Effect of the Floating Seedling Technique on Banana Seedling Growth

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Abstract. Floating seedling technology was used to propagate banana seedlings. The effects of different substrates, such as wood bran, vermiculite, and Murashige and Skoog (MS) nutrient solution, at different concentrations on the survival rate of banana floating seedlings and the growth of seedling stem, leaf, and root systems were compared. The results showed that banana seedlings treated with MS nutrient solution at one-half or one-third concentration or hydroponically with controlled slow-release fertilizer (0.5–0.6 g/plant) directly added to the wood bran substrate grew the fastest and had the largest number of roots. At 50 days after transplanting, these banana seedlings reached the standard of first-grade packaged seedlings, with the number of expanded leaves reaching 6.6 to 7.6, the width of leaves reaching 6.5 cm to 7.3 cm, and the root system relatively developed. The comprehensive characteristics of the seedlings were all better than those of other treatments. The results of this study have certain reference significance for accelerating seedling growth in greenhouses and large-scale production of disease-free banana seedlings. The banana floating seedling system we developed did not need watering every day and may be simpler than other seedling raising methods.

Seedling propagation is an important aspect of the production process in the banana industry, which is one of the most important fruit industries in tropical and subtropical regions. Bananas are a major food crop in some countries in Africa and Central and South America (Köberl et al., 2017; Li and Fang, 2008; Li et al., 2019). This industry ranks second among the dominant fruit industries in south and southwest China, and has a key role in promoting rural economic growth and revitalization in south and southwest China (Yang et al., 2003). However, at present, the banana industry is seriously threatened by fusarium wilt, which recently has led to a gradual decline in cultivation in the traditional banana producing regions of China (Sun et al., 2020). Fusarium wilt is a vascular disease transmitted mainly through soil and is caused by *Fusarium oxysporum* f. sp. *cubense* (Dita et al., 2018; Pegg et al., 2019). The pathogenic strain that causes fusarium wilt in major banana-producing regions globally is *F. oxysporum* f. sp. *cubense* race 4 (Foc4). The incidence of yellowing and

wilting of banana plants caused by Foc4 infection is typically 10% to 40%, and the associated severe morbidity in perennial banana plantations is more than 90% (He et al., 2010). The occurrence of fusarium wilt and the damage caused by it are closely related to variety characteristics, plant robustness, cultivation management measures, and soil microbial diversity (Deng et al., 2015; Köberl et al., 2017; Zhou et al., 2019). One of the key factors is whether the seedlings are diseased or robust when transplanting to the field. Banana plants would be bound to fusarium wilt if the seedlings carry the Foc4. Field-planted banana seedlings are usually produced through the cultivation process involving first-generation and second-generation seedlings. First-generation seedlings are cultivated through tissue culture and rapid propagation under sterile conditions (Banerjee and Langhe, 1985; Cronauer and Krikorian, 1985; Kuang et al., 2016; Lin, 2005; Xia and Guo, 2009). Second-generation banana seedlings are usually grown in soil or substrates (Kuang et al., 2016). Soil or substrate culturing produces seedlings that are prone to infection by exposure to pathogens in the soil (Warman and Aitken, 2018). Therefore, to prevent seedling exposure, some researchers and seedling manufacturing companies have adopted a scaffolding structure to cultivate seedlings; this involves placing bagged seedlings in a substrate on an iron or wooden frame to prevent contact with ground soil. That can effectively prevent pathogens in the soil from infecting banana seedlings, thus producing sterile seedlings. However, because the moisture in the nutrient bags can evaporate easily when the bags are off the ground, additional sprinklers must be installed. Frequent watering is necessary to

prevent seedling death caused by drought. As a result, both soil-planted and trellis cultivation techniques are associated with the high costs of cultivating and managing seedlings, and seedling growth is relatively slow. These techniques are not conducive to cultivating strong and healthy banana seedlings.

