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Impact of Gibberellic Acid and Organic Growth Media on Seed Germination and Seedling Development of Rubber (*Hevea brasiliensis*)

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ABSTRACT

One of the best interventions to hasten seed germination and seedling development is the exogenous application of phytohormone. This study evaluated the germination and seedling development of rubber seeds in response to gibberellic acid (GA_3) and organic growth media under nursery condition. The experiment was arranged in a 4x3 factorial experiment in completely randomized design (CRD) with three replications. Growth media (including: top soil, TS; TS + rice hull, RH; TS + chicken dung, CD; TS + vermicast, VC) were considered as Factor A, and different concentrations of GA_3 (0, 50, and 100 ppm) were comprised as the Factor B. Newly fallen seeds (with no defects) from existing 'RRIM 600' rubber tree plantation were collected and used in the experiment. Seeds were subjected to float in GA_3 solutions based on corresponding treatments for 18 h prior to sowing in different organic growth media. GA_3 (100 ppm) led to the earliest emergence, highest germination percentage and normal seedlings with least mortality. The TS+VC was the best growth media influencing germination. The TS+VC media regardless of the concentration of GA_3 caused 100% normal seedlings. Likewise, the combination of TS+CD as growth media and 100 ppm of GA_3 also produced promising percentage of normal seedlings of rubber with 97.33%. Seedling mortality was lessen using the combination of TS+VC as growth media and 100 ppm GA_3. Generally, application of 100 ppm GA_3 and top soil mixed with vermicast as growth media provoked better seed germination and early seedling development in rubber.

Introduction

The choice to venture in rubber production is not only for its economic importance, but this is also aligned with the current reforestation, environmental conservation and water management programs of the Philippine government in addressing the local and global issues and concerns regarding climate change and agriculture production. Aside from

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generating employment in the rural areas, planting rubber in idle hilly lands and uplands enhances environmental rehabilitation, being a good plant species in the sequestration of carbon dioxide (Southern Mindanao Integrated Agricultural Research Center [SMIARC], 2007).

Rubber seedling production is a good business to venture nowadays in the Philippines because of its increasing annual plantations (Fig. 1). To help attain the annual demands, there is also a need to invest in research and development of such commodity. Rubber is generally propagated by seed or by vegetative or clonal reproduction, which results in the generation of true-to-type trees, early development and establishment of plantations of genetically superior genotype in terms of productivity, quality of latex, resistance to the pest and diseases and other interesting traits. Seeds are sown for rootstock purposes. However, seeds do not germinate at the same time. These are gathered and accumulated first to reach a certain target quantity before field transplanting. In many cases, seeds lose their viability since rubber seeds are recalcitrant. This is one of the problems that needs to be addressed to help rubber nursery operators in the country.

There are several ways to hasten seed germination. But the most convenient method is the treatment using phytohormone. Gibberellic acid (GA_3) treatment has the potential to enhance germination of seeds according to Hartman and Kester (2000). As seeds germinated, there is also a need to sow them in appropriate growth media or substrates.

At present, limited studies have been performed on the use of gibberellic acid treatment and substrate compositions or growth media on the germination of rubber. The lack of information and technologies on the propagation of rubber seedlings under local condition necessitates to research on this topic. So far, no study has been reported on the evaluation of germination of rubber in response to gibberellic acid treatment under different substrate compositions. Therefore, this study was conducted to 1) evaluate the seed germination of rubber in response to different concentrations of GA_3, 2) compare the seed germination of rubber in different substrate compositions, and 3) identify the optimum level of GA_3 and substrate compositions that will lead to optimum seed germination of rubber.

Materials and Methods

The experiment was conducted in a private nursery at Dologon, Maramag, Bukidnon, Philippines from October 2010 to August 2011 and was carried out in 4x3 factorial arrangement in Complete Randomized Design (CRD). Factor A consisted of four substrate compositions including: top soil (TS) as control, TS + vermicast (VC), TS + chicken dung (CD), TS + rice hull (RH). Three concentrations of GA_3 [0 (control), 50 and 100 ppm) were considered as Factor B. All treatment combinations were replicated three times.

