Research Article

Micropropagation of Livingstone Potato (*Plectranthus esculentus* N.E.Br)

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Livingstone potato (*Plectranthus esculentus* N.E.Br) is an underutilised indigenous root vegetable grown by communal farmers in the eastern provinces of Zimbabwe. It is vegetatively propagated using unimproved retained tubers from the previous season. The risk of disease carryover is therefore high, leading to poor yields. The objective of the study was to exploit the tissue culture technique of micropropagation to produce a mass supply of healthy planting material for improved productivity. Two experiments were conducted: firstly, to determine the best explant type and secondly, to determine the best landrace and plant growth regulators for the growth of plantlets. The landraces, namely, *Ndurwe*, *Musande*, *Chibanda*, and *Chizambezi*, were sourced from communal farmers in the stated production areas. Naphthalene acetic acid (NAA) and benzyl amino purine (BAP) were the auxin and cytokinin used, respectively. The first experiment was laid out as a randomized complete block design (RCBD) with two factors: landrace and explant type (shoot tips, nodes, and leaves). After culturing the explants on a plain Murashige Skoog (MS) medium for ten weeks, the best explant was the node with regards to the number of nodes, shoots, and roots of the plantlets which were significant ($P < 0.05$). The second experiment was laid out as a RCBD with two factors: landraces and the plant growth regulator combinations. The nodes were subcultured on an MS medium supplemented with the 16 combinations of plant growth regulators (0 mg/l, 0.5 mg/l, 1 mg/l, and 2 mg/l BAP concentrations: 0 mg/l, 0.2 mg/l, 0.5 mg/l, and 1 mg/l NAA concentrations), respectively. *Chizambezi* performed best and is, therefore, highly recommended for the rapid multiplication of Livingstone potato. Results from this study have clearly demonstrated that the addition of NAA: BAP at varying concentrations was significant and is essential for optimizing the growth media for micropropagation of Livingstone potato in Zimbabwe. Commercial production of plantlets can, therefore, be carried out to provide healthy planting material for the communal farmers for improved productivity while preserving the germplasm of the underutilised crop at the same time.

1. Introduction

Livingstone potato or wild potato (*Plectranthus esculentus* N.E.Br) is a perennial herbaceous plant, which belongs to the Lamiaceae family and is grown for its edible tubers. The crop can be propagated sexually using seed, but the development of the crop is slow and the offspring is heterogeneous [1]. Communal farmers in Zimbabwe produce the crop asexually using small tubers which are retained from last season's crop [2, 3]. Although this ensures the maintenance of lines, the risk of disease carryover into the next season is very high and eventually leads to a decline in potential yield. Another

disadvantage of using this method is the low multiplication rate of the planting material [4]. For root vegetable crops like Livingstone potato, the availability of adequate disease-free planting material is a constraint to increased productivity, and as such, the crop remains neglected and underutilised in Zimbabwe [2].

Underutilised crops such as Livingstone potato are characterized by low yields [1–3]. However, they still remain relevant as they will produce harvestable yields where major food crops may fail [5]. Tindall [6] stated that the yield of Livingstone potato can be as low as two to six tonnes per hectare, while Schippers [1] highlighted yield potentials of

up to 60 t/ha under optimal experimental conditions using tissue culture produced plantlets in South Africa [7].

Biotechnology has been used effectively as a tool for the improvement of several vegetatively propagated crops. Tissue culture techniques are deemed as the easiest to implement initially, and micropropagation is a valuable and much used technique for the multiplication of many vegetatively propagated food crops like sweet potato, yam (*Dioscorea* spp), potato (*Solanum tuberosum*), and banana and plantain (*Musa* spp) [8]. Advances in tissue culture techniques have facilitated the production, multiplication, and maintenance of disease-free plants for these crops [9]. The rapid multiplication of plant tissues, however, requires the optimization of plant growth regulator (PGR) concentrations in the Murashige and Skoog media. The PGR requirements tend to vary with species since they determine the course of morphogenesis [10].

Research into the micropropagation of the *Plectranthus* species has been conducted [11–14]; however, there has been no reported research conducted on the micropropagation of the Livingstone potato landraces indigenous to Zimbabwe. The option of using the rapidly produced disease-free planting material is, therefore, currently not available for the producers. The high yields (60 t/ha) obtained under experimental conditions in South Africa [7] clearly demonstrate a need for optimizing the current traditional production methods in Zimbabwe with regards to the planting material. The objective of this study was to explore the micropropagation of Livingstone potato landraces found in Zimbabwe as a step in providing communal farmers with healthy planting material, thus improving productivity for the underutilised crop. Existing micropropagation protocols for the Livingstone potato were employed in this study to enable optimization for the indigenous landraces found in Zimbabwe.