Floating seedling technology was first studied and applied for tobacco seedling propagation in the United States in the 1990s (Anderson et al., 1997). In the mid-1990s, it was introduced into Chinese tobacco production for experimental research (Peng et al., 2010). At present, this technology has been widely studied and utilized for cultivating tobacco and vegetable crops because of its simple management and easy prevention and control of diseases and pests (Deng et al., 2015; Zhang et al., 2018; Zhao et al., 2016). For tobacco production in China, floating seedlings are used in ≈85% of the plantation area (Peng et al., 2010). However, no related research and applications in banana production have been reported. To simplify and innovate the methods used for the cultivation of banana seedlings, and to produce robust and disease-free second-generation seedlings, we studied the banana floating seedling technique to provide the technical support for its popularization and application.

Materials and Methods

Test materials. ‘Williams B6’ bagged seedlings (purchased from Guangxi Plants Tissue Culture Seedling Co. Ltd.) were used as test materials.

Test methods. The experiment was conducted from July to Oct. 2020, in the greenhouse at the agricultural base of the College of Agriculture at Guangxi University, Nanning, China. Three materials, namely vermiculite, wood bran, and wood bran with controlled-release fertilizer, were used as nursery substrates and placed in foam trays. Floating seedlings were raised using 84-cell foam trays with a cell size of 4.3 cm × 4.3 cm × 6.5 cm (length × width × depth). The nutrient solutions used for seedling growth were fresh water (tap water used as a control), and MS nutrient solutions were applied at concentrations of the full amount, one-half the amount, and one-third the amount, respectively. With the other treatment, seedlings supported by wood bran with 0.5 to 0.6 g controlled-release fertilizer (in each cell) were grown in fresh water. Nine treatments were included in this experiment, and 20 seedlings were transplanted for each treatment. The experiment was repeated four times. Specific details and tags corresponding to the treatments are shown in Table 1.

In a glass greenhouse, seedling pools were dug with dimensions of 1.2 m × 1.2 m × 0.25 m (length × width × depth). The pools were shaped as an inverted trapezoid, with a wide top and a small bottom. The interiors of these pools were covered with an impermeable membrane; then, 50 L of each nutrient solution in different treatments was added to different pools. Substrates specific to each

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Table 1. Different treatments and their markers.

Substrate + nutrient solution	Treatment marker
Wood bran + water	MCK
Wood bran + 1 × MS	M1MS
Wood bran + 1/2 × MS	M1/2MS
Wood bran + 1/3 × MS	M1/3MS
Vermiculite + water	ZCK
Vermiculite + 1 × MS	Z1MS
Vermiculite + 1/2 × MS	Z1/2MS
Vermiculite + 1/3 × MS	Z1/3MS
Wood bran with controlled-release fertilizer	MKCK

1 × MS = the full MS concentration; 1/2 × MS = one-half the MS concentration; 1/3 × MS = one-third the MS concentration; MS = Murashige and Skoog.

treatment were added to the foam tray. The banana seedlings were transplanted to the substrates in the foam tray. Then, this tray was placed in the pool to cultivate floating seedlings (Fig. 1). Seedling growth and pool water evaporation were observed. Tap water was added to the pool once per week so that the water content of the pool was maintained as that of the initial water content to maintain the concentration of nutrient solutions. When seedlings were infested with *Prodenia litura*, the insecticide cypermethrin was sprayed with the 0.025% a.i. concentration. In the process of growing seedlings, 1/2 × MS nutrient solution was sprayed once per week when seedling leaves showed nitrogen deficiency.

Determination of growth indications. To determine the survival rate after transplantation, the number of seedlings surviving each treatment was investigated during the first and second weeks after transplantation, and the survival rate was calculated. The growth indicators included the height of banana seedlings, the length and width of the second unfolded leaf on top, and the number of green leaves on the plant. These were measured once per week starting from the third week after transplantation and on July 25, Aug. 2, Aug. 9, and Aug. 25. Seedling height was measured from the surface of the substrate to the tip of the uppermost leaf. The length of the second unfolded leaf was measured from the base of the leaf to its tip, and the width of the leaf was the width of the widest part in the

Fig. 1. Banana floating seedling system. Wood bran and vermiculite were placed separately into the holes of foam board and banana plantlets were transplanted onto the substrate. The foam board with banana plantlets was then placed in a pool of nutrient solution for seedling cultivation.

middle portion of the leaf. Indicators of banana seedling growth, namely, plant height, girth of the basal pseudostem, length and width of the leaf, and number of green leaves, were determined at the time of transplanting to the field. The fresh and dry weights of seedlings and the number, volume, and fresh weight of roots were measured. After 60 d of cultivation, the seedlings were transplanted to the field, and their survival and growth were observed and recorded. The growth indicators were determined after 1 week of survival.

