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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 adaptibity 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 GA_3 50 ppm seed treatment (T_5), followed by GA_3 60ppm for 30 minutes on cotyledons (T_{14}). When comparing the results, seeds soaked overnight in GA_3 50ppm (T_5) 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 lowers 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 5ºC 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 GA_3 which indicates that GA_3 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, GA_3 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.

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

	Treatments
T_1	Control (dry seeds)
T_2	24 hour water treatment
T_3	48 hour water treatment
T_4	Mechanical scarification
T_5	GA_3 treatment 50ppm
T_6	Seed coat removal and GA_3 treatment 10ppm, 10min
T_7	Seed coat removal and GA_3 treatment 10ppm, 20 min
T_8	Seed coat removal and GA_3 treatment 10ppm, 30 min
T_9	Seed coat removal and GA_3 treatment 30ppm, 10 min
T_{10}	Seed coat removal and GA_3 treatment 30ppm, 20 min
T_{11}	Seed coat removal and GA_3 treatment 30ppm, 30 min
T_{12}	Seed coat removal and GA_3 treatment 60ppm, 10 min
T_{13}	Seed coat removal and GA_3 treatment 60ppm, 20 min
T_{14}	Seed coat removal and GA_3 treatment 60ppm, 30 min
T_{15}	Seed coat removal and GA_3 treatment 90ppm, 10min
T_{16}	Seed coat removal and GA_3 treatment 90ppm, 20 min
T_{17}	Seed coat removal and GA_3 treatment 90ppm, 30 min

GA_3 treatment

In GA_3 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 GA_3 (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 arc sine 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 seeds undergo dormancy under unfavorable conditions and maintain its seed viability. The germination period of cassava occurs between 16 to 20 days depending upon cultivars, storage duration and climatic conditions. Newly harvested seeds exhibit physiological dormancy and require three to six months of storage at ambient temperature for germination. (Jennings and Iglesias, 2002). Cassava seeds can remain viable for up to one year, although germination percentage may decline substantially after six months (Rajendran et al., 2000). Cassava seeds are orthodox seed type which is tolerant to the removal of free water as well as bound water (Vertucci and Farrant, 1995) and the longevity of seeds to germination increased with reduced bound water (Roberts and Ellis, 1989).

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 GA_3 (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, GA_3 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 (T_2 and T_3). Started germination from sixth day onwards and attained 20 and 27% germination respectively on 10^{th} day (Fig.1; Fig.2). The effect of mechanical scarification (T_4) on seed germination was tested and recorded a percentage germination of 36.6. By comparing the effects of GA_3 seed treatment with water treatment and scarification, direct soaking of seeds in 50ppm GA_3 overnight (T_5) started germination from the second day onwards and showed 60% germination percentage on 10^{th} day. The effect of different concentration of GA_3 (10, 30, 60, 90ppm) in different time intervals (10, 20, 30 minutes) on the cotyledons was assessed and found that the germination percentage of GA_3 at 10 ppm and 30 ppm for 10 and 20 minutes (T_6, T_7, T_{10}, T_{11}) were on par and could attain a germination within the range of 15-30% within 10 days after treatment. The effect of GA_3 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 GA_3 for 30 minutes (T_{14}). It was found that 90 ppm GA_3 in three different time

Table 2. Retransformed values of seed germination at 20 days with different seed pre-treatments