The seeds of RRIM 600 clone of rubber which is the highly recommended clone in the country were used as plant materials in this study. It has high latex yield, and resistant to pests and diseases. Seeds were collected from 30 year-old rubber trees in Rubber Project of Resource Generation and Management Office (formerly known as University Income Generation Program) at Central Mindanao University, Musuan, Bukidnon, Philippines.

Soil from 20 cm below ground level was collected and used as the TS. Collected soil was sieved to remove boulders. The sieved soil was then mixed in each of the organic substrate (VC, CD, and RH) at 1:1 ratio based on volume. The choice of growth media was based on practice of various rubber nursery operators and their availability.

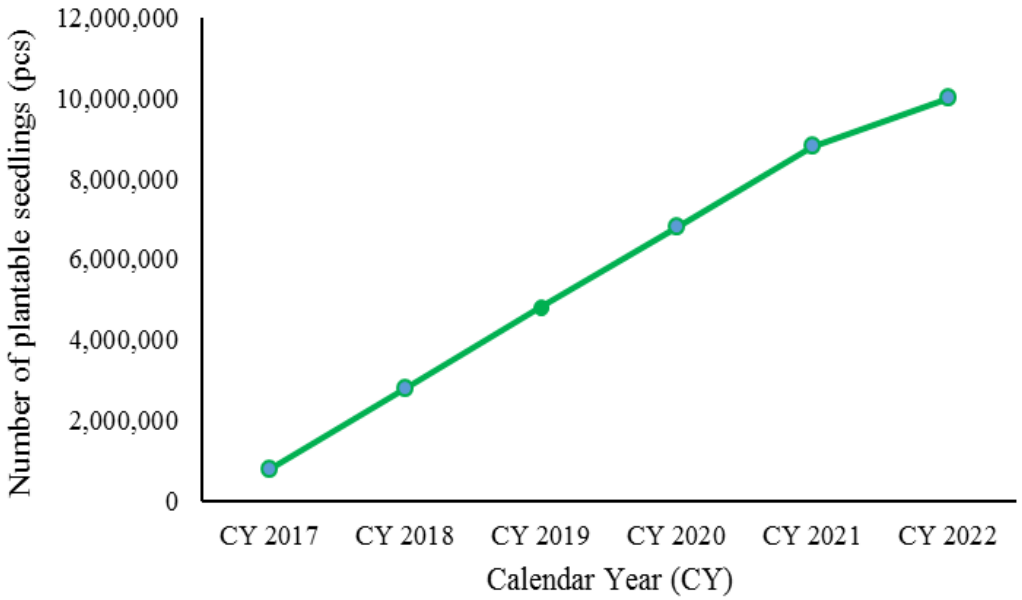

Fig. 1. Six-year requirement of budded rubber seedlings (Source: PHLRubber Technical Working Group, 2017) for new plantation establishment in the Philippines

GA_3 of 50 and 100 ppm concentrations were prepared from a 5000 ppm stock solution. The stock solution was diluted to obtain the desired concentrations, which served as the treatments. All seeds were standardized using only those seeds of similar size, shell color and well-formed shapes. Seeds were soaked in GA_3 for 18 h before sowing. It has been reported by Ayaz et al. (2019) that dipping bitter gourd seeds in gibberellic acid for 18 h improves its germination and seedling survival. After seed treatments, 30 seeds per plot in previously prepared beds were sown at 33.30 cm distance between rows and 15 cm between hills. Seeds were arranged in a single layer with their flat grooved side facing downward. Seeds were gently pressed into the bed until the top portion leveled from the surface of the growth media.

Watering was done regularly at equal amount to prevent the soil media from drying and compacting, and ease in removing the growing weeds. Manual weeding was done frequently to avoid competition for nutrients that could hinder the normal growth of rubber seedlings. Rubber seedlings were sprayed with Fungoran–OH and Thiodan twice a month up to the termination of the study preventing the possible attack of pest and diseases on newly germinated seedlings.

Data acquisition

Emergence Time (days): Seeds germinated were monitored and counted daily. The number of days from sowing to seedling emergence was determined when 50% of seeds have emerge in each experiment unit.