Statistical analyses. Excel 2010 was used to analyze the data. Statistical software SPSS 10.0 was used to conduct an analysis of variance of the data. Duncan's multiple range test was used to perform multiple comparisons and assess the significance of differences.

Results and Discussion

Effects of different substrates and nutrient concentrations on the survival of transplanted banana seedlings. Banana tissue culture plantlets were transplanted onto foam trays with different substrates and then placed into MS nutrient solutions of different concentrations for seedling cultivation. The survival of the seedlings was affected by the composition and characteristics of various substrates, and the concentrations of MS nutrient solution differed considerably. As shown in Fig. 2, the survival rate 1 week after transplantation was the highest for seedlings treated with MCK and M1/3MS (survival rate, 100%), followed by those treated with ZCK and Z1/2MS (survival rate, 98.2%). Those treated with MKCK had a survival rate of 97.6%. Two weeks after transplantation, the survival rates of seedlings treated with MCK, M1/3MS, ZCK, and MKCK reached more than 95%. The survival rates of seedlings subjected to different treatments decreased to varying degrees 1 week after transplanting. Survival decreased the most for seedlings that received the Z1MS treatment, with a reduction of 62.5% (from 89.3% to 26.8%). This was followed by a decrease of 14.3 to 19.6% for

seedlings that received Z1/2MS and Z1/3MS treatments with vermiculite as a substrate. The highest decline in the survival rate of seedlings using wood bran as a substrate was noted after M1MS treatment (from 94.6% to 82.1%, indicating a decline of 12.5%). The decline in the survival rate after all other treatments was 1.8 to 3.6%. The growth and increment of the stems and leaves of banana seedlings cultivated using wood bran were all greater than those of seedlings cultivated using vermiculite with the same concentration of MS nutrient solution. Therefore, the survival rate of banana seedlings cultivated using wood bran as the substrate was higher than that of seedlings cultivated in the vermiculite substrate. When using wood bran or vermiculite, the survival rates of seedlings treated with 1/2 and 1/3MS nutrient solutions were higher than those treated with 1MS nutrient solution. Additionally, banana seedlings treated with vermiculite as a substrate and 1MS within 1 to 2 weeks after transplantation had rotten roots and dead seedlings.

The results indicated that wood bran is more conducive to the survival and growth of seedlings. The reason was presumed to be the softer texture and lower mineral element content of wood bran compared with the relatively compact texture and higher mineral content of vermiculite. Banana seedlings do not tolerate the high osmotic pressure in hydroponics because of their relatively tender tissues and weak osmotic stress resistance. Some studies have shown that vermiculite is composed of layered silicates, aluminum, and magnesium and is rich in exchangeable cations, such as magnesium, calcium, and potassium (Tian and Zhen-Hong, 2019). The release of these mineral cations from vermiculite into the nutrient solutions can increase the concentration of mineral ions, resulting in the osmotic pressure of the solution up, and affect the water potential balance of the cells of banana seedling roots, thereby inhibiting the growth and absorptive properties of the roots. In addition, because most of the tested seedlings died and the number was

Fig. 2. Comparison of survival rates of banana seedlings transplanted with different treatments at 1 and 2 weeks after transplantation.

insufficient, Z1MS treatment was removed from subsequent studies.

Effects of different substrates and concentrations of nutrient solution on the growth of banana seedlings. The growth rate and robustness of banana seedlings after transplantation were closely related to the height of seedlings, number of green leaves, and size of unfolded leaves. In general, seedlings with normal growth were taller and had more green leaves, with the leaf size gradually increasing from the bottom to the top. As shown in Fig. 3A, the height of seedlings with different treatments increased gradually with transplantation time. Fifty days after transplanting, seedlings that received M1/2MS, M1/3MS, and MKCK treatments reached a height more than 30 cm. The tallest seedlings were reported with the M1/2MS treatment, with a height of 39.31 cm, followed by those with the M1/3MS