	Treatments	20th day retransformed value
T_1	Control (dry seeds)	70.00(0.9912)^{a}
T_2	24 hour water treatment	47.24(0.7578)^{bdac}
T_3	48 hour water treatment	53.86(0.8241)^{bac}
T_4	Mechanical scarification	46.50(0.7504)^{bdac}
T_5	GA3 treatment 50ppm	63.40(0.9211)^{ba}
T_6	Seed coat removal and GA_3 treatment 10ppm, 10min	34.78(0.6308)^{bdac}
T_7	Seed coat removal and GA_3 treatment 10ppm, 20 min	18.91(0.4499)^{ebdac}
T_8	Seed coat removal and GA_3 treatment 10ppm, 30 min	16.35(0.4163)^{ebdac}
T_9	Seed coat removal and GA_3 treatment 30ppm, 10 min	17.95(0.4376)^{ebdac}
T_{10}	Seed coat removal and GA_3 treatment 30ppm, 20 min	28.54(0.5637)^{ebdac}
T_{11}	Seed coat removal and GA_3 treatment 30ppm, 30 min	15.72(0.4077)^{ebdc}
T_{12}	Seed coat removal and GA_3 treatment 60ppm, 10 min	11.61(0.3478)^{ebdc}
T_{13}	Seed coat removal and GA_3 treatment 60ppm, 20 min	13.00(0.369)^{ebdc}
T_{14}	Seed coat removal and GA_3 treatment 60ppm, 30 min	50.15(0.7869)^{bac}
T_{15}	Seed coat removal and GA_3 treatment 90ppm, 10min	3.68(0.1932)^{ed}
T_{16}	Seed coat removal and GA_3 treatment 90ppm, 20 min	0^{e}
T_{17}	Seed coat removal and GA_3 treatment 90ppm, 30 min	6.69(0.2618)^{edc}

*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.

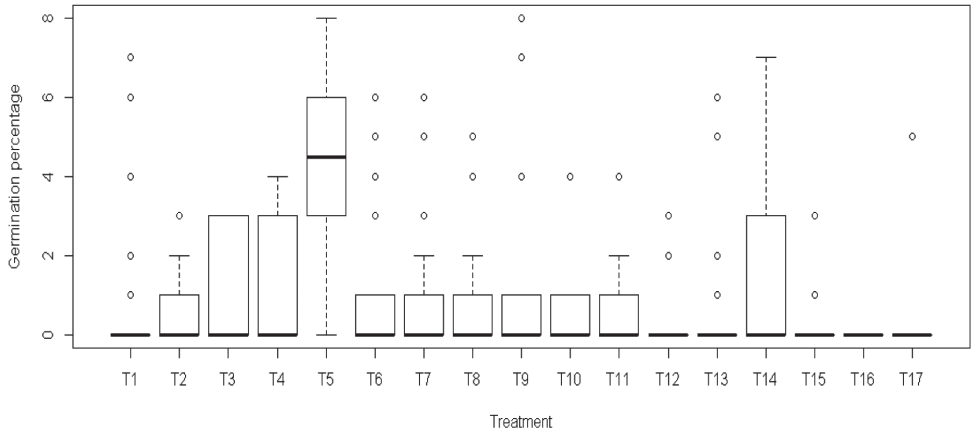

Fig.1. Effect of different treatments on germination of cassava seeds (1-10 days)

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_2 and T_3) which attained almost 50 % germination on 15th day and found to be on par with the control on 29th day (~65% - 70%). The germination percentage of mechanical scarification (T_1) was found to be 43.3 on day15 and was on par with the control on 29th day (Fig.3 and Fig.4). The germination percentage of GA_3 treatment 50 ppm (T_5) had increased to 66.67 on day 15 followed by 60 ppm GA_3 treatment for 30 min on cotyledons (T_{14}).