2. Materials and Methods

2.1. Source of Livingstone Potato Landraces and Preparation. Four Livingstone potato landraces, namely, HRCTzD0001 (*Ndurwe*), HRCTzS0011 (*Chizambezi*), HRCTzD0003 (*Chibanda*), and HRCTzD0007 (*Musande*) were harvested from farmers in the in the Marondera district of the Mashonaland East province of Zimbabwe. Marondera district lies in Natural Region IIb of the agro ecological zones of Zimbabwe. The region is characterized by an annual average rainfall of 850 mm and severe dry spells during the rainy season. Soils are greyish brown sands and sandy loams derived from granitic rocks [15]. The farmers produce the crop on ridges in wetlands and with no application of fertilizers. The tubers are ready for harvest after six to nine months [1–3].

For each landrace, the tubers were obtained from one single plant weighing approximately 1.3 kg [13]. The freshly harvested tubers were then taken to the University of Zimbabwe in Harare, where they were kept at room temperature for a period of four weeks in the laboratory to enable the healing of minor cuts and bruises and thicken the skin of the tubers. The tubers were then washed in a

detergent solution (one part sunlight liquid detergent to nine parts water) and thoroughly rinsed in distilled water. Small slits were cut on the tubers which were then soaked in a 5% gibberellic acid solution (5 ml-GB/litre of distilled water) for 10 minutes to encourage sprouting. The cuts were made to enable the rapid absorption of the GA [4]. The tubers were then air dried and left on trays in dark cupboards until sprouting occurred. Different parts of the sprouts (which were left to grow to a length of 5 cm) were then used as the sources of the explants. Two experiments were conducted in the laboratory at the University of Zimbabwe Crop Science Department, which has an average temperature of 25°C and a relative humidity between 50–70%.

2.2. Experiment 1: Effect of Explant Type on Root and Shoot Development of Livingstone Potato

2.2.1. Culture Medium Preparation. The aim of the experiment was to determine a suitable explant type (shoot tips, nodes, and leaves) for the micropropagation of Livingstone potato. A standard culture medium containing the full strength of macronutrients and micronutrients, as well as vitamins as described by Murashige and Skoog [16], was used in the study. The Murashige and Skoog (MS) medium was supplemented with 30 g of sucrose and vitamins, and the pH was adjusted to 5.6–5.8 using 0.5 M sodium hydroxide. Three grams per litre of Gelrite™ was added as the solidifying agent, and the sterilized culture vessels were filled 20 ml each with the MS medium. These were then autoclaved for 15 minutes at 121°C and 15 kPa and left to cool down and solidify at room temperature.

2.2.2. Surface Sterilization of Explants. The explants (shoots tips, nodes, and leaves) cut from the sprouted tubers were washed in a weak soapy water solution to remove any plant debris adhering to the explants. The explants were placed in a beaker with two drops of Tween-20 and agitated to reduce surface tension and then left under running water overnight to create an isotonic solution inside the segments. After the washing procedure, the explants were then soaked in 10% concentration of sodium hypochloride for 15 minutes and then rinsed three times in sterile distilled water to remove the disinfectant. This procedure was performed under aseptic conditions in a laminar airflow hood. The basal end tips of the explants were trimmed to remove bleach damaged tissue. A 70% ethanol solution was prepared and used for the surface sterilization of the apparatus and the laminar airflow cabinet [17].

2.2.3. Capturing of the Explants. The explants (leaves, nodes, and shoot tips) which were approximately 10 mm were excised using a sterile scalpel on a white tile and placed in sterile culture vessels with solidified MS medium using a pair of sterilized forceps. The culture vessel caps were sealed with Parafilm™ [13]. The cultures were placed in the growth room on top of polystyrene sheets where the temperature was kept at $27 \pm 2^\circ\text{C}$, and a 16 hour light: 8 hour dark photoperiod was

maintained [18]. The cultures were checked for contamination three days after capturing for bacterial infections and six days after capturing for fungal infections. All infected materials were then discarded.

