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Micropropagation of medicinally important enset (Ensete ventricosum (Welw.) Cheesman) landraces

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Abstract

Cultivated enset (*Ensete ventricosum* (Welw.) Cheesman) is commonly propagated vegetatively due to various reasons. This conventional mode of propagation is vulnerable to transmission of devastating diseases like bacterial wilt, and results in poor propagation rate. Most of the landraces used in traditional medicine are susceptible to diseases, and vulnerable to various other constraints. Micropropagation of two landraces (*guarye* and *kibnar*) used in traditional medicine was conducted using shoot tip explants on Murashige and Skoog medium. At the initiation stage, significantly highest systemic contamination (17.67%) was recorded on MS medium without antibiotics (cefotaxime) as compared to the treatments that contain 250 and 500 mg l^{-1} cefotaxime. The highest mean shoot number (8.8±1.40) per shoot tip was regenerated on MS medium containing 5.0 mgl^{-1} BAP in combination with 1.0 mg l^{-1} IBA. From the two enset landraces, higher mean shoot number (6.88) per shoot tip was obtained from *guarye*. The highest root number (3.0) per shoot was obtained from both full and half-strength MS medium containing 3.0 mgl^{-1} IBA and 2.0 mgl^{-1} IBA, respectively. The protocol can be employed for rapid propagation and conservation of the landraces as well as for its future genetic improvement using biotechnological tools.

Key Message

Efficient micro-propagation protocol has been developed for medicinally important landraces of enset (*Ensete ventricosum*), to overcome poor propagation rates and diseases transmission problems of conventional method.

Introduction

Ensete ventricosum is one of the oldest cultivated crops domesticated as early as 10 000 years ago (Brandt et al. 1997), and Ethiopia is an independent primary centre of origin for *Ensete ventricosum* (Vavilov 1951). Enset is mainly produced for carbohydrate-rich human food stored in its pseudostem and underground corm, and contributes to the food security and rural livelihoods of about 20 million people in Ethiopia (Yemataw et al. 2016). It has also a significant contribution for animal feed especially in dry season. Since enset can tolerate prolonged drought and flooding, it is a priority crop in most parts of southern nations, nationalities and peoples region of Ethiopia, where it is produced as a staple food. Enset contributes to the improvement of local environment through soil nutrient balance (Elias et al. 1998), providing shade, and being part of farming systems with high biodiversity (Tesfaye 2008). The industrial use of enset products including; Bulla as a gelling agent in place of agar for in vitro propagation (Ayenew et al. 2012; Mengesha et al. 2012), and enset starch as a pharmaceutical applications (Wondimu et al. 2014) were also reported.

Some enset landraces play essential role in traditional medicine. Corms of a number of enset landraces are reported to have medicinal importance in the treatment of bone fracture or other bone related human ailments, and to cure similar disorders in domestic animals (Tsehaye and Kebebew 2006; Daba and Shigeta 2016). This could be probably related to their superiority in phosphorus, zinc and tannin contents which are known to have beneficial effects on bone growth or healing of wound (Nuraga et al. 2019a). The highest arginine content of medicinal landraces, which involves in collagen formation, tissue repair and wound healing (Tamrat et al 2020) can be the other reason for use of these landraces selectively. It was reported that most of the landraces are susceptible to diseases like bacterial wilt, and drought (Nuraga et al. 2019b). Moreover, they are highly preferred by wild animals like porcupine and wild pig (Negash 2007). Due to these reasons the production of medicinal enset landrace is highly constrained.

Cultivated enset is commonly propagated vegetatively due to the poor germination rate of the seed and harvested before flowering of the crop (Negash, 2000), and probably to get a true to type clones. Unlike banana cultivated enset do not produce suckers naturally, instead it must be induced artificially by cutting down the immature, about 2–4 years of old plant and removing the apical meristem. Using this method, variable number of sucker production (10 to 100) per plant was reported (Gebre-Mariam 1996; Diro and Tabogie 1994; Shumbulo et al. 2012; Yemataw et al. 2018). Rotting of some of the buried corms in a soil during propagation, and the production of very few suckers is common, especially for the landraces used in traditional medicine (personal communication), indicating poor regeneration performance of these landraces.