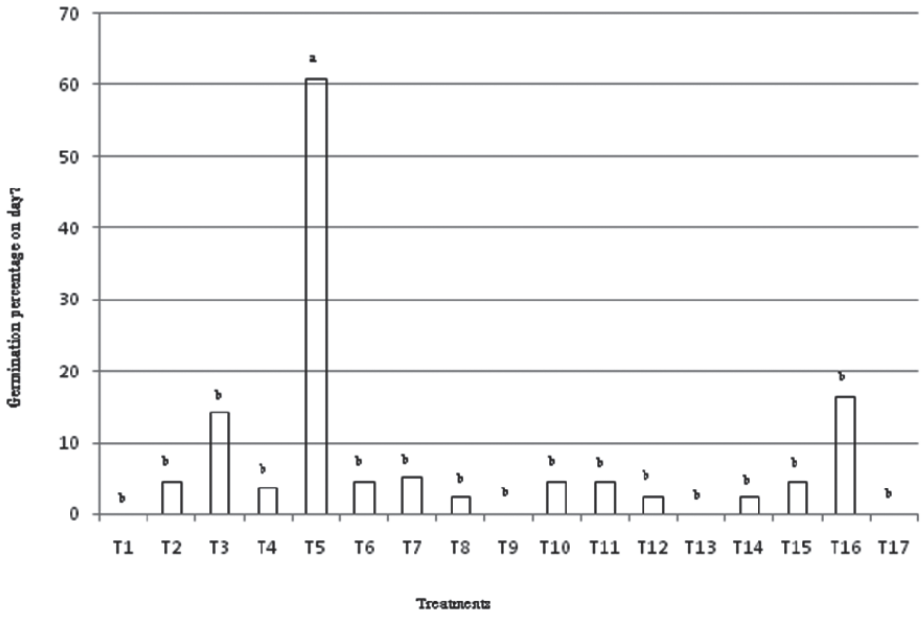

Fig.2. Germination percentage of pre-treated cassava seeds at 7 days of treatment

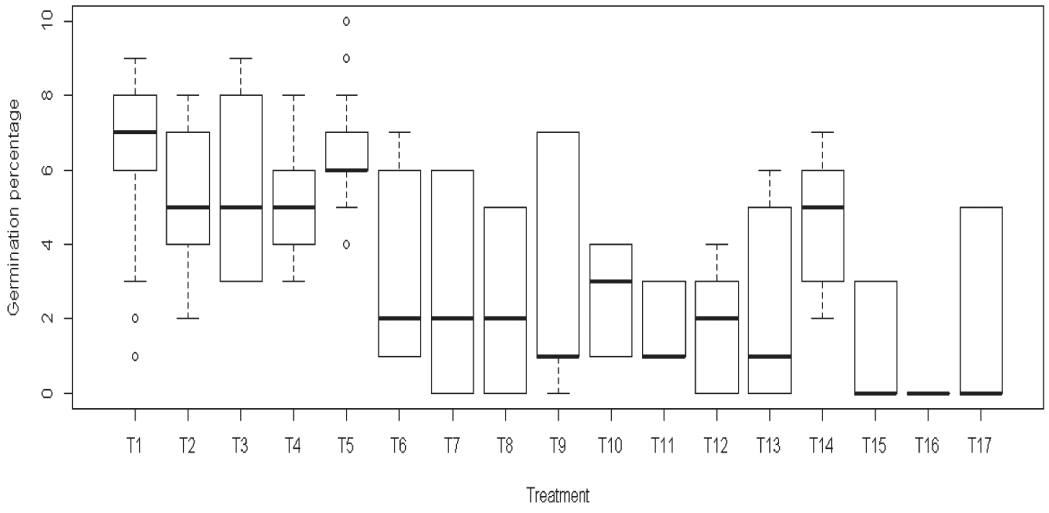

Fig.3. Effect of different treatments on germination of cassava seeds (11-29 days)

The effect of different treatments on three different time intervals (7^{th}, 15^{th}, 20^{th} day) was analyzed and was noted that 50 ppm GA3 (T_2) attained 60% germination on 7^{th} day attained nearly 70% germination on 15^{th} day and maintained the same on 20^{th} day, followed by T_{14} attained 50% germination on 15^{th} day. It was found that seed soaked in 24 hours water treatment (T_2), 48 hour water treatment (T_3) and overnight water treatment and seed coat removed (T_5) attained 43, 50 and 43% germination on 15^{th} 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 GA_3 (T_5) showed significantly higher germination percentage than other treatments and attained 60% germination on 5^{th} day itself.