2.2.4. Experimental Procedure and Data Analysis. The experimental design was a 4×3 factorial laid out in a randomized complete block design (RCBD). The experiment had two factors, three blocks, and twelve treatments replicated three times in each block. The first factor was the landrace at four levels (*Ndurwe*, *Musande*, *Chibanda*, and *Chizambezi*), and the second factor was the explant type at three levels (shoot tips, nodes, and leaves). The treatments were blocked against light and distance from the door. The cultures were observed on a daily basis, and the number of days to root and shoot initiation was monitored and recorded. After ten weeks of culturing, the shoot length was measured using a ruler, and shoot number, leaf number, and number of nodes on the shoot were physically counted and recorded. The data collected were then subjected to analysis of variance (ANOVA) using the Genstat Statistical package version 18. Means were separated using 5% Fisher's least significant difference (LSD) test. The best explant (nodes) according to the results was then used in the second experiment.

2.3. Experiment 2: Effect of Landrace and Plant Growth Regulators on the Growth of Livingstone Potato Plantlets

2.3.1. Experimental Procedure. To optimize the medium for the development and growth of Livingstone potato plantlets from nodes, different combinations of auxin: cytokinin were tested. The auxin used was naphthalene acetic acid (NAA), while benzyl amino purine (BAP) was the cytokinin used. Different combinations of NAA and BAP growth regulators were added to the MS medium to make up the respective 64 treatments, as shown in Table 1. The 0 mg/l of NAA: BAP was used as the control and contained the MS medium only. The experimental design was a 4×16 factorial laid out in a RCBD with two factors and three blocks with three replications. The first factor was the landraces at four levels (*Ndurwe*, *Musande*, *Chibanda*, and *Chizambezi*), and the second factor was the NAA: BAP combinations (NAA at 0 mg/l, 0.2 mg/l, 0.5 mg/l, and 1 mg/l concentrations: BAP at 0 mg/l, 0.5 mg/l, 1 mg/l, and 2 mg/l concentrations) at sixteen levels (Table 1).

2.3.2. Medium Preparation and Subculturing. Standard stock solutions (1 mg/ml) of the growth regulators BAP and NAA were prepared by dissolving 50 mg of each of the growth regulators in two separate beakers with a few drops of ethanol and then adding distilled water to bring the volume to 50 ml. The MS medium was prepared as described in Section 2.2.1 and poured into 16 separate beakers where the different plant growth regulator combinations (BAP: NAA), shown in Table 1, were then added. The pH, sucrose, and Gelrite™ were adjusted and added, respectively, as

TABLE 1: NAA: BAP combinations.

NAA (mg/l)	BAP (mg/l)			
	0	0.5	1	2
0	A (0:0)	B (0:0.5)	C (0:1)	D (0:2)
0.2	E (0.2:0)	F (0.2:0.5)	G (0.2:1)	H (0.2:2)
0.5	I (0.5:0)	J (0.5:0.5)	K (0.5:1)	L (0.5:2)
1	M (1:0)	N (1:0.5)	O (1:1)	P (1:2)

described in Section 2.2.1 including the pouring of the prepared media into the culture vessels. Ten millimetre-long nodes which were the best explant from the first experiment were obtained from the four landraces. These were cut using a sterile blade and then subcultured onto the solid MS medium with different concentrations of the NAA and BAP using sterile forceps into the culture vessels. This process was performed under aseptic conditions in a laminar airflow cabinet. After capturing, the cultures were incubated in a growth room, and the environmental conditions were maintained as described in Section 2.3.3 including the contamination checks.

2.3.3. Data Collection and Analysis. Data collection was as described in Section 2.2.4. *Ndurwe* explants cultured using the NAA: BAP combinations J (0.5:0.5), L (0.5:2), and P (1:2) did not show any signs of growth during the 10 week period of the study; therefore, they were removed from the analysis. The data were, therefore, treated as an unbalanced ANOVA using GenStat Statistical package version 18. Graphs were constructed using R statistical package. Means were separated using 5% Fisher's least significant difference (LSD) test.

3. Results

3.1. Experiment 1: Effect of Explant Type Shoot Development of Livingstone Potato

3.1.1. Effect of Landrace and Explant Type on the Average Shoot Number. The landrace, explant type, and the landrace $\times$ explant type interaction effects were significant ($P < 0.05$) on the mean shoot number. The interaction resulted in *Musande* nodes producing the highest mean shoot number which was not significantly different ($P > 0.05$) from that of the *Musande* shoot tip. The *Ndurwe* leaf explant produced the least number of shoots which performed statistically the same with the leaf and shoot tips from the other landraces as well as the nodes from *Chibanda* and *Chizambezi* (Table 2).

3.1.2. Effect of Landrace and Explant on the Average Number of Nodes per Shoot. The effect of the explant type was not significant ($P > 0.05$), while the landrace and the landrace $\times$ explant type effects were significant ($P < 0.05$) on the average number of nodes per shoot. *Musande* nodes produced shoots with the highest number of nodes which was statistically similar to that of the *Ndurwe* shoot tip. The *Ndurwe* leaf explant produced the least number of nodes per shoot, and this was not significantly different to the leaf and shoot

TABLE 2: Effect of landrace and explant on the average shoot number.