This conventional mode of propagation is also vulnerable to transmission of devastating diseases like bacterial wilt. As far as we know, this disease has no treatment and no resistant variety is developed so far. According to McKnight CCRP, 2013, as cited by Yemataw et al. (2017), up to 80% of enset farms in Ethiopia are infected with this disease, which indicates that the chance of getting disease free planting material is very low. In addition to that, waiting two to four years in order to get a mother plant capable of producing optimum number of suckers for multiplication is another problem in the traditional propagation method. In this regard, micropropagation can have a great role in facilitating mass propagation and overcome some of the limiting factors related to conventional approaches, especially for those landraces used in traditional medicine which are more vulnerable due to a number of natural and human related factors (Nuraga et al. 2019).

Few studies are reported on micropropagation of enset using different sources of explants (Zeweldu and Lüdders 1998; Negash et al. 2000; Diro 2003; Birmeta and Welander 2004 and Diro and Staden 2004). Some of the attempts were failed due to high oxidation of phenols which resulted in the death of explants. While in others only partial success or low rate of multiplications were reported. Moreover, micropropagation of only few economically important enset landraces was reported in the previous studies. As genotype plays a significant role in different phases of micropropagation, the developed protocols may not be appropriate for other valuable landraces (Negash et al. 2000). Therefore, this study was conducted to develop a protocol for micropropagation of selected medicinally important enset landraces (*guarye* and *kibnar*) using shoot tip explants.

Materials And Methods

Plant material

Two types of enset (*Ensete ventricosum*) landraces (*guarye* and *kibnar*), which are important in traditional treatment of bone fracture and other diseases were used in this experiment. Three to four-year-old plants, which were obtained from farmers' backyard at Gurage zone, Cheha district were used to multiply suckers as a source of explants. To have enough number of explants, the source plants were multiplied using traditional method in a greenhouse at Wolkite University. The sprouts were emerged after 2 months, and the suckers were separated and transplanted individually in to 16 cm diameter polyethylene bags 2 months after emergence as shown in Fig. 1. The suckers that were grown for 2 months after transplanting were used as starting material in the micropropagation experiment.

Culture Initiation

From the suckers of greenhouse grown *guarye* and *kibnar* enset landraces, shoot tips with some portion of corms having 4 to 5 cm in length and 2 to 3 cm in diameter were excised using a stainless steel knife and placed separately into labelled beakers. In order to clean the explants from dirt, and to remove phenol exudates that have detrimental effect on the growth and development, the explants were washed under running tap water and detergents for fifteen minutes. The explants were trimmed to a length of 2.5 to 3.0 cm, and around 1.5 cm in diameter, and rinsed three times with sterile distilled water. The explants were then surface sterilized by 70% (v/v) ethanol for 10 minutes. After rinsing three times with sterile distilled water, further sterilization was carried out using 40% (v/v) sodium hypochlorite (NaOCl) solution (2% active chlorine) containing three drops of Tween-20 for 25 min, and then rinsed three times again with sterile distilled water.

After the final sterilization, the explants were trimmed to a final size of 1.0 cm length and 1.0 cm diameter shoot tip explants with subtended corm. The explants were then splitted in to half longitudinally based on recommendations of Diro and Staden (2005). Then each half of the shoot tips was cultured into glass jars containing 60 ml MS medium supplemented with 30 g l^{-1} sucrose, 1.5 g l^{-1} activated charcoal and 1.5 mg l^{-1} BAP and solidified with 8 g l^{-1} agar. In all cases the pH of the media were adjusted to 5.8 before autoclaving at a temperature of 121°C and, pressure of 105 kPa for 15 min. In order to study the effect of antibiotic in controlling the endogenously born bacterial contamination, two different concentrations (250 and 500 mg l^{-1}) cefotaxime, sterilized using 0.22 mm sieve were incorporated to the autoclaved MS medium, after cooling in laminar air-flow hood, while the control treatments were devoid of the cefotaxime.

The cultures were maintained in a dark, at room temperature for 15 days until the new shoots emerge, then after, placed in a growth shelf under light intensity of 22 µmol m^{-2} s^{-1} and 16 h photoperiod provided by cool-white fluorescent lamps. They were sub-cultured in 23–25 days in to the same medium compositions so that the corms at the base swollen and ready for multiplication experiment. The two

types of enset landraces and three concentration of cefotaxime formed 6 (2x3) treatments, which were replicated 20 times and arranged in a complete randomized design (CRD).