The treatment 50 ppm GA_3 (T_5) was found to be the best which started germination on second day onwards and attained 60% germination on 5^{th} day itself and thus this

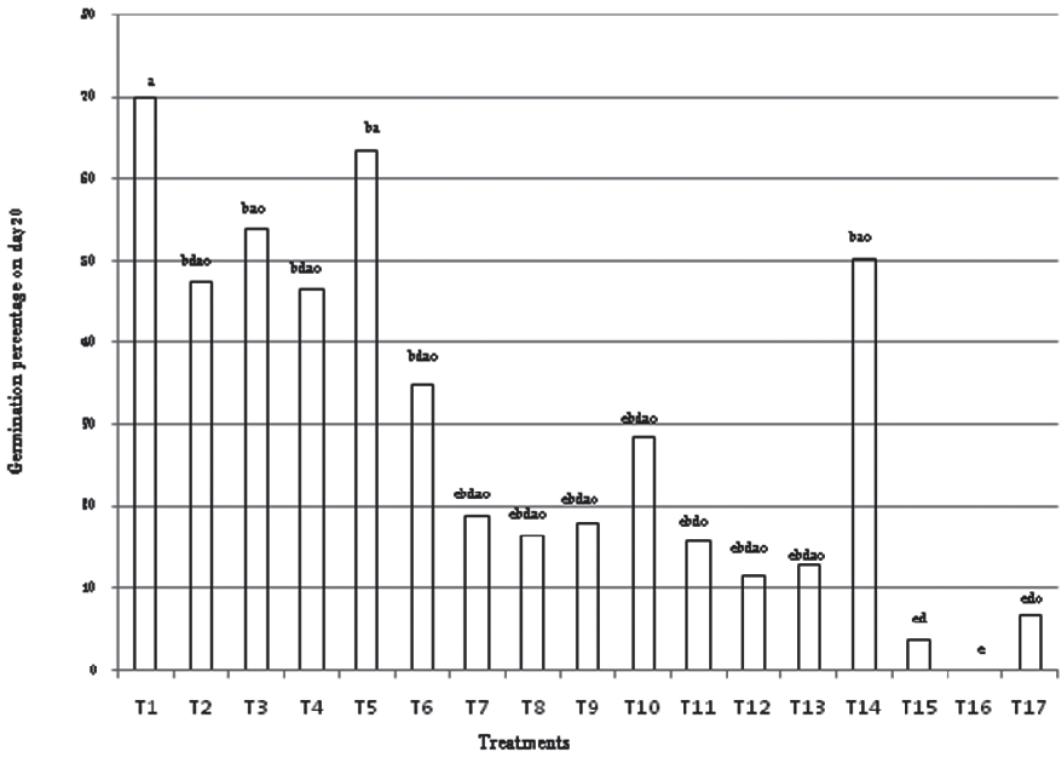

Fig.4. Germination percentages of different treatments on 20th day

treatment can be recommended for early germination. But considering cost effectiveness, seed soaked in water for 24 hour (T_2), and 48 hours (T_3) can be also used. Overnight seed soaked in water and seed coat removal (T_4) 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 GA_3 and other hormones that help in the expansion of embryo and consequently seedling emergence. Overnight soaking in water and seed coat removed (T_4) 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).

GA_3 is one of the most important plant hormone involved in the promotion and maintenance of seed germination. GA_3 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 gibberellic acid application (Geneve, 2003) and promotes seed germination (Baskin and Baskin, 1971). In the present study, the effect of GA_3 on germination was tested

with 13 different GA_3 treatments in different time intervals. Treating the seeds overnight in GA_3 50 ppm (T_5) 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_3 and obtained 60% germination on day 17 in 300 ppm GA_3 for 24 hours soaking. All other GA_3 treatments (T_6 to T_{17}) 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 GA_3 for 30 minutes (T_{14}) and gave 50% germination on 10th day, followed by T_6, T_7, T_8 (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 GA_3 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 GA_3 treatment had a significant effect on the germination of cassava seeds and it varies with the concentration of GA_3. The best concentration of GA_3 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 GA_3 and the higher germination (60%) response was between 5 to 15 days.