treatment (height, 34.06 cm) and those with the MKCK treatment (height, 31.65 cm). M1MS, Z1/2MS, and Z1/3MS treatments resulted in seedling heights of 20 to 30 cm. The MCK and ZCK treatments resulted in the slowest growth and the shortest seedlings (9.96 and 10 cm, respectively). Except for the MCK and ZCK treatments, the difference in seedling height among the other treatments was significant ($P = 0.05$) or extremely significant ($P = 0.01$). Therefore, the most favorable conditions for seedling growth included wood bran as a substrate with MS nutrient solution at concentrations of 1/2MS and 1/3MS, followed by the use of wood bran with controlled-release fertilizer. Seedlings subjected to these three treatments showed the fastest growth, and the resulting seedling height was significantly higher than that of the others.

The number of green leaves and leaf size of banana seedlings are important factors affecting photosynthesis. With more green leaves and larger leaf areas, the photosynthetic performance of seedlings is high, implying rapid growth. Throughout the process of seedling cultivation, the number of green leaves of seedlings under all treatments, except for the MCK treatment, increased with the seedling cultivation time. Fifty days after transplanting, the number of green leaves of seedlings subjected to M1/2MS, M1/3MS, and MKCK treatments was more than six. Among these treatments, MKCK resulted in the highest number of green leaves (7.7 leaves), and this resulting number of green leaves was significantly higher than that of other treatments. Seedlings with six to seven green leaves were noted with M1MS and Z1/3MS, and the number of green leaves was not significantly

Fig. 3. Comparison of growth characteristics of banana seedlings with different treatments at different times after transplantation. The plant height (A), leaf number (B), and area of the second expanding leaf (C) of banana seedlings with different treatments at different times after transplantation are shown. The uppercase and lowercase letters on the bars are significantly different at $P \leq 0.01$ (uppercase) and $P \leq 0.05$ (lowercase). The probability levels among different treatments and the numbers 1 to 4 indicate the measured time at 20, 27, 34, and 50 d after the plantlets were transplanted.

different between the two treatments. Seedlings with less than five green leaves were noted with MCK, ZCK, and Z1/2MS treatments. Among treatments, MCK resulted in the lowest number of green leaves, and this resulting number was significantly lower than that resulting from other treatments (Fig. 3B).

Because of the inconsistent timing of leaf emergence, the most newly unfolded leaves of banana seedlings are usually not similarly mature and exhibit different photosynthetic performances. In contrast, the second unfolded leaf is fully mature and better exposed to light with higher photosynthetic performance. Therefore, the second unfolded leaf was used for investigation during this study. During seedling cultivation, the area of the top second unfolded leaf of seedlings with different treatments increased gradually as the leaf position rose. Fifty days after transplanting, the area of the second unfolded leaf was highest among seedlings subjected to the M1/2MS treatment (103.578 cm²), and it was significantly higher than that of other treatments. These results were followed by those resulting from the M1/3MS treatment (87.576 cm²) and the MKCK treatment (82.97 cm²); these two areas did not differ significantly. The second unfolded leaves of seedlings subjected to other treatments were relatively small, with areas smaller than 45 cm². There were no significant differences between the leaf areas of seedlings subjected to the M1MS, Z1/2MS, and Z1/3MS treatments. However, the leaf areas of seedlings subjected to MCK and ZCK treatments were the smallest (10.208 and 10.986 cm², respectively) (Fig. 3C).

The results showed that the growth of banana floating seedlings was significantly affected by the concentration of nutrient solution and substrate. The root growth and respiration of banana seedlings were affected by the ventilation performance of the substrate, and the growth of banana seedlings was affected by the growth of roots (Tian et al., 2020). When the humidity was high, vermiculite was relatively compact, and the ventilation was worse than that of wood bran, thus inhibiting the root development of banana seedlings. Therefore, the growth of banana seedlings in the vermiculite substrate was worse than that of wood bran. MS is a nutrient solution with a high inorganic salt concentration and high contents of ammonium nitrate and potassium nitrate. Banana seedlings would suffer from osmotic stress and ammonium salt toxicity when the concentration of the culture solution was 1 times MS because the concentration of inorganic salt was too high, especially the ammonium ion. When the substrate was vermiculite with 1 times MS, the osmotic pressure of the culture solution would be increased by the ions of magnesium, calcium, and potassium released from vermiculite. Therefore, the growth of roots, stems, and leaves of banana seedlings was limited, and the root growth and nutrient absorption of banana seedlings were inhibited because of high osmotic pressure. In general, the growth of banana seedlings in 1 times of MS and the vermiculite substrate was worse than that in 1/2 and 1/3 times of MS and

wood bran substrate during the whole seedling growth process.