Regeneration of Multiple Shoots

The initiated shoots were cultured for 15 days on the same medium except antibiotics, to avoid carryover effect of cefotaxime. After 15 days, the explants were cultured on bud proliferation medium: MS medium supplemented with different concentration of BAP (0.0, 2.5, 5.0 and 7.5) alone and in combination with IBA (0.0, 0.5, 1and 2), 30 g l^{-1} sucrose, 1.5 g l^{-1} activated charcoal and solidified with 8 g l^{-1}agar. During culturing, the external dark/brown portions of corms of initiated shoots were trimmed with a sterilized blade. The cultures were then maintained at room temperature in the dark for 15 days, until multiple buds start to regenerate, and then maintained under 16 h photoperiod, provided by cool-white fluorescent lamps at room temperature. The two enset landraces and 10 concentrations of PGRs formed 20 (2x10) treatments and they were replicated 5 times, and arranged in CRD. After 23 to 25 days, 75% of the cultures were used for rooting experiment, while the remaining were successively sub-cultured on the best performed bud proliferation medium (MS medium supplemented with 5.0 mg l^{-1}BAP in combination with 1.0 IBA) for further multiplication of the shoots.

Rooting of and acclimatization

Well grown individual shoots with expanded leaves were excised from shoot clusters and transferred to full and half salt strength MS medium each containing 5 different concentrations of IBA (0, 1, 2, 3 and 4 mgl^{-1}), 30g l^{-1} sucrose, 1.5g l^{-1} activated charcoal which were solidified by 8 g l^{-1}agar. The two enset landraces, the 10 media combinations formed 20 (2x10) treatments, which were replicated 5 times and arranged in CRD. The pH of the medium and the growth condition were as indicated in shoot multiplication stage.

The rooted plantlets were removed from the culture vessels and the roots were washed gently under running tap water followed by planting on perforated seed trays containing forest soil and sand in a ratio of 2:1, respectively. The trays were labelled and covered with polyethylene bags and kept moist under shade in a greenhouse to ensure high humidity and to reduce the direct sun light effect. The humidity was gradually reduced by reducing watering frequency and by reducing the shade. Then the shade was totally removed after 21 days and the plantlets were kept in an open space, in the greenhouse, and they were transferred to polythene bags filled with forest soil and sand in a 2:1 ratio, respectively.

Data collection and statistical analysis

The data on number of contaminated explants due to endogenous microorganisms (those occurred at the base of the explants in the culture, after 7th day), number of shoots per shoot, shoot length, number of roots per shoot, length of roots, and performance of plantlets during acclimatization in the greenhouse were recorded. In all cases the data were then subjected to analysis of variance using SAS statistical software, version 9.2 (SAS, 2002), and significant treatment means were separated using Fisher's least significant difference (LSD) test at a 5% probability.

Results

Surface sterilization and control of phenolic compounds

Surface sterilization of shoot tip explants using 70% (v/v) ethanol for 10 min, and 40% sodium hypochlorite (NaOCl) for 25 min, resulted in 96% contamination free explants in the first 7 days, a period at which exogenous contamination usually appears. On the other hand, the inclusion of 1.5 g l^{-1} activated charcoal in the culture medium was found to be very effective in controlling blackening of explants during culture initiation. Death of explants due to blackening was only 2% (data not shown), and no adverse effect was visible by using the activated charcoal.

Effect of cefotaxime on systemic microbial contamination

MS medium that does not contain cefotaxime showed significantly ($p < 0.05$) highest systemic contamination (17.67%), while the remaining two treatments that contain 250 mg l^{-1} and 500 mg l^{-1} showed less contamination (Table 1, Fig. 2a). The contamination level of *kibnar* enset landrace (13.56%) was statistically similar to *guarye* (12.78%). It was also recognized that there was no phytotoxicity (necrotic) effect due to the application of cefotaxime during the initiation stage.