Treatment	Shoot number			
<i>Ndurwe</i> leaf	0.56 ^a			
<i>Chibanda</i> leaf	0.67 ^a			
<i>Musande</i> leaf	0.78 ^a			
<i>Ndurwe</i> shoot tip	0.89 ^{ab}			
<i>Chibanda</i> shoot tip	0.89 ^{ab}			
<i>Chizambezi</i> node	0.89 ^{ab}			
<i>Chizambezi</i> leaf	0.89 ^{ab}			
<i>Chibanda</i> node	1.00 ^{abc}			
<i>Chizambezi</i> shoot tip	1.22 ^{abc}			
<i>Ndurwe</i> node	1.56 ^{bc}			
<i>Musande</i> shoot tip	1.67 ^{cd}			
<i>Musande</i> node	2.33 ^d			
	<i>P</i> value	SED	LSD _{0.05}	
Landrace	0.002	0.198	0.395	
Explant	<0.001	0.172	0.342	
Explant $\times$ landrace interaction	0.044	0.345	0.684	

Means followed by the same letters in a column are not significantly different at LSD (0.05)

tips of other landraces as well as nodes from *Ndurwe* and *Chizambezi* (Table 3).

3.2. Experiment 2: Effect of the Plant Growth Regulators on the Growth of Livingstone Potato Plantlets

3.2.1. Effect of Landraces and NAA: BAP Combination on Days to Root Initiation. A significant landrace $\times$ NAA: BAP interaction ($P < 0.001$) on days to root initiation was observed. *Chibanda* and *Chizambezi* each initiated roots in the shortest time when cultured using the NAA: BAP combination K (0.5:1), while *Musande* took the longest time for root initiation when no plant growth regulators were added (NAA: BAP combination A (0:0)), as shown in Figure 1. *Ndurwe* showed no root initiation during the 10 week time frame for the NAA: BAP combinations of J (0.5:0.5), L (0.5:2) and P (1:2).

3.2.2. Interaction of Landraces and the NAA: BAP Combination on Days to Shoot Initiation. The landrace, NAA: BAP combination, and the landrace $\times$ NAA: BAP interaction effects were significant ($P < 0.05$) on the time taken to shoot initiation. The interaction resulted in the landraces responding differently to the NAA: BAP combinations, as presented in Figure 2. *Chibanda* and *Chizambezi* both had the least number of days to shoot initiation as the concentration of the plant growth regulators increased. *Chizambezi* also had the same result at the highest NAA: BAP combinations although the time taken was not significantly different ($P > 0.05$) from the NAA: BAP combinations D, H, and L. *Musande* took the longest time to initiate shoots when cultured using the NAA: BAP combination of E (0.2:0) (Figure 2). *Ndurwe* showed no shoot initiation during the time frame of 10 weeks for the same NAA: BAP combinations of J (0.5:0.5), L (0.5:2), and P (1:2).

3.2.3. Effect of Landraces and the NAA: BAP Combination on the Shoot Number. The landrace and NAA: BAP combination effects were significant ($P < 0.05$) on the shoot number. A significant interaction ($P < 0.001$) was observed between the landrace and NAA: BAP combination on the mean shoot number. *Musande* and *Ndurwe* produced the highest mean shoot numbers which were the same statistically when both landraces were cultured using the NAA: BAP (0.2:1) combination. However, the shoots were noticeably small structurally and short (0.7 cm-0.5 cm, respectively). *Ndurwe* did not produce any shoots when the explants were cultured using the A (0:0), C (0:1), J (0.5:0.5), and L (0.5:2) NAA: BAP combinations (Figure 3).

3.2.4. Effect of Landraces and the NAA: BAP Combination on the Leaf Number. There was a significant ($P < 0.001$) landrace $\times$ NAA: BAP interaction on the leaf number. *Ndurwe* produced the highest mean leaf number at NAA: BAP combination M (1:0) which was not significantly different ($P > 0.05$) from that of *Chizambezi* at the same concentration. A noticeable trend was that as the concentration of the plant growth regulators was increased, *Chizambezi* produced high leaf numbers which were not significantly different ($P > 0.05$) amongst 5 different NAA: BAP combinations (C, E, I, J and M) (Figure 4). *Ndurwe* and *Chibanda* were also not significantly different with the gradual increase for three NAA: BAP combinations each (B, C, D, E, L, and M). Treatments J, L, and P had no leaf number readings as there was no growth for *Ndurwe* (Figure 4).