Seed Germination Percentage: Seeds germinated per experiment unit were counted and recorded at daily interval. To determine the seed germination percentage, the following equation was used:

$$\text{Seed Germination Percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of sown seeds}} \times 100$$

Normal Seedlings: Normal seedlings were described as erect, with complete leaves without defoliated parts or twisted roots. Number of normal seedlings was counted and computed as percentage using the following equation:

$$\text{Normal seedlings } (\%) = \frac{\text{Number of normal seedlings}}{\text{Number of sown seeds}} \times 100$$

Seedling Mortality: Mortality of seedlings was recorded at 30 days after sowing and was computed using the following equation:

$$\text{Normal seedlings } (\%) = \frac{\text{Number of seedling mortality}}{\text{Number of sown seeds}} \times 100$$

Results

Effects of GA_3 and organic growth media on emergence time of rubber

Significant interaction between growth media and GA_3 concentration was not shown in the days to seedling emergence of rubber data (Fig. 2). However, results showed that the number of days to emergence of rubber seedling was highly affected by either growth media (Fig. 3) or GA_3 concentration (Fig. 4).

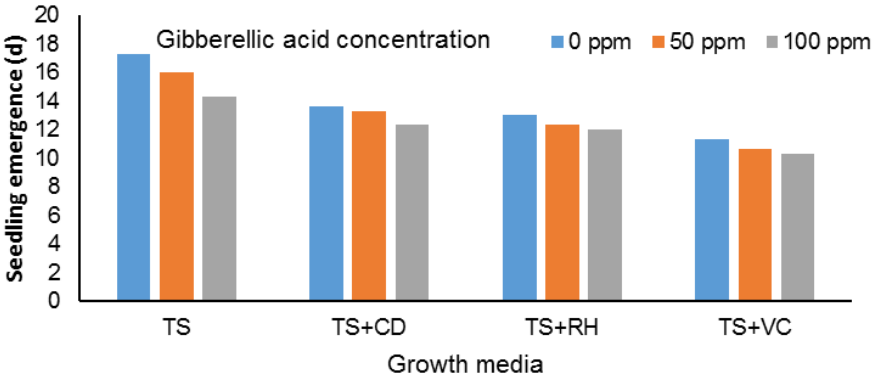

Fig. 2. Effects of gibberellic acid and organic growth media on the days to seedling emergence of rubber. Top soil (TS) as control, TS + chicken dung (CD), TS + rice hull (RH), TS + vermicast (VC). Treatment means are not significant at 5% level DMRT

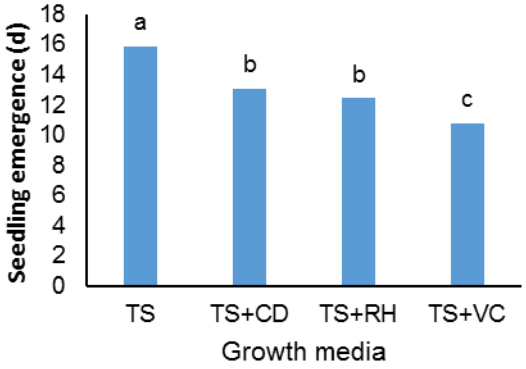

Fig. 3. Effect of growth media on the days to seedling emergence of rubber. Top soil (TS) as control, TS + chicken dung (CD), TS + rice hull (RH), TS + vermicast (VC). Treatment means are not significant at 5% level DMRT

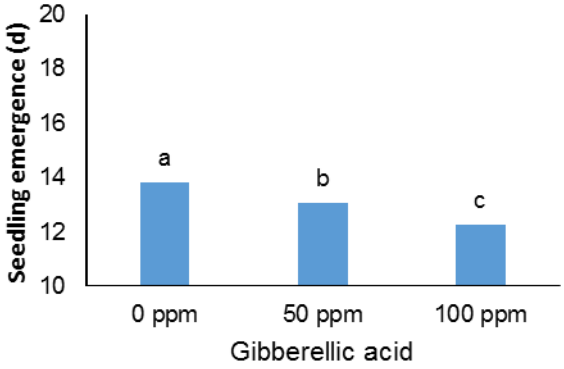

Fig. 4. Effect of gibberellic acid concentration on the days to seedling emergence of rubber. Treatment means are not significant at 5% level DMRT

Rubber seeds sown in TS in combination with VC emerged in the earliest time at an average of 10.78 days. Those seeds sown in TS mixed with either RH or CD had comparable seedling emergence of 12.44 and 13.11 days, respectively. However, seeds sown in TS alone (control) had significantly delayed emergence of 15.88 days, which significantly differed from all the other treatments.