Comparative analysis of the growth of stems and leaves when banana seedlings grow to the planting standard. There were significant or very significant differences in the growth of stems and leaves of banana seedlings subjected to different treatments because of varying substrate structures and nutrient concentrations. As seen in Fig. 4, after 60 d of provisional planting, banana plantlets grew to the planting standard for the field and M1/2MS treatment resulted in the most rapid growth of stems and leaves of banana seedlings, as determined by indicators, including plant height (20.26 cm), girth of the pseudostem (4.99 cm), leaf area of newly unfolded leaves (236.56 cm²), and fresh and dry weights of seedlings (30.38 and 1.5 g, respectively), which were all significantly higher than those resulting from other treatments. The rates of seedling growth with other treatments were as follows: M1/3MS > MKCK > Z1/3MS > Z1/2MS > M1MS. The growth of stems and leaves was the slowest for banana seedlings treated with MCK and ZCK, with plant heights and stem girths of only 3.517 to 3.853 cm and 1.633 to 1.76 cm, respectively. Indicators of seedlings of these two treatments, including the number of green leaves, leaf area, and fresh and dry weights of seedlings, were significantly lower than those of other treatments. The results indicated that different substrates and concentrations of MS nutrient solution had a significant effect on the growth of stems and leaves of banana seedlings aboveground. Using the floating seedling technique and with wood bran as substrate for the cultivation of banana seedlings, the most favorable treatments for seedling growth involved growing seedlings in 1/2MS or 1/3MS nutrient solution and in tap water containing an appropriate amount of controlled-release fertilizer. The growth indicators in stems and leaves of banana seedlings in these treatments were all better than those resulting from other treatments.

There was a correlation between the growth of banana seedling parts aboveground and underground. The more leaves and the larger the leaf area of the plant, the more

organic matter that was produced during photosynthesis, which promoted the growth of roots and increased their number and absorption area. Moreover, the higher the number and absorptive capacity of the roots, the more nutrients they provided for the growth of stems and leaves above ground, thereby increasing the growth rate of banana seedlings. Therefore, the seedlings were stronger and more robust.

Comparative analysis of banana root growth when seedlings were grown to the planting standard. The root system is the organ that absorbs the water and mineral nutrients of crops. The growth rate of crop stems and leaves is affected by growth conditions and the absorption capacity of the root system. The growth of banana seedling roots varied considerably with different treatments using the floating seedlings technique. Notably, banana seedlings treated with MKCK had the best root development, highest number of roots (10.6) in a single plant, longest root length (51.86 cm), largest root volume (14.82 cm³/plant), and the highest fresh and dry weights (10.29 and 0.566 g/plant, respectively), with all root variables being significantly higher than those of other treatments. For treatments with wood bran as the substrate and different concentrations of the MS nutrient solution, the indicators, including the longest root on a single plant, root volume, and fresh and dry root weights, increased with the decreasing concentration of the MS nutrient solution, and the number of roots was the highest on seedlings treated with m1/2MS. At different concentrations of the MS nutrient solution, the root indicators of seedlings treated with M1MS, M1/2MS, and M1/3MS were significantly higher than those of seedlings treated with the control MCK; the root indicators of M1/2MS and M1/3MS were significantly higher than those of M1MS. The differences in the root indicators were not significant between M1/2MS and M1/3MS treatments, except for a very significant difference in root length. Among the treatments with vermiculite as the substrate and different MS concentrations, the treatment that yielded the highest values of all root indicators, except root length, was Z1/2MS, followed by Z1/

Fig. 4. Comparison of growth traits of banana seedlings with different treatments grown to the planting standard for the field at 60 d after transplantation.

