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Minituber production potential of selected Potato (*Solanum tuberosum* L.) genotypes in different propagation media

Sibongile C. Zimba*, Joyce P. Njoloma, Jacinta A. Nyaika, Weston F. Mwase, Moses F. Maliro, James M. Bokosi and Moses B. Kwapata

Lilongwe University of Agriculture and Natural resources, Bunda College, P.O. Box 219, Lilongwe, Malawi.

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Potato farmers in Malawi and other developing countries lack healthy and quality potato seed. This is mainly due to limited seed multiplication programmes to provide farmers with clean potato seed. A study to evaluate minituber production potential for selected genotypes in different media types would assist in planning for future selection of good high yielding varieties. It will also assist in planning for rapid seed multiplication programme of pathogen free planting materials to increase production of potato in the country. Three different propagation media (vermiculite, sand and sawdust) and seven different genotypes (two local genotypes- *Magalabada* and *Rosita,* five introduced genotypes-Up to date, Buffelspoort, Van der plank, Lady Rosetta and Bp 1 2007) were investigated. Plantlets grown on vermiculite performed better with higher mini tuber yield of 1740 g/m^2 for all genotypes while 850 and 292 g/m^2 in sand and sawdust, respectively. Among the genotypes 'Up to date' and Lady Rosetta produced more tubers of between 12 tubers/plant and 10 tubers/plant. Thus, vermiculite increases the number and size of Minitubers which is a very important step for rapid multiplication of potato seed. The study findings provide valuable information for potential genotypes and propagation media to assist in scaling up seed multiplication programmes for pathogen free planting materials.

Key words: Propagation media, genotype, potato, minituber.

INTRODUCTION

Potato (*Solanum tuberosum* L.) plays nutritional, economic and industrial roles in Malawi and it can supplement the food needs in the country in a substantial way (FAO, 2008). Despite potato being an important food and cash crop in Malawi, the actual yield and quality is low. The present average yield ranges from 7 to 10 tonnes/ha, but yields up to 40 tonnes/ha is achievable (MoAIFS, 2007; FAO, 2008). A major limiting factor to realizing high yields and good quality of potatoes in Malawi is inadequate availability of quality seed. This is due to limited multiplication programme to provide farmers with clean potato seed of high yielding varieties

*Corresponding author. E-mail: sibozim@yahoo.co.uk, sibozimba87@gmail.com. Tel: (265) 999 044 484.

suitable for different agro ecological zones. Consequently, farmers use low quality and sometimes infected seed from previous potato crop and without proper selection. This practice has led to high degeneration of seed tubers due to systemic viral and bacterial diseases resulting in poor quality and quantity of potato tubers (Demo et al., 2007; Vanaei et al., 2008). As a result national potato production is below market demand of 88 kg per capita (FAO, 2008). One strategy towards improving the quality and quantity of potato tubers in Malawi is to develop a seed system that will increase multiplication of clean potato seed. Tissue culture, through micropropagation, offers a feasible propagation technique. Micropropagation is a technique of regenerating plants using small pieces of plants or tissues that are cultured on an artificial media under controlled environment and sterile conditions. Micropropagation enables the rapid multiplication of large number of potato seed tubers; in contrast with conventional means of propagation, micropropagation is faster and plants produced through micropropagation are usually free from infection by pathogens (Singh, 2002; Altindal and Karadogan, 2010).

Planting materials from tissue culture can be further multiplied in growth media for minituber seed production under greenhouse conditions. Although, potato minituber production can be useful for potato seed multiplication, response may vary among different genotypes (Sharma and Pandey, 2013). Minitubers are small seed potato tubers produced after acclimatization of plants propagated *in vitro* and planted at high density in the glasshouse in seed beds or in containers using different propagation media. Minitubers can be produced throughout the year and are principally used for the production of pre-basic or basic seed by direct field planting (Lommen and Struik, 1999; Ritter et al., 2001). The size of minitubers may range from 5 to 25 mm although in current systems larger minitubers have become common. This size range correlates to a weight range of 0.1 to 10 g or more (Struik, 2007). The quality of the transplant is very important for the production of minitubers (Jami-Moeini et al., 2001; Struik, 2007).