There was a significant ($P < 0.001$) landrace $\times$ NAA: BAP interaction on the node number with *Chizambezi* having the highest mean node number at the NAA: BAP combination of I which was not significantly different at M for the same landrace. This result was statistically the same for *Chibanda* cultured at NAA: BAP combinations A, C, D, and I, for *Ndurwe* at E and M and for *Musande* at NAA: BAP combination O. On the other hand, *Ndurwe* at C had no nodes (Figure 5).

3.2.5. Effect of Landraces and the NAA: BAP Combination on the Shoot Length. The landrace $\times$ NAA: BAP interaction effects on shoot length were significant ($P < 0.05$). *Musande* produced plantlets with the longest shoots at NAA: BAP combination F (0.2:0.5) which was not significantly different from that of *Musande* at O (1:1), as well as *Ndurwe* at D (0:2), E (0.2:0), and M (1:0). No shoot lengths were recorded for *Ndurwe* at NAA: BAP combinations J, L, and P due to the absence of growth (Figure 6).

3.2.6. Summary of Landrace Performance due to Application of Plant Growth Regulators. A summary of the effects of landraces cultured in MS with different PGRs combinations cultured over a period 10 weeks is shown in Table 4. For each parameter, the best performing landraces and the corresponding NAA: BAP combinations are stated. The NAA: BAP concentrations are stated from the lowest concentration to the highest for the best performing landrace. For all

TABLE 3: Effect of landrace and explant on the average number of nodes per shoot.

Treatment	Number of nodes per shoot		
<i>Ndurwe</i> leaf	1.67 ^a		
<i>Chizambezi</i> shoot tip	1.78 ^a		
<i>Chibanda</i> leaf	2.22 ^{ab}		
<i>Chibanda</i> shoot tip	2.56 ^{abc}		
<i>Ndurwe</i> node	2.67 ^{abc}		
<i>Chizambezi</i> node	3.11 ^{abcd}		
<i>Chizambezi</i> leaf	3.11 ^{abcd}		
<i>Musande</i> leaf	3.67 ^{bcd}		
<i>Musande</i> shoot tip	4.00 ^{cd}		
<i>Chibanda</i> node	4.11 ^{cd}		
<i>Ndurwe</i> shoot tip	4.11 ^{cd}		
<i>Musande</i> node	4.56 ^d		
	<i>P</i> value	SED	LSD _{0.05}
Landrace	0.024	0.5	0.993
Explant	0.098	0.43	NS
Explant $\times$ landrace interaction	0.054	0.87	1.72

Means followed by the same letters in a column are not significantly different at LSD (0.05).

FIGURE 1: Mean days to root initiation of the Livingstone potato landraces in response to NAA: BAP in mg/l application. $P < 0.001$, $LSD = 3.22$. Error bars represent standard errors of the differences. Plant growth regulators key: A (0 mg/l: 0 mg/l), B (0 mg/l: 0.5 mg/l), C (0 mg/l: 1 mg/l), D (0 mg/l: 2 mg/l), E (0.2 mg/l: 0 mg/l), F (0.2 mg/l: 0.5 mg/l), H (0.2 mg/l: 2 mg/l), I (0.5 mg/l: 0 mg/l), J (0.5 mg/l: 0.5 mg/l), J (0.5 mg/l: 0.5 mg/l), K (0.5 mg/l: 1 mg/l), L (0.5 mg/l: 2 mg/l), M (1 mg/l: 0 mg/l), N (1 mg/l: 0.5 mg/l), O (1 mg/l: 1 mg/l), and P (1 mg/l: 2 mg/l).

the growth parameters measured, the addition of PGRSs gave positive results, and the only exception was the *Chibanda* landrace which had a high response on node numbers when no PGRSs were used (Table 4). While all the landraces managed to produce plantlets, *Chizambezi* was evaluated as the best landrace for micropropagation with good results most of the parameters measured using a range of 0–1 mg/l of NAA: 0.5–2 mg/l of BAP combinations (Table 4).