Rubber seeds soaked in 100 ppm GA_3 were

emerge earlier (12.25 days) followed by those applied with 50 ppm (13.08 days), than the controls. In contrary, seeds not soaked in GA_3 had significantly delayed emergence of 13.83 days.

Effects of GA_3 and organic growth media on seed germination percentage of rubber

No significant interaction was found between growth media and GA_3 concentration for seed germination percentage of rubber (Fig. 5). However, significant effects of growth media (Fig. 6) and GA_3 concentration (Fig. 7) were found for seed germination percentage of rubber. Seeds sown in TS + VC media had the highest seed germination (84.44%), followed by those in TS in combination with either CD (75.56%) or RH (68.15%) but were statistically comparable. The control (TS) gave the lowest germination percentage of 54.69%.

Application of 100 ppm GA_3 concentration on seeds caused the highest germination of 78.33%, which differed from those treated with 50 ppm GA_3 concentration with 71.11%, and the untreated seeds which had the least germination percentage of 62.69%.

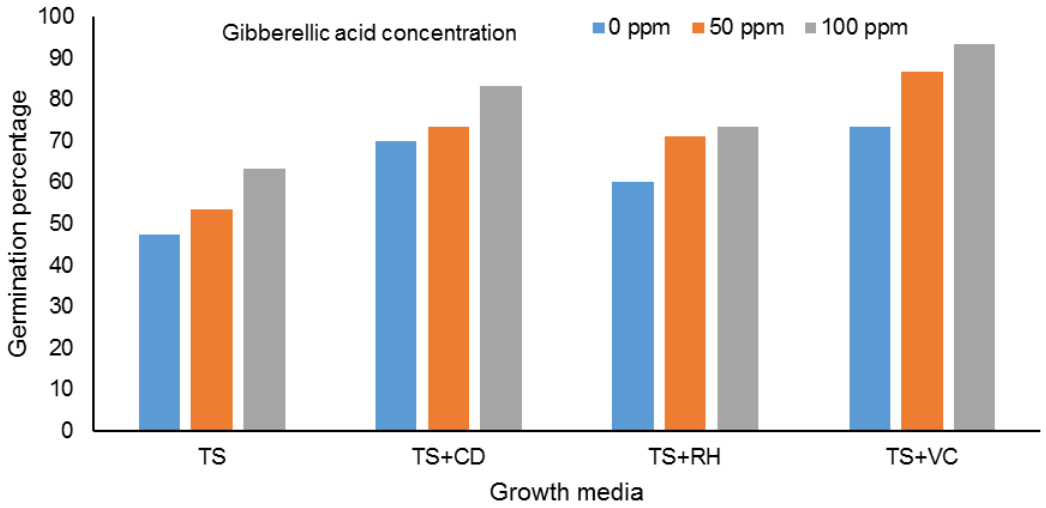

Fig. 5. Effects of gibberellic acid and organic growth media on rubber seed germination percentage. Top soil (TS) as control, TS + chicken dung (CD), TS + rice hull (RH), TS + vermicast (VC). Treatment means are not significant at 5% level DMRT

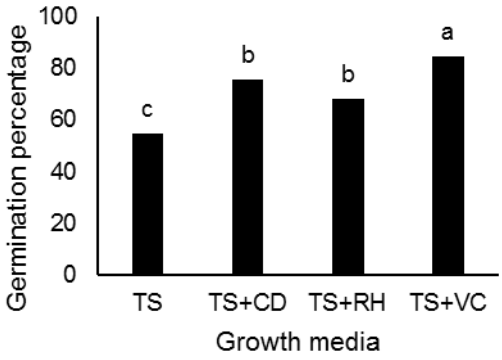

Fig. 6. Effect of growth media on rubber seed germination percentage. Top soil (TS) as control, TS + chicken dung (CD), TS + rice hull (RH), TS + vermicast (VC). Treatment means are not significant at 1% level DMRT