Table 1
Effect of cefotaxime and type of landrace on level of contamination at initiation stage of enset micropropagation on MS medium

	Treatments	Percentage of contamination
		Mean ± SD
Cefotaxime concentration (mg/l)	0	17.67 + 4.13a
	250	11.50 + 3.83b
	500	10.33 + 3.61b
Type of	*Kibnar*	13.56 + 4.90a
landrace	*Guarye*	12.78 + 5.19a
Notes: SD = standard deviation. Means followed by the same letter are not significantly different at 5% probability level		

The effect of plant growth regulators and type of enset landrace on number and height of in vitro regenerated enset shoots

Significantly ($p < 0.05$) highest mean number of shoots (8.8 + 1.40) per shoot tip was regenerated from MS medium supplemented with 5 mg l^{-1} BAP in combination with 1.0 mg l^{-1} IBA, while the lowest average shoot number (3.4 + 0.97) was recorded on MS medium devoid of plant growth regulators (Table 2, Fig. 2b and c). From the two types of enset landraces, significantly highest mean numbers of shoots (6.88) per shoot were regenerated from *guarye*, while 6.08 shoots per shoot were regenerated from

kibnar landrace (Table 2). Moreover, from two successive sub-culturing of shoots on the best performed medium (MS medium supplemented with 5.0 mg l^{-1} BAP + 1.0 mg l^{-1} IBA), an average of 8 new shoots per shoot tip could be regenerated per month.

Table 2
Effect of plant growth regulators (PGRs) and type of landrace on number and height of in vitro regenerated shoots of enset on MS medium

	Treatments		Shoot number	Shoot height (cm)
			Mean ± SD	Mean ± SD
	0.0 BAP	0.0 IBA0	3.4 + 0.97d	5.13 + 0.7b
	2.5 BAP	0.0 IBA	5.2 + 1.40c	6.56 + 0.81 a
	2.5 BAP	0.5 IBA	4.8 + 1.03c	6.53 + 0.99 a
	05 BAP	0.0 IBA	7 + 1.70b	6.50 + 1.21 a
	5.0 BAP	0.5 IBA	7.6 + 1.26b	6.31 + 1.55a
PGRs (mg/l)	5.0 BAP	1.0 IBA	8.8 + 1.40a	6.25 + 1.62 a
	5.0 BAP	1.5 IBA	7.2 + 1.93b	6.44 + 1.73 a
	7.5 BAP	0.0 IBA	7 + 1.05b	6.47 + 1.82 a
	7.5 BAP	1.5 IBA	7 + 1.41b	6.31 + 0.98 a
	7.5 BAP	2.0 IBA	6.8 + 1.03b	6.16 + 0.89 a
Type of landrace	*Guarye*		6.88 + 2.18a	6.10 + 1.32a
	Kibnar		6.08 + 1.66b	6.43 + 1.30a

Notes: SD = standard deviation. Means followed by the same letter within the same column are not significantly different at 5% probability level

On the other hand, all levels of plant growth regulators combinations resulted in statistically similar average shoot height, ranging from 6.10 cm to 6.56 cm, while PGRS free medium produced the lowest average shoot height (Table 2). Similarly, the two types of enset landrace didn't show a significant difference in shoot height.

Rooting and acclimatization

Roots could be initiated on both full and half-strength MS medium containing different concentrations of IBA (Fig. 2d), but some level (15%) shoot-tip necrosis was observed, of which, 11% were died. Significantly ($p < 0.05$) equal highest mean root number (3.00 + 0.67 and 3.00 + 0.47 mg l^{-1}) per shoot was regenerated from full MS and half salt strength MS medium containing 3 mg l^{-1} IBA and 2 mg l^{-1} IBA respectively (Table 3; Fig. 2e). It was followed by full MS medium containing 2.0 and 4.0 mg l^{-1} IBA,

and half-strength MS with 3.0 mg l^{-1} IBA. The lowest mean root numbers per shoot (0.80 + 0.42 and 1.20 + 0.63), which are statistically at par, were recorded from half salt strength MS and full salt strength MS medium devoid of plant growth regulators respectively. On the other hand, statistically similar average numbers of roots per shoot (2.00 + 1.01 and 1.86 + 0.81) were regenerated from the two types of enset landrace, *guarye* and *kibnar*, respectively (Table 3).