4. Discussion

Micropropagation is regarded as a fast method of multiplying plants and has great potential to develop high-quality and disease-free plants. Advancements in this field have led to the development of several techniques for the rapid multiplication and improvement of a wide range of horticultural crops and their production systems [19]. Amongst

FIGURE 2: Mean days to shoot initiation of the Livingstone potato landraces in response to NAA: BAP in mg/l application. $P < 0.001$, $LSD = 2.82$. Error bars represent standard errors of the differences. Plant growth regulators key: A (0 mg/l : 0 mg/l), B (0 mg/l : 0.5 mg/l), C (0 mg/l : 1 mg/l), D (0 mg/l : 2 mg/l), E (0.2 mg/l : 0 mg/l), F (0.2 mg/l : 0.5 mg/l), H (0.2 mg/l : 2 mg/l), I (0.5 mg/l : 0 mg/l), J (0.5 mg/l : 0.5 mg/l), K (0.5 mg/l : 1 mg/l), L (0.5 mg/l : 2 mg/l), M (1 mg/l : 0 mg/l), N (1 mg/l : 0.5 mg/l), O (1 mg/l : 1 mg/l), and P (1 mg/l : 2 mg/l).

these horticultural crops are underutilised and neglected vegetables such as sweet potato and taro (*Colocasia esculenta*) [20] whose micropropagation and germplasm preservation have been successful [21–25]. In this study, the Livingstone potato was also subjected to micropropagation techniques, and the results are discussed here.

While the micropropagation of the Livingstone potato has been previously carried out [13, 26], genetic differences within the *Plectranthus* species call for the optimization of the regeneration protocol. The plant material used in this study was from Livingstone potato landraces *Chibanda*, *Ndurwe*, *Chizambezi*, and *Musande* which are indigenous to Zimbabwe. The explant source has been proved to be an important factor for *in vitro* growth and development of plant species, affecting callus induction and adventitious bud induction as well as shoot regeneration [27]. Physiologically, younger plant tissue is generally regarded to be more responsive *in vitro* [28] and this was taken into account in this study by using the sprouts of the tuberos roots. The different explants were cultured in the MS medium which is the most widely used culture medium that most plant cell cultures react to favourably [29].

Kyesmu [11] developed the original basic protocol for the micropropagation of Livingstone potato and used shoots and nodes as the explants. In previous research carried out by Allemann [13], Livingstone potato sprouts were used as

the source of explant material where axillary buds and shoots were cultured and successfully produced plantlets. In this study, shoot tips, nodes, and leaves were used to determine the best explant on the four landraces. Plant regeneration was successfully achieved by all the explant types. This can be explained by the aspect of totipotency, a cell characteristic which plant tissues have that aids them to retain regeneration potential from the adult organism. Organogenesis allowed roots and shoots to be induced and differentiate in the respective explants that were used in the study [29]. However, the least shoots, nodes, and roots were recorded when leaf explants were used because leaves have fewer axillary buds that can develop into shoots. In addition, the levels of endogenous growth regulators in leaves which would promote root and shoot development are also low. The results of this study are in agreement with those that were obtained by Addae-Frimpomaah et al. [18] and Doliński and Olek [22] on *in vitro* propagation of sweet potato node explants.

The genetic differences within the species are demonstrated in the different responses exhibited by the four landraces of Livingstone potato using the different types of explants. The results are in agreement with Ogero [30], who showed that differences existed between two sweet potato varieties with regards to their response to micropropagation. The best explant across the landraces was the node, thus

FIGURE 3: Mean shoot number of the Livingstone potato landraces in response to NAA: BAP in mg/l application. $P < 0.001$, $LSD = 2.57$. Error bars represent standard errors of the differences. Plant growth regulators key: A (0 mg/l: 0 mg/l), B (0 mg/l: 0.5 mg/l), C (0 mg/l: 1 mg/l), D (0 mg/l: 2 mg/l), E (0.2 mg/l: 0 mg/l), F (0.2 mg/l: 0.5 mg/l), H (0.2 mg/l: 2 mg/l), I (0.5 mg/l: 0 mg/l), J (0.5 mg/l: 0.5 mg/l), J (0.5 mg/l: 0.5 mg/l), K (0.5 mg/l: 1 mg/l), L (0.5 mg/l: 2 mg/l), M (1 mg/l: 0 mg/l), N (1 mg/l: 0.5 mg/l), O (1 mg/l: 1 mg/l), and P (1 mg/l: 2 mg/l).

FIGURE 4: Mean leaf number of the livingstone potato landraces in response to NAA: BAP in mg/l application. $P < 0.001$, $LSD = 2.7$. Error bars represent standard errors of the differences. Key: A (0 mg/l: 0 mg/l), B (0 mg/l: 0.5 mg/l), C (0 mg/l: 1 mg/l), D (0 mg/l: 2 mg/l), E (0.2 mg/l: 0 mg/l), F (0.2 mg/l: 0.5 mg/l), H (0.2 mg/l: 2 mg/l), I (0.5 mg/l: 0 mg/l), J (0.5 mg/l: 0.5 mg/l), J (0.5 mg/l: 0.5 mg/l), K (0.5 mg/l: 1 mg/l), L (0.5 mg/l: 2 mg/l), M (1 mg/l: 0 mg/l), N (1 mg/l: 0.5 mg/l), O (1 mg/l: 1 mg/l), and P (1 mg/l: 2 mg/l).

