ treatment 10ppm, 10min</td>
</tr>
<tr>
<td>T₇ Seed coat removal and GA₃ treatment 10ppm, 20 min</td>
</tr>
<tr>
<td>T₈ Seed coat removal and GA₃ treatment 10ppm, 30 min</td>
</tr>
<tr>
<td>T₉ Seed coat removal and GA₃ treatment 30ppm, 10 min</td>
</tr>
<tr>
<td>T₁₀ Seed coat removal and GA₃ treatment 30ppm, 20 min</td>
</tr>
<tr>
<td>T₁₁ Seed coat removal and GA₃ treatment 30ppm, 30 min</td>
</tr>
<tr>
<td>T₁₂ Seed coat removal and GA₃ treatment 60ppm, 10 min</td>
</tr>
<tr>
<td>T₁₃ Seed coat removal and GA₃ treatment 60ppm, 20 min</td>
</tr>
<tr>
<td>T₁₄ Seed coat removal and GA₃ treatment 60ppm, 30 min</td>
</tr>
<tr>
<td>T₁₅ Seed coat removal and GA₃ treatment 90ppm, 10min</td>
</tr>
<tr>
<td>T₁₆ Seed coat removal and GA₃ treatment 90ppm, 20 min</td>
</tr>
<tr>
<td>T₁₇ Seed coat removal and GA₃ treatment 90ppm, 30 min</td>
</tr>
</tbody>
</table>
GA₃ treatment

In GA₃ treatment, seeds were directly soaked with GA₃ 50 ppm for 12 hours.

Combinatorial treatments

In these combinatorial treatments, first the seeds were soaked in water for 12 hours in night and then seed coat was removed manually using dissection needle. The cotyledons were then treated with different concentrations of GA₃ (10, 30, 60 and 90 ppm) for different time intervals (10, 20 and 30 min).

Control

Dry seeds without any treatment were used as control for the pretreatment experiment.

After the treatment, the seeds were sown in raised bed for germination and seedling nursery was maintained in glass house. Data were recorded for one month to find out percentage and rate of germination along with the control.

Statistical analysis

The percentage of germination of each treatment was calculated, and the values were transformed using arcsine transformation and data was statistically analyzed by one-way analysis of variance (ANOVA) using SAS system version 9 (SAS, 2010) to compare the effect of different treatments. The mean values were compared using the Duncan’s Multiple Range Test (DMRT). The distribution of the percentage of germination obtained under different treatment were graphically displayed by box plot, drawn using the package R- environment for statistics. (R, 2013).

Results and Discussion

Cassava belongs to Euphorbiaceae family and exhibit physiological dormancy that may be non-deep, intermediate or deep depending upon the condition of seed storage. If the seed dormancy is non-deep or intermediate physiological dormancy, the dormancy can be broken through cold stratification (Carpita et al., 1983), temperature (Junttila, 1973) or light (Scheibe and Lang, 1965) and darkness (Rajendran et al., 2000) as well as by GA₃ (Baskin and Baskin, 1971). Seeds permanently undergo deep physiological dormancy with longer seed storage at low temperature and cannot be overcome through pre-treatments. In the present study, mechanical scarification, water treatment, GA₃ treatment and combinatorial treatments were used to break dormancy.

Seed germination response in cassava was varied significantly among different treatments. The effect of each treatment on the germination was evaluated for about one month and were analyzed in two sections (from 1-10 days and 11-29 days) and presented in Table 2.

Effect of different treatments on germination of seeds at 1-10 days.

The effect of different treatments on germination of seeds was assessed from 1-10 days. It was found that the seeds treated with water in two different time intervals, 24 and 48 hours (T₂ and T₃). Started germination from sixth day onwards and attained 20 and 27% germination respectively on 10th day (Fig.1; Fig.2). The effect of mechanical scarification (T₄) on seed germination was tested and recorded a percentage germination of 36.6.