Media is one of the most important factors to consider for clean seed tuber (minituber) production. Due to the relatively shallow depth and limited volume of a container, potting media must be amended to provide the appropriate physical and chemical properties necessary for plant growth. Field soils are generally unsatisfactory for the production of plants in containers. Albajes et al., (2002) stated that this is primarily because soils do not provide the aeration, drainage and water holding capacity required. To improve this situation several "soilless" potting media gained more and more importance because they eliminate or reduce the need for soil disinfestations. Reaching a commercial scale in soilless culture is motivated by a potential for increasing the crop productivity and efficiency. Among soilless substrates, rock wool has been widely used in northern Europe, while in the tropics and sub-tropics cheaper substrates have been exploited (Mobini et al., 2009). Widespread adoption of soilless potting media production in global food and plant production has been reported as the technical solution for problems including root diseases, root zone oxygen deficiency, fertility control which occurs in other systems. Jami-Moeini et al. (2001) emphasized that the advantages of container production systems over ground production systems are greater water and nutrient efficiencies, with more food production, better cropping with higher salinity levels than soil grown.

Although, research has shown that different media (perlite, pumice, sand, styroform, compost, peat and sphagnum) can be used to grow *in vitro* potato plantlets in the greenhouse the most preferred media used is vermiculite (Balali et al., 2008). However, vermiculite is very expensive because of energy cost for heating to prepare suitable product. Due to high cost associated with vermiculite other alternative can be used such as sand, rice hulls and sawdust. Among these sand is a cheap media and easy to use.

Therefore, the aim of the study was to evaluate the growth and seed yield response of micropropagated potato planting materials of locally adapted and newly introduced potato genotypes in different propagation media in order to optimize clean potato seed production and increase availability to farmers in Malawi.

MATERIALS AND METHODS

The study was conducted in the greenhouse at Bunda College of Agriculture in Lilongwe, Central Malawi. The experiment was conducted from April 2011 to November 2011 (eight months).

Plant materials

Healthy (clean) tubers of locally adapted genotypes (Kwapata et al., 2007, unpublished): Rosita and Magalabada were collected from different growing areas in Malawi (Jenda in Mzimba, Dedza, Ntcheu, Bvumbwe and Mulanje). These genotypes were cleaned and certified as disease free plantlets using ELISA test. Certified seed stock of newly introduced genotypes in plantlets form were obtained from Agriculture Research Council (ARC-LNC) in South Africa they were certified as disease free plantlets using ELISA test by suppliers. These genotypes include: Van der plank, Lady Rosetta, Bp 1 2007, Buffel spoort07 and Up to date.

Bp1 2007 is a popular cultivar in South Africa and Lesotho. It produces oval tubers with white flesh and firm texture suitable for making chips. Van Der plank, a white fleshed, pear shaped and firm textured. Good for roasting, chips and stews. Lady Rosetta is used mostly by chips industries or factories because it absorbs less oil. Buffelspoort has a cream flesh with firm texture tubers and is suitable for chips and roasting. Up to date, a white fleshed with floury used mainly for mashed and baked potatoes (ARC.LNC, 2010?). Among local genotypes, Rosita and Magalabada are popular genotype in Malawi and most preferred by farmers (Demo et al., 2007). They have cream flesh with pinkish texture, oval shaped and are good for making chips.

Plant materials used in this study were raised in tissue culture at

Table 1. Characteristics of different media.

Substrate	pH	% organic carbon	% Nitrogen
Vermiculite	7.5	0.65	0.084
Sand	7.3	0.89	0.042
Saw dust	7.6	20.05	0.070

Bunda College and were sub cultured for multiplication using nodal cutting technique. The media was prepared using full strength MS mixture powder, containing all the required basal salts as outlined by Murashige and Skoog (1993), 4.4/l g MS powder. It was supplemented with 25 g/l sucrose and 7 g/l agar (a gelling agent). The medium was measured for its pH and adjusted to 5.7 (with NaOH or HCl). Four weeks old *in vitro* plantlets were subjected to hardening off for about 10 days to prepare them for *in vivo* environment. During hardening off, the following environmental factors were regulated: increase in temperature, increase in light intensity and humidity was reduced. Since plantlets were transferred to plastic magenta tubes, relative humidity (RH) was reduced by making small holes on the lid and keeping them in a sterile place for few days before transferring them to a green house. This gradually reduced the relative humidity (ARC.LNR, 2010). After hardening off, the *in vitro* produced plantlets were then used in the green house for production of minitubers.

Propagation media collection, sterilization and analysis

River sand was collected from shores of Lake Malawi to obtain pure sand. Vermiculite was sourced from South Africa and saw dust was collected from carpentry shops around Lilongwe, Malawi. Each type of media was filled in the covered aluminium jars and autoclaved at 121°C for 1 h. After autoclaved each propagation media was then analyzed for pH, total nitrogen and Organic Matter in the laboratory (Table 1).