FIGURE 5: Mean node number of the Livingstone potato landraces in response to NAA: BAP in mg/l application. $P < 0.001$, $LSD = 1.05$. Error bars represent standard errors of the differences. Plant growth regulators key: A (0 mg/l: 0 mg/l), B (0 mg/l: 0.5 mg/l), C (0 mg/l: 1 mg/l), D (0 mg/l: 2 mg/l), E (0.2 mg/l: 0 mg/l), F (0.2 mg/l: 0.5 mg/l), H (0.2 mg/l: 2 mg/l), I (0.5 mg/l: 0 mg/l), J (0.5 mg/l: 0.5 mg/l), J (0.5 mg/l: 0.5 mg/l), K (0.5 mg/l: 1 mg/l), L (0.5 mg/l: 2 mg/l), M (1 mg/l: 0 mg/l), N (1 mg/l: 0.5 mg/l), O (1 mg/l: 1 mg/l), and P (1 mg/l: 2 mg/l).

agreeing with findings by Kyesmu and Mantell [31]. Similar results were also obtained for sweet potato [18, 22] which is also a tuberous root like the Livingstone potato. Overall, the *Musande* landrace performed the best for all the growth parameters. The node which was the best explant was then used in the second experiment to determine the suitable PGR combination for the production of the plantlets.

Growth regulators used in tissue culture are synthetic chemical versions of natural plant growth regulators that are applied to plants to produce a desired effect such as shoot and root proliferation [32]. The most common plant growth regulators used in micropropagation include gibberellins, auxins, and cytokinins. Previous research has shown that the use of plant growth regulators is not necessary in the micropropagation of vegetatively propagated tuber crops [13]. However, other researchers have extensively demonstrated that there is merit in using these chemicals to enhance the growth and development of plants during micropropagation. In this study, naphthalene acetic acid (NAA) and benzyl amino purine (BAP) at different combinations were used for the micropropagation of the Livingstone potato so as to determine if the culture medium needed to be optimized for the local landraces in Zimbabwe.

4.1. Root and Shoot Initiation. *Chizambezi* and *Chibanda* initiated roots and shoots in the shortest time at high and low concentrations of NAA: BAP, while *Musande* took the

longest time to initiate roots and shoots when NAA and BAP were absent from the MS culture medium. Liljana et al. [33] also achieved successful root and shoot initiation on potato with the addition of the same plant growth regulators added to the MS culture medium. A similar result was also reported by Ezeibekwe et al. [34], on their work on white yams (*Dioscoera rotundata* L.). According to Schmülling [35], the ratio of cytokinins to auxins *in vitro* determines the differentiation of cultured plant tissues to either shoots or roots. In this study, a combination of NAA and BAP produced the best results for shoot initiation for *Chizambezi* and *Chibanda* compared to the use of BAP acting alone in the MS medium culture. BAP is regarded as the best phytohormone for shoot initiation [36, 37]. This result is supported by findings by Kabir [38], on the research on cassava. *Chizambezi* and *Chibanda* both benefitted from the addition of the plant growth regulators even in low quantities, thus disproving the findings of Allemann [13], who worked on the same *Plectranthus* species. Genetic differences also accounted for the difference in performance amongst the four landraces.

4.2. Shoot, Leaf, and Node Number of the Plantlets. The highest shoot number was recorded in treatments that had a combination of NAA: BAP at 0.2 mg/l: 1 mg/l for *Musande* and *Ndurwe*. However, the shoots were structurally small and short. Masekesa et al. [39] showed that high BAP

FIGURE 6: Mean shoot length of the livingstone potato landraces in response to NAA: BAP in mg/l application. $P < 0.001$, $LSD = 1.53$. Error bars represent standard errors of the differences. Plant growth regulators key: A (0 mg/l : 0 mg/l), B (0 mg/l : 0.5 mg/l), C (0 mg/l : 1 mg/l), D (0 mg/l : 2 mg/l), E (0.2 mg/l : 0 mg/l), F (0.2 mg/l : 0.5 mg/l), H (0.2 mg/l : 2 mg/l), I (0.5 mg/l : 0 mg/l), J (0.5 mg/l : 0.5 mg/l), J (0.5 mg/l : 0.5 mg/l), K (0.5 mg/l : 1 mg/l), L (0.5 mg/l : 2 mg/l), M (1 mg/l : 0 mg/l), N (1 mg/l : 0.5 mg/l), O (1 mg/l : 1 mg/l), and P (1 mg/l : 2 mg/l).