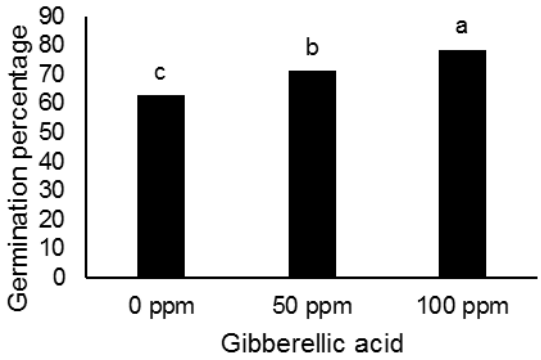

Fig. 7. Effect of gibberellic acid concentration on rubber seed germination percentage. Treatment means are not significant at 1% level DMRT

Effects of GA_3 and organic growth media on development of normal seedlings in rubber

The percentage of normal seedlings in response to growth media and GA_3 concentration was shown in Figure 8. The percentage of normal seedlings was highly influenced by the growth media and GA_3 concentration. Moreover, highly significant interaction effects were detected between growth media and GA_3 concentration. Among the growth media, the TS + VC induced the development of 100% normal seedlings, which was basically higher than other treatments. Plants grown in TS mixed with CD and RH ranked second and third with 91.69% and 89.17% normal seedlings, respectively. Seeds germinated in solely TS significantly showed the least percentage of 81.84% normal seedlings.

Application of high concentration of GA_3 highly affected the percentage of normal seedlings. Seeds treated with 100 ppm GA_3 concentration caused the highest percentage of normal seedlings (93.75%), followed by those treated with 50 ppm GA_3 (90.68%), while the control had the lowest percentage of normal seedlings (87.59%).

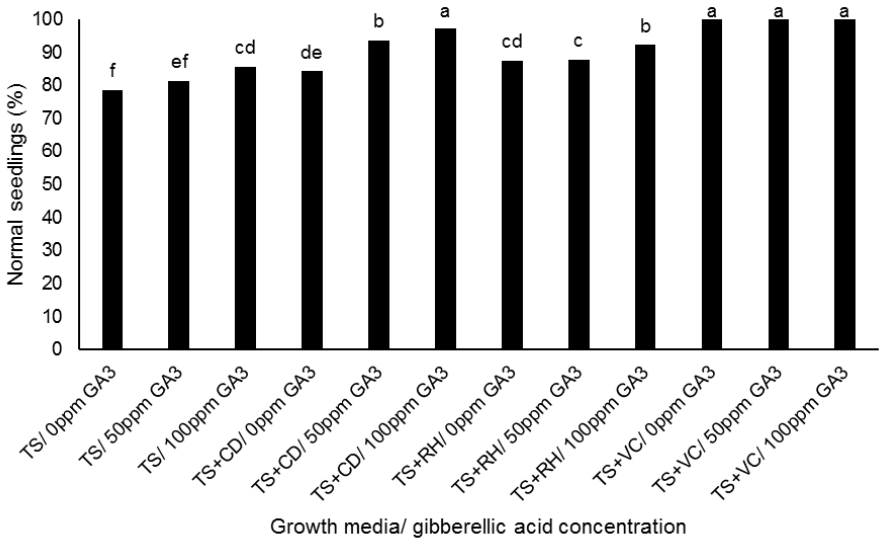

Fig. 8. Effects of gibberellic acid and organic growth media on percentage of normal seedling in rubber. Top soil (TS) as control, TS + chicken dung (CD), TS + rice hull (RH), TS + vermicast (VC). Treatment means are not significant at 1% level DMRT

Effects of GA_3 and organic growth media on the seedling mortality of rubber

The mortality of rubber seedlings sown in different growth media applied with various GA_3 concentration is presented in Figure 9. Results revealed that both the independent and interaction effects of the growth media and GA_3 concentration highly influenced this parameter. The least mortality was observed in seedlings grown in TS + VC with 14.81%, which differed significantly from the rest of the media treatments. The highest mortality of 45.31% was observed in seeds sown in TS alone.

The results showed that the higher the GA_3 concentration, the lower mortality percentage. Untreated (0 ppm GA_3 concentration) seeds showed the highest mortality percentage of 36.20%, which significantly differed from those seeds treated with 50 ppm and 100 ppm with lower mortality of only 29.44 and 21.67%, respectively.