Potato minituber production in greenhouse

The rooted *in vitro* plantlets were transplanted to a greenhouse in trays of 28 L volume filled with different substrates (sand, vermiculite and sawdust) at a volume of 15 L. The plantlets were spaced at 10 cm between plants and 10 cm between rows, corresponding to 40 plants per tray as a gross plot and 20 plants were used as net plot. The irrigation unit was placed at the top of the tray. The inlet of this unit was connected to the water pump through a solenoid valve. The nozzles of the drip irrigation unit were fixed 10 cm equidistant to direct water and nutrient solution towards the root zone at required time intervals (Farran and Mingo-Castel, 2006; CIP, 2008; Chiipanthenga et al., 2012). NPK fertilizer was used (23:21:0 +4S), at the rate of 1.2 g per plant every 10 days throughout the plant growth. The rate was adopted from Alsadon and Knutson (1994). The day and night temperatures in the green house were regulated and the average day temperatures were 20 ± 2°C and night temperatures of 18 ± 2°C.

Statistical analysis

The study had two factors; one factor composed of three propagation media (sand, vermiculite and saw dust) also referred to as growth media or substrate. Second factor composed of seven genotypes: Bp1 2007, Van der plank, Lady Rosetta, Buffelspoort, Up to date, Rosita and Magalabada. These treatments were arranged in a randomized complete block design (RCBD) with three replicates (each block represent a replicate containing 21 treatments/trays). Data collected include; shooting percent which was determined by counting the number of plants that have survived and established against the total plantlets two weeks after transplanting. Days to mini tuber setting was determined by counting number of days plant took to start forming tuber. Tuber set was checked by lifting the media on a net plot and putting the propagation media back. Harvesting of mini-tubers started 9 weeks after transplanting when the mini tubers showed its maturity. The skin of the mature mini-tuber was thick and easy to skin off. Minituber diameter was determined by measuring the width of the tuber using a calliper and minituber yield was determined by measuring the weight of tubers in grams per square metre. Analysis of Variance (ANOVA) was performed using GenStat Statistical package version 13, using General Linear Model procedure. Mean values were compared using the least significance difference (LSD) method at the 5% level of significance.

RESULTS AND DISCUSSION

Effect of media type and genotype on the survival and the establishment of tissue culture plantlets planted in three different growing media 2 weeks after transplanting

Results have shown highly significant ($P \leq 0.001$) variation on survival and establishment percentage among the three propagation media. Higher plant establishment percentage of 95% was observed in genotypes planted in vermiculite than in sand (77%) and in sawdust (49%). There was a significant ($P \leq 0.01$) variation observed among the seven genotypes on shooting percentage. BP 1 2007, Rosita and Lady Rosetta had highest percentage of plants to shoot at two weeks after transplanting (Table 2). There was no media x genotype interaction with respect to plant survival and establishment (Table 2). The variation in plant survival and establishment percentage in different growth media agrees with the findings by Hassanpanah and Khodadadi (2009) who reported the advantage of vermiculite medium against other media which led to the maximum tuber yield under greenhouse conditions. Vermiculite has excellent ex-change and buffering capacities as well as the ability to supply potassium and magnesium (Badon and Chauhan, 2010). Among the growth media variations could be attributed to difference in the composition and characteristics of media. For instance organic carbon in sawdust was about 20% (Table 1). The results are supported by Öztürk and Yildirim (2010) who found that the C: N ratio of sawdust is such that it is not readily decomposed possibly and the conditions are not conducive for the growth of microbes.

Table 2. Effect of media type and genotype on the survival and the establishment of tissue culture plantlets planted in three different growing media, 2 weeks after transplanting.

Genotype	Propagation media			
	Sand	Vermiculite	Sawdust	Mean for genotypes
Magalabada	75.00±0.00	90.0±0.00	53.0±1.67	72.80[bc]
Rosita	73.00±3.33	93.3±5.78	55.0±8.82	76.10[ab]
Lady Rosetta	75.00±5.74	100.0±0.00	50.0±8.66	75.00[ab]
Bp 1 2007	83.30±1.67	100.0±0.00	56.7±4.41	80.00[a]
Up to date	78.00±4.41	91.7±1.67	36.7±1.67	68.90[c]
Van der plank	80.00±5.00	88.3±4.41	45.0±2.87	71.10[bc]
Buffelspoort	73.33±1.67	93.3±5.78	50.0±1.67	72.20[bc]
Mean	76.90[b]	94.52[a]	49.52[c]	

Values in the table are presented as mean ± standard error, Means in the main effects have been compared separately, Means with different letter superscripts within the same column and rows are significantly different at P<0.05.