References

AL-Helal, A.A. 1989. Degradation of storage protein of *Acacia laeta L.* seeds. *Phyton*, **50**, 103-107.

Alves, M.C.S., Medeiros-Filho S., Andrade-Neto M., and Teófilo, E.M. 2000. Superação da dormência em sementes de *Bauhinia monandra* Britt. e *Bauhinia ungulata* L. - Caesalpinoideae. [Overcoming dormancy in seeds of Bauhinia monandra Britt. and Bauhinia L. ungulata – Caesalpinoideae]. *Brazilian Journal of Seeds,* **22:** 139-144.

Asrar, A.A. 2009. Effect of growth media on vegetative growth and flowering of *Rumex vesicarius* L. (Hommaidh).*Alexandria. J. Agric. Res.,* **54**: 73-80.

Bahrani, M.J., Gask, M.R., Shekafandeh A. and Taghvaei, M. 2008. Seed germination of wild caper (*Capparis spinosa* L. var. *parviflora*) as affected by dormancy breaking treatments and salinity levels. *Seed Sci. Technol.,* **36**: 776-780.

Baskin, J.M. and Baskin, C.C. 1971. Germination ecology and adaptation to habitat in Leavenworthia spp. (Cruciferae). *Am. Midl. Nat.,* **85**: 22-35.

Bewley, J.D. and Black 1994. Physiology of Development and Germination. *Seeds,* **9:** 445.

Black, M. 1972. Control Processes in Germination and Dormancy. In *Oxford Biology*, Head, J.J. and Lowenstein E.O. (Eds.) 3-16.

Booth, D.T. and Sowa, S. 2001. Respiration in dormant and non-dormant bitterbrush seeds. *J. Arid Environ.,* **48**: 35-39.

Carpita, N.C., Skaria, A., Barnett, J.P. and Dunlap, J.R. 1983. Cold stratification on growth of radicles of loblolly pine (*Pinus tardo*) embroyos. *Physiol. Plant.,* **59**: 601-606.

Deng, Z.J., Cheng, H.Y. and Song, S.Q. 2010. Effects of temperature, scarification, dry storage, stratification, phytohormone and light on dormancy-breaking and germination of *Cotinus coggygria* var. *cinerea* (Anacardiaceae) seeds. *Seed Sci. Technol.,* **38**: 572-584.

Elias, M. 2000., McKey, D., Panaud, O., Anstett, M.C. and Robert, T. 2001a. Traditional management of cassava morphological and genetic diversity by the Makushi Amerindians (Guyana, South America) perspectives for on-farm conservation of crop genetic resources. *Euphytica,* **20**: 143-157

Food and Agricultural Organization. 2015. Cassava production data, 2014.

Fukuda, W.M.G., Costa, I.R.S., Vilarinhos, A.D. and Oliveira, R.P. 1996. Banco de germoplasma de mandioca: manejo, conservacao e caracterizacao. [Cassava germplasm bank: management, conservation and characterization.]. *EMBRAPA-CNPMF Documents EMBRAPA-CNPMF*, **68**: 103.

Geneve, R.L. 2003. Impact of temperature on seed dormancy. *Hort. Sci.,* **38**: 336-341.

IITA. (1974, 1977, 1978, 1980). Annual reports of the International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria.

Itamara Mezzalira., Caroline Jacome Costa., Eduardo Alano Vieira., Josefino de Freitas Fialho., Marilia Santos Silva., Marcelo Luiz Denke. and Karina Nascimento da Silva. 2013. Pre-germination treatments and storage of cassava seeds and their correlation with emergence of seedlings. *J. Seed Sci.,* **35**: 113-118.