3MS. The number of roots on a single plant was significantly or very significantly higher for seedlings treated with Z1/2MS than for those treated with Z1/3MS or the control treatment ZCK. However, the remaining root indicators did not differ significantly (Fig. 5). The results of the study showed that different seedling substrates and MS nutrient solution concentrations had an important effect on the growth and development of banana seedling roots. Therefore, the most suitable condition for root growth and development of banana floating seedlings involved cultivating seedlings in wood bran substrate containing the right amount of controlled-release fertilizer in clear water. In addition, banana seedlings supported by the wood bran substrate were cultivated in 1/2 and 1/3 MS nutrient solutions, and the root indicators of these banana seedlings were also significantly better than those of seedlings cultivated with vermiculite as the substrate.

The results showed that the roots of banana seedlings in the floating culture system extended and grew normally under immersion conditions, but the number, length, volume, and fresh weight of roots were greatly affected by the nutrient concentration in the culture solution. When the growth environment was rich in nutrients, the amount of banana seedling root growth was relatively high, such as in MKCK, 1/2MS, and 1/3MS treatments. When the nutrient content was too high or too low, such as in M1MS, MCK, and ZCK treatments, the root growth of banana seedlings was inhibited, resulting in the decreased number, length, and volume of roots. When the nutrient content in the culture solution was too high, the osmotic pressure was too high and the root system of banana seedlings could not absorb water normally. Moreover, when the ammonium nitrogen content in the solution was too high, the toxic effect of ammonium on the root system was induced; therefore, the whole growth of the seedlings was inhibited, and the root number, root length, and root-to-shoot ratio were decreased. This may be similar to ammonium poisoning in the soil (Li et al., 2010; Yi et al., 2020) because the absorption of NH_4^{++} by plants is accompanied by the release of H^+ (Schubert and Yan, 1997), leading to plant rhizosphere acidification, root cell expansion (Cosgrove, 1999) and water conduction were

Fig. 6. Survival rate of banana seedlings with different treatments transplanted to the field.

inhibited (Kamaluddin and Zwiazek, 2004). Because NH_4^{++} and K^+ have the same charge, their ionic radius, hydration energy, and transmembrane conduction mode are very similar (Wang et al., 1996). NH_4^{++} could enter the cytoplasm from potassium ion channels (Bittsánszky et al., 2015). Therefore, the uptake of potassium in plants is usually inhibited by ammonium stress (Ten et al., 2010). Furthermore, the absorption of calcium, magnesium, manganese, and other cations by plants was reduced (Liu et al., 2014), resulting in toxic effects of ammonium stress. However, when the concentration of MS is too low, it can cause poor growth of roots, stems, and leaves because of insufficient nutrient supply, thus prolonging the time to reach the transplanting standards. Therefore, an appropriate substrate and concentration of nutrient solution must be selected with the floating seedling process to produce robust banana seedlings within a short time.

Comparative analysis of the survival rate of banana seedlings under different treatments after field transplantation. The number of roots and viability of crop seedlings were important factors affecting the restoration of growth after transplantation. Banana seedlings under different treatments had varying numbers of roots and growth rates, which affected their survival after transplantation to differing degrees. As shown in Fig. 6, banana seedlings that achieved a 100% survival rate at 1 week after transplantation were those that received one of the following five treatments: MCK, M1MS, M1/2MS, M1/3MS, or MKCK. The survival rate of those receiving the Z1/3MS treatment was 88.9%, whereas the survival

rate of seedlings receiving ZCK or Z1/2MS was only 66.7%. These results showed that the survival rate of transplanted banana seedlings was significantly associated with the seedling substrate and growth. The survival rate of banana seedlings with vigorous stem and leaf growth and a high number and volume of roots reached 100%, and seedlings cultivated using wood bran after transplantation had a higher survival rate than those cultivated using vermiculite as a substrate.

Whether the substrate of seedlings is wood chaff or vermiculite, which can be easily scattered during transplanting to the field, the root system of banana seedlings may be exposed out. However, the survival rate of banana seedlings will not be affected if transplantation is performed at an appropriate time, such as on a cloudy day or in the morning and evening when the solar radiation is low and which will cause less damage to banana seedlings. Before transplanting, yellow mud can be used to wrap the roots to avoid root water loss and root drying, thus improving the survival rate of transplants. If the transplanting technique is performed well, then all banana seedlings can survive.

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