By comparing the effects of GA₃ seed treatment with water treatment and scarification, direct soaking of seeds in 50ppm GA₃ overnight (T₅) started germination from the second day onwards and showed 60% germination percentage on 10th day. The effect of different concentration of GA₃ (10, 30, 60, 90ppm) in different time intervals (10, 20, 30 minutes) on the cotyledons was assessed and found that the germination percentage of GA₃ at 10 ppm and 30 ppm for 10 and 20 minutes (T₆, T₇, T₁₀, T₁₁) were on par and could attain a germination within the range of 15-30% within 10 days after treatment. The effect of GA₃ 60 ppm on cotyledons for 10, 20, 30 minutes was analyzed and found that the germination percentage increases with the increase in time intervals and reached an average of 30 per cent germination for cotyledons soaked in 60 ppm GA₃ for 30 minutes (T₁₄).

It was found that 90 ppm GA₃ in three different time
Table 2. Retransformed values of seed germination at 20 days with different seed pre-treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>20th day retransformed value</th>
</tr>
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<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; Control (dry seeds)</td>
<td>70.00(0.9912)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt; 24 hour water treatment</td>
<td>47.24(0.7578)&lt;sup&gt;bdac&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; 48 hour water treatment</td>
<td>53.86(0.8241)&lt;sup&gt;bac&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; Mechanical scarification</td>
<td>46.50(0.7504)&lt;sup&gt;bdac&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt; GA3 treatment 50ppm</td>
<td>63.40(0.9211)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 10ppm, 10min</td>
<td>34.78(0.6308)&lt;sup&gt;bdac&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 10ppm, 20 min</td>
<td>18.91(0.4499)&lt;sup&gt;bdbc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 10ppm, 30 min</td>
<td>16.35(0.4163)&lt;sup&gt;bdc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;9&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 30ppm, 10 min</td>
<td>17.95(0.4376)&lt;sup&gt;bdc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;10&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 30ppm, 20 min</td>
<td>28.54(0.5637)&lt;sup&gt;bdc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;11&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 30ppm, 30 min</td>
<td>15.72(0.4077)&lt;sup&gt;bdc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;12&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 60ppm, 10 min</td>
<td>11.61(0.3478)&lt;sup&gt;bdc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;13&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 60ppm, 20 min</td>
<td>13.00(0.369)&lt;sup&gt;bdc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;14&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 60ppm, 30 min</td>
<td>50.15(0.7869)&lt;sup&gt;bdc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;15&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 90ppm, 10min</td>
<td>3.68(0.1932)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;16&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 90ppm, 20 min</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;17&lt;/sub&gt; Seed coat removal and GA&lt;sub&gt;3&lt;/sub&gt; treatment 90ppm, 30 min</td>
<td>6.69(0.2618)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*The Mean values with same alphabet in the superscript in each column do not differ significantly.

*The Values in the parenthesis are the arc sine transformed mean values.

Fig.1. Effect of different treatments on germination of cassava seeds (1-10 days)

intervals (10, 20, 30 minutes) has least effect on germination when compared with other treatments (Fig 2).

**Effect of different treatments on germination of seeds at 11-29 days**

The effects of different treatments on the germination of seeds were assessed between 11-29 days. It was found that seed soaked in water for 24 and 48 hours (T<sub>2</sub> and T<sub>3</sub>) which attained almost 50% germination on 15<sup>th</sup> day and found to be on par with the control on 29<sup>th</sup> day (~65% - 70%). The germination percentage of mechanical scarification (T<sub>4</sub>) was found to be 43.3 on day 15 and was on par with the control on 29<sup>th</sup> day (Fig.3 and Fig.4). The germination percentage of GA<sub>3</sub> treatment 50 ppm (T<sub>5</sub>) had increased to 66.67 on day 15 followed by 60 ppm GA<sub>3</sub> treatment for 30 min on cotyledons (T<sub>14</sub>).
The effect of different treatments on three different time intervals (7th, 15th, 20th day) was analyzed and was noted that 50 ppm GA3 (T5) attained 60% germination on 7th day attained nearly 70% germination on 15th day and maintained the same on 20th day, followed by T14 attained 50% germination on 15th day. It was found that seed soaked in 24 hours water treatment (T2), 48 hour water treatment (T3) and overnight water treatment and seed coat removed (T5) attained 43, 50 and 43% germination on 15th day respectively and increased to 47%, 54% and 47% on day 20 and all other treatments showed less than 30% germination. It was also noted that 50 ppm GA3 (T5) showed significantly higher germination percentage than other treatments and attained 60% germination on 5th day itself.