Jennings, D.L. and Iglesias, C. 2002. Breeding for crop improvement. *In Cassava: Biology, production, and utilization*, (eds R.J. Hillocks, J.M. Thresh, and A. Bellotti), 149–166.

Junttila, O. 1973. The mechanism of low temperature dormancy in mature seeds of Syringa species. *Physiol. Plant.,* **29**: 256-263.

Karam, N.S. and Al-Salem, M.M. 2001. Breaking dormancy in *Arbutus andrachne* L. seeds by stratification and gibberellic acid. *Seed Sci. Technol.,* **29**: 51-56.

Lozano, J.C. and Nolt, B.L. 1989. Pest And pathogens of cassava. In *Plant Protection and Quarantine: Selected Pests and Pathogens of Quarantine Significance*, **2**: 174-175.

McKey, DL., Emperaire., Elias, M., Pinton, F., Robert, T., Desmouliere, S. and Rival, L. 2001. Gestions locales et dynamiques regionales de la diversite varietale du manioc en Amazonie Genetique. [Local and regional managements dynamics of varietal diversity of cassava in the Amazon Genetics]. *Selection and Evolution*, **33:** 465-490.

Pallais, N.E., Nelly, Y., Espinola., Rosario, M., Falcon, M. and Garcia, R.S 1991. Improving seedling vigor in potatoes: II. Genotype, dormancy, and pre-sowing treatments. A*m. J. Potato Res.,* **67**: 109-119.

Pujol, B., Gigot, G., Laurent, G., Pinheiro-Kluppel, M., Elias, M., Hossaert-McKey, M. and McKey, D. 2002. Germination ecology of cassava (*Manihot esculenta*) in traditional ecosystems: Seed and seedling biology of a vegetatively propagated domestic plant. *Econ. Bot.,* **56:** 366–379.

R. (2013). R Version 3.02. The R foundation for Statistical Computing

Rajendran, P.G., Ravindran, C.S., Nair, S.G. and Nayar, T.V.R. 2000. True cassava seeds (TCS) for rapid spread of the crop in non-traditional areas. Technical Bull. Series 28. Central Tuber Crops Research Institute (Indian Council of Agricultural Research), Sreekariyam, Thiruvananthapuram, Kerala, India.

Roberts, E.H. and Ellis, R.H. 1989. Water and seed survival. *Ann. Bot.,* **63**: 39–52.

SAS. (2010). SAS Institute Inc., Cary, NC, USA.

Scheibe, J. and Lang, A. 1965. Lettuce seed germination: Evidence for a reversible light-induced increase in growth potential and for phytochrome mediation of the low temp effect. *J. Plant Physiol.,* **40**: 485-492.

Stenzel, N.M.C., Murata, I.M. and Neves, C.S.V.J. 2003. Superação da dormência em sementes de atemóia e fruta-do-conde. [Overcoming dormancy in seeds and fruit atemóia-the-count]. *Brazilian Journal of Fruit Crops*, **25**: 305-308.

Vertucci, C.W. and Farrant, J.M. 1995. Acquisition and loss of desiccation tolerance. *Seed development and germination*, Kigel J. and Galili G. (Eds.), Marcel Dekker Press, New York. 237-271 pp.

Zeinalabedini, M., Majourhat, K., Khayam-Nekoui, M., Hernández, J.A. and Martínez-Gómez, P. 2009. Breaking seed dormancy in long-term stored seeds from Iranian wild almond species. *Seed Sci. Technol.,* **37**: 267-275.

Zeng, Y.J., Wang, Y.R., Zhang, J. and Li, Z.G. 2010. Germination response to temperature and dormancy breaking treatments in *Nitraria trangutorum* Bobr. and *Nitraria sibirica* Pall. *Seed Sci. Technol.,* **38**: 537-550.