Figure 1. Bp 1 2007 genotype at five weeks after transplanting in green house (a) vermiculite, (b) sand, (c) sawdust.

The high cellulose and lignin content along with insufficient Nitrogen supplies creates depletion problems which can severely restrict plant establishment and growth. However, supplemental applications of nitrogen at the required rate can reduce this problem. In this study, all the media used where supplemented with the similar rate and type of fertiliser. However, it can be observed that probably fertiliser used and the rate could not be enough for sawdust media to retain the required nutrients to the plants and causing poor growth of plants. Apart from being influenced by the genetic constitution, high plant shooting percentage is also influenced by the type of the media used among other environmental factors (Kuria et al., 2008).

Observation on shooting percentage explains the establishment of the plants in the growth media which include well developed roots and shoots (Figure 1). Other reports show that a well-established root and shoot system is important for subsequent growth which later influence tuber bulking stage in potato plant (Dwelle and Love, 2000). In this study the results implies that genotypes Bp 1 2007, Rosita and Lady Rosetta were well established in the propagation media than other genotypes.

Number of days to tuber formation as affected by propagation media and genotype

There was significance differences (P≤ 0.05) observed on interaction between propagation media and genotype (Table 3) on number of days to tuber formation. Plants in vermiculite started forming tubers earlier than plants in sand and sawdust. Among the genotypes BP 1 2007 and Buffelspoort started forming tubers at an average of 33 and 34 days after transplanting, respectively. Rosita (41 days) and Magalabada (40 days) were late in forming tubers than the introduced genotypes. The variations in tuber formation could be attributed to the media types and probably genetic constitution of the genotypes. According to a review on South Africa genotypes Black (2008) found that some genotypes were bred for early maturing others medium or late maturing depending on the agro-ecological zone which might influence time for

Table 3. Effect of Media and genotype on number of days to tuber set.

Genotype	Propagation media			Mean for genotypes
	Sand	Vermiculite	Sawdust	
Magalabada	41±0.00[c]	40±0.33[c]	45±0.00[a]	42[a]
Rosita	42±0.33[b]	41±0.01[c]	45±0.67[a]	43[a]
Lady Rosetta	37±0.00[ef]	36±0.00[fg]	38±0.00[de]	37[b]
Bp 1 2007	36±0.00[fg]	33±0.67[h]	38±0.00[de]	35[c]
Up to date	36±0.00[fg]	35±0.33[g]	39±0.00[d]	36[b]
Van der plank	37±0.00[ef]	36±0.00[fg]	37±0.00[ef]	36[b]
Buffelspoort	35±0.00[g]	34±0.33[h]	37±0.00[ef]	35[c]
Mean for media	37[b]	35[c]	40[a]	

Values in the table are presented as mean ± standard error, Means in the main effects have been compared separately, Means with different letter superscripts within the same column and rows are significantly different at P<0.05.

Table 4. Minituber yield per genotype and propagation media on yield grams per m^2 at harvest.

Genotype	Propagation media			Mean
	Sand	Vermiculite	Sawdust	
Magalabada	954[f]	1059[d]	259[i]	757[d]
Rosita	962[f]	1490[b]	262[i]	904[c]
Lady Rosetta	640[g]	2146[a]	244[i]	1010[b]
Bp 1 2007.1	1136[e]	2140[a]	431[h]	1238[a]
Up to date	643[g]	2012[a]	291[hi]	982[bc]
Van der plank	708[g]	1263[c]	318[hi]	779[d]
Buffelspoort	700[g]	1364[bc]	240[i]	770[d]
Mean	820[b]	1740[a]	292[c]	

Means in the main effects have been compared separately.

tuber set in a green house. Other studies have further indicated that tuberisation may be influenced by several factors such as media type, genotype, nutrition among others (Alexopoulos et al., 2007). Khodadadi et al. (2011) reported that under appropriate growth conditions, the tips of stolons will “hook” and begin to swell, resulting in initiation of new tubers. For many cultivars, including Van der plank and up to date, this occurs during early flowering. Dwelle and Love (2000) further illustrates that although there is no causal relationship between the two events. Potatoes need moderate availability of nitrogen and cool nights for good tuber growth. The results in the study indicate that media influence potato tuber formation and bulking. Therefore, good selection of media and genotype would assist in mass potato mini tuber production.