Interaction of the two factors showed that seedlings sown in TS + VC media applied with 50 ppm and 100 ppm GA_3 concentrations exhibited the least and lower mortality rates of 13.33% and 6.67%, respectively.

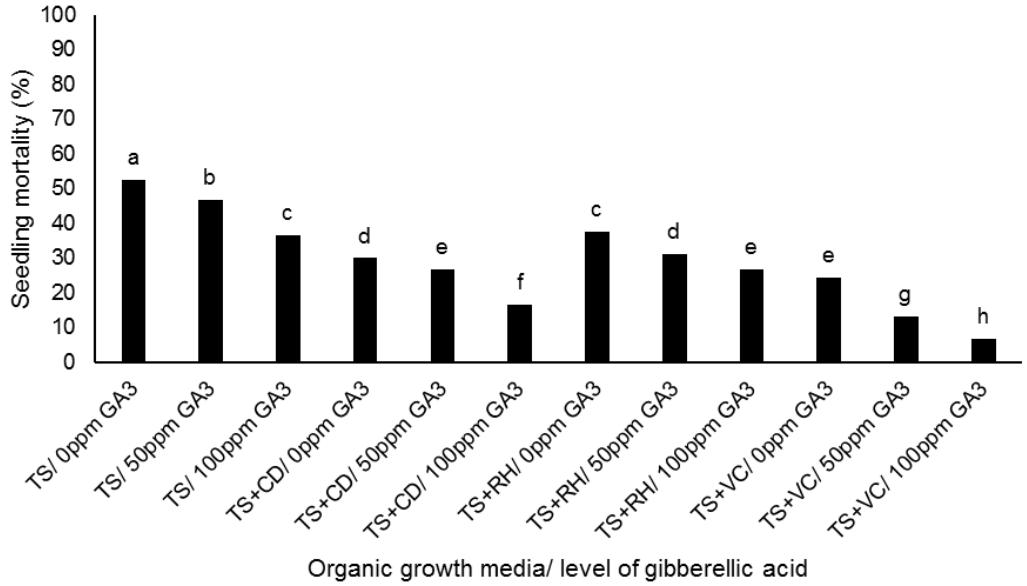

Fig. 9. Effects of gibberellic acid and organic growth media on percentage of seedling mortality in rubber. Top soil (TS) as control, TS + chicken dung (CD), TS + rice hull (RH), TS + vermicast (VC). Treatment means are not significant at 1% level DMRT

Discussion

Effects of gibberellic acid and organic growth media on emergence time of rubber

The mixture of TS and VC in equal proportions accelerated seedling emergence of rubber seeds. The use of VC as germinating and growth media has several advantages. This effect may be due to the high nitrate content which hastens seed germination. Hartman and Kester (2000) reported that the use of VC provides the seed with sufficient moisture, aeration, resistance from pest and disease-causing microorganisms. Cicero et al. (1986) conveyed that rubber seeds complete germination in 10 to 15 days after sowing and produce hypogeal seedlings, which are in agreements with the findings of the present study.

On the other hand, earliest seedling emergence (12.25 days) was exhibited by rubber seeds soaked in 100 ppm GA_3 followed by those applied with 50 ppm (13.08 days). Seeds not soaked in GA_3 had significantly delayed emergence of 13.83 days. The early emergence of seedling in this study could be explained by the mechanism of action of gibberellins through their effects on the synthesis of α-amylase and through the stimulation of the activity of these enzymes particularly in grains (Vieira et al., 2002; Amri et al., 2016). The rapid activity of enzymes on stored food of moist seeds in the endosperm brings about the conversion of the complex substances into simple forms and be assimilated in the embryonic axis to induce seed germination and seedling growth.

Effects of gibberellic acid and organic growth media on seed germination percentage of rubber

The effect of organic growth media on the seed germination of rubber is similar with the findings of Rahman et al. (2007), who determined the effects of different soil media on germination and seedling growth of peach. Different growth media used for seed germination of peach were farm yard manure (FYM), saw dust, canal silt, FYM + sawdust, FYM mixed with canal silt, and FYM + saw dust + silt. Data showed that sawdust in combination with canal silt caused maximum germination of peach seeds followed by silt and farm yard. The control treatment showed the lowest germination. These results

proved the superiority of organic media treatments for induction of germination. This superiority can be attributed to the fact that in mixed soil media, the structure improved, which in turn increases the metabolic activity leading to better germination of seedlings (Rahman et al., 2007).