The treatment 50 ppm GA3 (T5) was found to be the best which started germination on second day onwards and attained 60% germination on 5th day itself and thus this.
Pre-treatments on enhancing seed germination in cassava
treatment can be recommended for early germination. But considering cost effectiveness, seed soaked in water for 24 hour (T2), and 48 hours (T3) can be also used. Overnight seed soaked in water and seed coat removal (T4) is also an effective method for enhancing seed germination, but it is very difficult and tedious to remove the seed coat.

Cassava seeds have very hard seed coat and the chemicals that accumulate during seed development can act as germination inhibitors. Some of the substances associated with inhibition are various phenols, coumarins and abscisic acid (Booth et al., 2001). In the present study, seeds were first soaked in water and after few hours water turned into brown color indicating the presence of chemical substance in the seeds. Based on the observation, it was clear that there are some inhibiting chemicals present in the seed and that inhibited the seed germination. Cassava seeds have thick seed coat which prevents proper imbibitions of water. So the removal of seed coat helps in the proper imbibitions of water and also releases hormones like GA3 and other hormones that help in the expansion of embryo and consequently seedling emergence.

Overnight soaking in water and seed coat removed (T4) treatment recorded a 43.3% germination on 15th day. These results are in agreement with those reported by Teles et al., (2000); Rodolfo-Junior et al., (2009) in which the seeds of Manihot glaziovii and white leadtree [Leucaena leucocephala (Lam) de Wit] was subjected to mechanical scarification and found to be efficient in promoting seedlings emergence speed and also agreed with the results of Itamara et al., (2013).

GA3 is one of the most important plant hormone involved in the promotion and maintenance of seed germination. GA3 are known to obviate the requirement of seeds for various environmental cues, promote germination and counteract with cytokinins (Bewley and Black, 1994). It is one of the major plant hormones involved in the control of mobilization of food reserves from the endosperm or cotyledons, most especially enzymatic production (Black, 1972). The physiological component of dormancy determines the dormancy level response to external gibberelic acid application (Geneve, 2003) and promotes seed germination (Baskin and Baskin, 1971). In the present study, the effect of GA3 on germination was tested.

Fig. 4. Germination percentages of different treatments on 20th day
with 13 different GA₃ treatments in different time intervals. Treating the seeds overnight in GA₃ 50 ppm (T₆) gave above 50 per cent germination from 6th day onwards and attained nearly 70% germination on 15th day. Similar type of experiment was conducted by Rajendran et al., 2000 with 100 ppm, 300 ppm and 500 ppm GA, and obtained 60% germination on day 17 in 300 ppm GA₃ for 24 hours soaking. All other GA₃ treatments (T₇ to T₁₇) were done on mechanically scarified seeds and the germination percentages of each treatment were calculated. The seed germination was observed to be enhanced in seeds treated with 60 ppm GA₃ for 30 minutes (T₁₆) and gave 50% germination on 10th day, followed by T₆, T₇, T₈ (35 to 40%). Similar type of study was reported by Stenzel et al., (2003) on Cherimoya and Alves et al., (2000) on orchid tree and mountain ebony and found that the seedling emergence speed was quicker after mechanical scarification followed by immersion in gibberellic acid. The results were in agreement with the study conducted by Itamara et al., 2013, in which mechanically scarified seeds were immersed in 100 ppm and 200 ppm solutions of GA₃ for 24 hrs and found that lower emergence mean time than the seeds of control treatments. Based on the above experimental results, it is concluded that GA₃ treatment had a significant effect on the germination of cassava seeds and it varies with the concentration of GA₃. The best concentration of GA₃ that promotes early germination was between 50 ppm to 60 ppm on seed after removal of seed coat.

**Conclusion**

Based on the study it is concluded that the optimum condition for getting early germination in cassava is treating the seeds overnight with 50 ppm GA₃ and the higher germination (60%) response was between 5 to 15 days.

**References**