Minituber yield as affected by genotype and propagation media (yield grams per m^2) at harvest

There was a significant interaction between genotype and propagation media with respect to yield. Lady Rosetta, Bp 1 2007 and Up to date genotypes transplanted in vermiculite produced relatively higher yields of 2146, 2140 and 2012 g/m^2, respectively, among the seven genotypes tested. However, the trends were different for genotypes planted in sand Bp 1 2007, Lady Rosetta and Magalabada outperformed other genotypes having higher yields of 1136, 962 and 954 g/m^2, respectively (Table 4). The variation in yield per genotype could be attributed to the influence by media and genetic composition. Findings by Khurana et al. (2003) suggest that different potato genotype adapt differently to the planting bed. In addition, some external factor such as temperature may influence tuber initiation and bulking. The results on yields from this study are in line with findings of Akoumianakis et al., (2000); Khodadadi et al., (2011) who also reported yield range of 300 to 2000 g per square metre depending on the varieties and growth media under greenhouse conditions. Yields were lower in sawdust due to poor growth and this affects photosynthetic area which subsequently affects tuber formation of potato plant. In addition some studies have also indicated that the source of the sawdust compromise its quality and ability to return nutrients to the plants (Palacios et al., 2009). This obser-

Table 5. Number of minitubers per plant as affected by propagation media types and genotypes.

Genotype	Propagation media			Mean for genotype
	Sand	Vermiculite	Sawdust	
Magalabada	5±0.33^{fgh}	8±0.00^{cd}	2±0.00^{j}	5.02^{c}
Rosita	5±0.78^{fgh}	9±0.08^{bc}	2±0.33^{j}	5.32^{bc}
Lady Rosetta	6±0.17^{e}	10±0.79^{b}	3±0.44^{ij}	6.13^{b}
Bp 1 2007	4±0.22^{gh}	6±0.29^{ef}	3±1.33^{hi}	4.59^{c}
Up to date	7±0.29^{de}	12±0.77^{a}	3±0.68^{ij}	6.99^{a}
Van der plank	3±0.22^{ij}	4±0.11^{hi}	2±0.15^{j}	2.76^{d}
Buffelspoort	5±0.21^{efg}	7±0.17^{de}	2±0.47^{j}	4.60^{c}
Mean for Media	4.94^{b}	7.81^{a}	2.39^{c}	

Values in the table are presented as mean ± standard error, means in the main effects have been compared separately.

vation agreed with the findings by Donnelly et al. (2008) who reported that the species of tree from which sawdust is derived largely determines its quality and value for use in a growth media. In this study, saw dust that was used was a mixture from different trees which could have an effect on plant growth and tuber yield. The main role of physical characteristics of media is having suitable air-filled porosity for efficient oxygen diffusion and maintaining favourable water content for supplying water and nutrients and respiration of root (Richard et al., 2004). This implies that for high multiplication of minitubers in the greenhouse media is a very important factor to be considered as it affects both growth and minituber yield of potato.

Effect of propagation media and genotypes on number of tubers per plant

There was a significant (P≤ 0.001) interaction between genotype and propagation media with respect to number of tubers per plant. (Table 5). Up to date and Lady Rosetta genotypes planted in vermiculite registered the highest number of tubers per plant (12 and 10, respectively). Alsadon and Knutson (1994) reported that tuber number rather than weight is important for reporting yield under greenhouse conditions. Green house may permit an expression of tuber number potential but limitations in media space and possibly radiation may not permit maximum tuber size development. Up to date and Lady Rosetta recorded higher number of mini tubers per plant indicating superiority over other genotypes. This could be attributed to the interaction between genetic constitution of the genotypes and environment. High number of minitubers per plant indicates high multiplication potential of the genotypes under greenhouse conditions. The results agree with Black (2008) that Up to date and Lady Rosetta were bred for high yielding and are most popular varieties and make up 77% of the potatoes grown in South Africa. These findings by Black (2008) were also reflected in this study by having these two genotypes outperforming the other genotypes. The study findings imply that Up to date and Lady Rosetta demonstrated potential traits for high yield that would assist breeders for selection and improvement.

Asghari-Zakaria et al. (2009) reported that nutrient uptake of plant is determined by the growth media. Vermiculite despite being expensive is still the best media indicating superiority among the media tested. This could be attributed to its ability to hold and retain nutrients to the plants. Other studies suggest that generally the mixture of two or three different media is better (Khodadadi et al., 2011) This therefore indicates that with good manipulation and management of other alternative media tested such as sand could equally do well in minituber production as vermiculite and this can limit the use of this expensive media.