Gibberellic acid has been used to break seed dormancy and to attain synchronized seed germination of several crops (Karam & Al-Salem, 2001; Parvin et al., 2015; Ferreira et al., 2016; Torres-G, 2018). In this study, rubber seed germination percentage was increased with increasing GA_3 concentration applied as seed treatment. Seeds applied with 100 ppm GA_3 concentration significantly had the highest germination of 78.33%, which differed from those treated with 50 ppm (71.11%), and the untreated seeds which had the least with 62.69% germination. Results supported the statement of Achard et al. (2008) that gibberellins are endogenous growth factors of plants that have been described to play a key role in germination and their biosynthesis is activated with external cues linked with survival conditions appropriate for the seed to germinate. Ayaz et al. (2019) reported also that GA_3 treatment improved the seed germination of bitter gourd, which is in accordance with the result of this study.

Effects of gibberellic acid and organic growth media on development of normal seedlings in rubber

Normal seedlings had optimum morphological structures, which enhanced photosynthesis, absorption of essential nutrients from the soil and developed into normal plants (Ehiagbonare et al., 2008) with great field transplantable value. In this study, significant interaction effects showed that seeds grown in TS + VC developed 100% normal seedling regardless of applied GA_3 concentration. Similarly, seeds sown in TS + CD resulted in 97.33% normal seedling which is also showed no significant difference from the latter.

It was also observed that high GA_3 concentration (100 ppm) resulted in higher percentage of normal seedlings regardless of growth media compared to the rest of the treatments. According to Owen (2001), suitable growth media which provides proper aeration, sufficient water and nutrient availability promotes excellent roots system development which consequently give luxurious growth of plants. This study indicates that 100 ppm GA_3 concentration produced more robust seedlings however, GA_3 treatment formed spindly and elongated leaves with lanky structures. Hence, seedlings applied with 50-100 ppm GA_3 concentrations have percentage of 90.68 to 100% normal seedlings. Gibberellic acid application has been found to have positive effects on the growth of beans (Jaques et al., 2019) and *Cyclamen* (Cornea-Cipcigan et al., 2020), which supports the result of this study on the development of normal seedlings.

Effects of gibberellic acid and organic growth media on the seedling mortality of rubber

The mixture of TS + VC reduced the mortality percentage of rubber seedlings. This result is probably due to the release of available organic nutrients as products of decomposition which consistently absorbed by the seedlings. The result is comparable with the findings of Espejon (2019) that VC application improved the survival of *Calophyllum blancoi*. Organic nutrients like CD, VC, and RH organic nutrients have potentials in hastening the growth of seedlings. The result also suggested that 50 and 100 ppm GA_3 concentrations could increase the survival percentage of rubber seedlings. This result are in line with the work of Ayaz et al. (2019) on the effects of gibberellic acid treatment on bitter gourd. In their study, application of 60 ppm of GA_3 for 18 h prior to sowing resulted in higher survival percentage of bitter gourd.

According to Ehiagbonare et al. (2008), abnormal seedlings are grossly deficient in the

normality of morphological structures leading to severely impaired physiological processes which could be due to environmental stresses like water stress, or incomplete development of embryo due to diseases and other factors. The seedlings were with retarded growth and eventually died, therefore no field transplantable value.

Conclusions

The application of 100 ppm GA_3 concentration on seed germination of rubber enhances the earliest germination, germination percentage and production of normal seedlings with the minimum mortality. TS mixed with VC was found to be the best growth media influencing better germination of 'RRIM 600' rubber.

The use of TS mixed with VC media regardless of GA_3 concentration significantly promotes 100% normal seedlings. Likewise, the combination of TS + CD as growth media and 100 ppm GA_3 concentration also produces promising normal seedling production of rubber with 97.33%. Further, seedling mortality is lessen with the combination of TS + VC as growth media and 100 ppm GA_3 concentration.

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Conflict of Interest

The authors indicate no conflict of interest for this work.

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