TABLE 4: Summary of landrace performance due to application of PGRs.

Parameter	Landrace and NAA:BAP combination (mg/l)			
Days to root initiation	Chibanda and Chizambezi K (0.5 mg/l:1 mg/l)			
Days to shoot initiation	Chibanda and Chizambezi D (0 mg/l: 2 mg/l, H (0.2 mg/l: 2 mg/l) and L (0.5 mg/l: 2 mg/l)		Chizambezi P (1 mg/l: 2 mg/l)	
No. of shoots	Ndurwe and Musande G (0.2 mg/l:1 mg/l) shoots were structurally small and short			
No. of leaves	Chibanda, Chizambezi, and Ndurwe B-E (0 mg/l: 0.5 mg/l–0.2 mg/l: 0 mg/l)	Chizambezi I-J (0.5 mg/l: 0 mg/l–0.5 mg/l: 0.5 mg/l)	Ndurwe, Chizambezi, and Chibanda L-M (0.5 mg/l: 2 mg/l–1 mg/l: 0 mg/l)	
No. of nodes	Chibanda and Ndurwe A-E 0 mg/l: 0 mg/l–0.2 mg/l: 0 mg/l	Chizambezi I (0.5 mg/l: 0 mg/l)	Chizambezi, Ndurwe, and Musande M (1 mg/l: 0 mg/l)	Musande O (1 mg/l: 1 mg/l)
Length of shoots	Musande and Ndurwe D-F (0 mg/l: 2 mg/l–0.2 mg/l: 0.5 mg/l)	Ndurwe M (1 mg/l: 0 mg/l)	Musande O (1 mg/l: 1 mg/l)	

concentrations tend to produce shoots that are shorter in stature because cytokinins encourage growth mainly through promoting cell division at the expense of cell expansion. With regards to the leaf number, high numbers per main shoot were observed for *Chizambezi*, *Chibanda*, and *Ndurwe* under NAA alone, BAP alone, and when the two plant growth regulators were combined at varying concentrations. The variation in mean leaf numbers amongst the three landraces could be linked to the levels of endogenous cytokinins available in the buds of the explant instead of the influence of the ones added externally as reported by

Demeke et al. [40]. *Musande* had the lowest leaf number, a result which could be attributed to the fact that leaf number is a function of genotype specificity [41].

The number of nodes is an important aspect in the micropropagation, especially in this study where the node was the explant of choice. The higher the number of nodes produced, the faster the production of more plantlets will be carried out through subculturing. All four landraces responded well to the application of the PGRs at varying concentrations (0:0-1:1) mg/l by producing high node numbers on the shoots. This result is in line with findings by

[34] on white yams although at a concentration of NAA: BAP (0.5:0.2) mg/l.

4.3. Shoot Length of the Plantlets. *Musande* and *Ndurwe* produced the longest shoots compared to the other two landraces when cultured using the NAA: BAP at a concentration of range of 0-1 mg/l: 0-0.5 mg/l, respectively. This can be attributed to the synergistic effect of the NAA and the BAP when applied together at a low concentration [42]. This result is also supported by Demeke et al. [40] who achieved a similar result on nodal explants of cassava (*Manihot esculenta*). On the other hand, Ezeibekwe et al. [34] found contrasting results with the white yam where longer shoots were obtained without the addition of NAA and BAP to the MS culture medium. The influence of the genotype could also have impacted the results obtained amongst the landraces and their response to the PGRs.

5. Conclusions

The obtained results showed that the best explant type were nodes where the *Musande* landrace had the best results for this explant type. While all four landraces were able to produce plantlets from the nodes, *Chizambezi* was the best landrace with regards to the growth parameters measured under the different NAA: BAP concentrations. *Chizambezi* is highly recommended for the rapid multiplication of Livingstone potato using tissue culture. Results from this study also clearly demonstrated that the addition of PGRs is essential for optimizing the growth media in enhancing the micropropagation of Livingstone potato. Commercial production of plantlets can, therefore, be carried out in Zimbabwe using the optimized protocol to provide healthy planting material for the communal farmers for improved productivity. In addition, production of planting material using tissue culture ensures the preservation of the germplasm, a critical aspect considering that the crop is underutilised and is facing extinction in Zimbabwe.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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