Potato tuber diameter as affected by propagation media and genotype

There were significant (P≤0.05) variation among the interaction between genotype and media. The introduced genotypes transplanted in vermiculite had more tubers of bigger sizes than local genotypes (Figure 2). The same trend was experienced in sand and sawdust growth media.

However, Van der plank, Lady Rosetta and Bp 1 2007 outperformed all genotypes having more tubers of diameter up to 2 cm. It was observed that Van der plank had little number of tubers per plant (Table 3) but the tubers were of bigger sizes among the genotypes possibly due to compensatory growth. According to the results sand media has also a potential to produce minitubers of the average sizes and can still work in replacing vermiculite as the best media for potato production. Sand is relatively cheaper to source than vermiculite which makes it easier to use sand than vermiculite.

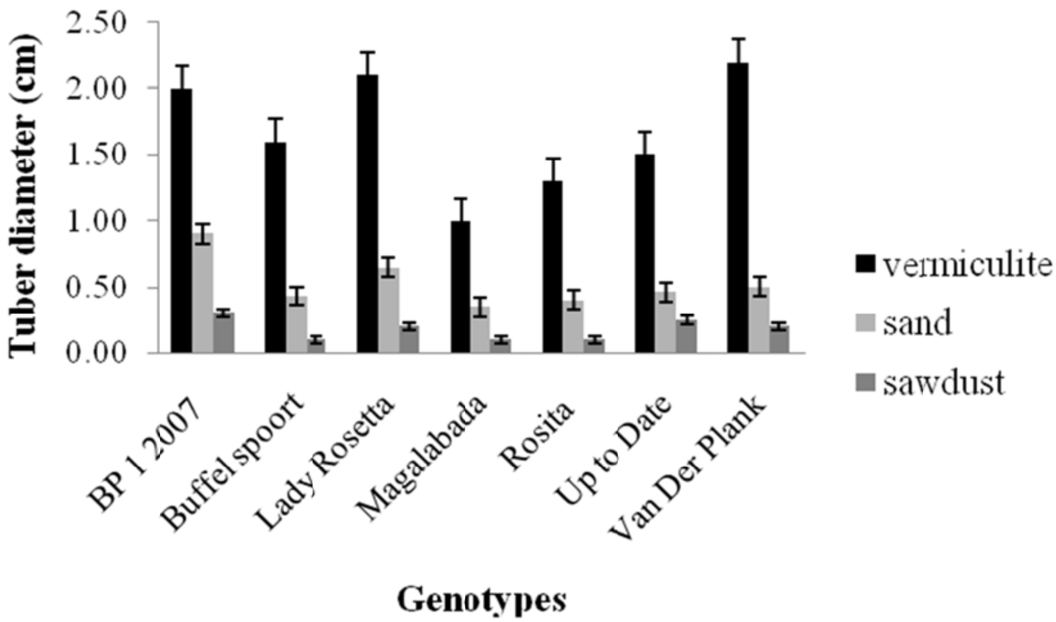

Figure 2. Tuber diameter for genotypes in different media in greenhouse at harvest.

Conclusion

The results have shown that minituber yield vary among local and introduced potato genotypes under greenhouse conditions. Genotypes with high shooting percentage and vigorous growth in a particular media represent a potential for establishment and production of minituber potatoes. Introduced genotypes grew vigorously indicating good establishment than the local genotypes. The findings of this study have revealed genotypes with a potential for minituber seed production of potato. Introduced genotype Bp 1 2007, Lady Rosetta and Up to date and Van der plank have demonstrated valuable characteristics that could assist in selection for multiplication and breeding purposes. The results have also shown that performance of potato genotypes vary with propagation media under greenhouse conditions. Among the propagation media tested vermiculite was still found to be superior in supporting the plants followed by sand. Thus, vermiculite has positive effect on potato growth and tuber formation. However, sand would also be a potential media if it can be manipulated and managed by either media combination or proper nutrient supplementation so that it replaces the use of vermiculite for optimum production of minitubers. The use of sawdust can be improved with high nutrient supplement, combined with other media and proper selection of tree species that may not have an effect to the plantlets. The study has provided valuable information on potential genotypes and propagation media which will be a bench mark on minituber production research and up scaling programmes for making available pathogen free seed tubers to increase potato production in Malawi.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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