Table 3
Effect of plant growth regulators (PGRs) and type of landrace on number and height of in vitro regenerated shoots of enset on MS medium

	Treatments	Root number	Root length (cm)
		Mean ± SD	Mean ± SD
Media (MS/1/2MS) + IBA (mg/l)	MS + 0.0 IBA	1.20 + 0.63cd	1.77 + 0.17d
	MS + 1.0 IBA	1.60 + 0.70c	2.25 + 0.18b
	MS + 2.0 IBA	2.10 + 0.57b	2.26 + 0.23b
	MS + 3.0 IBA	3.00 + 0.67a	2.50 + 0.18a
	MS + 4.0 IBA	2.30 + 0.48b	2.46 + 0.10a
	1/2MS + 0.0 IBA	0.80 + 0.42d	1.77 + 0.24d
	1/2MS + 1.0 IBA	1.50 + 0.53c	2.00 + 0.41c
	1/2MS + 2.0 IBA	3.00 + 0.47a	2.01 + 0.17c
	1/2MS + 3.0 IBA	2.20 + 0.42b	2.26 + 0.12b
	1/2MS + 4.0 IBA	1.60 + 0.52c	1.92 + 0.16cd
Type of landrace	*Guarye*	2.00 + 1.01a	2.08 + 0.39a
	Kibnar	1.86 + 0.81a	2.10 + 1.37a

Notes: SD = standard deviation. Means followed by the same letter within the same column are not significantly different at 5% probability level

Significantly the highest ($p < 0.05$) mean root length (2.50 + 0.18 cm and 2.46 + 0.10 cm) were regenerated from shoots cultured on MS medium supplemented with 3.0 mg l-1 and 4.0 mg l^{-1} IBA respectively (Table 3). They were followed by MS medium containing 1.0 mg l^{-1} or 2.0 mg l^{-1} IBA, or half-strength MS medium fortified with 3 mg l^{-1} IBA which produced statistically equal average root length. Whereas, statistically similar mean lowest root length (1.77 + 0.17 cm, 1.77 + 0.24 cm, 1.92 + 0.16 cm) were recorded from both PGRs free full and half-strength MS medium, and from half-strength MS medium supplemented with 4.0 mg l^{-1} IBA respectively (Table 3). However, no significant difference in mean root length was observed among the two enset landraces (Table 3).

Among the plantlets transferred to soil, 75% survived in a greenhouse after acclimatization, while the remaining 25% of the plantlets from both *guarye* and *kibnar* landraces were died during acclimatization. The plants individually transferred to polyethylene bag were well established, and no phenotypic variation was observed after two months (Fig. 2f).

Discussion

Although the plant materials are disinfected and all vessels and media are sterilized properly, microbial contamination in tissue culture, especially bacteria are reported (Leifert and Cassells 2001; Mona et al. 2019). Such contaminants resistant to surface disinfestations are likely to be endophytic (Reed et al. 1995), and they can be controlled by antibiotics (Gubišová and Gubiš 2019). The role of cefotaxime antibiotics in controlling bacterial contamination of enset shoot tip culture was investigated on the two landraces of enset, *guarye* and *kibnar*. The result showed that, the inclusion of two concentrations (250 mg l^{-1} and 500 mg l^{-1}) cefotaxime on MS medium resulted in less bacterial contamination than the control (the medium devoid of cefotaxime). Similarly, Kumari et al. (2015), who compared the effect of different antibiotics on micropropagation of sugarcane, reported that cefotaxime at a concentration of 250 mg l^{-1} and 500 mg l^{-1} was the most effective in reducing systemic bacterial contamination as compared to other antibiotics.

Cefotaxime is usually considered to be non-toxic to plant cells (Ogawa and Mii 2004). No toxicity effect was also visible in the current study. The average level of contamination during culture initiation of the current study (13.17) was much lower than the loss of explants (50–60%) reported due to internal contamination of enset shoot tip culture without the use of antibiotics (Birmeta and Welander 2004), which indicates that the use of cefotaxime in the growth media is advantageous for efficient micropropagation of enset.

The amount or plant growth regulators required for micropropagation usually depends on the genotype used. In the current study, however, the interaction effect of PGRs and genotype was not significant. Similarly, the highest shoot proliferation rate of 7 enset landraces was recorded from one type of formulated medium (Birmeta and Welander 2004). The highest mean number of shoots (8.8) regenerated in the current study was much higher than the shoot number (3.6) reported by Diro (2003) from splitted shoot tips of *oniya* enset landrace on MS medium containing different concentration of PGRs. Negash et al. (2000) also reported less values (2.5, 2.6 and 2.3) of shoot number from three enset landraces (*choro*, *nobo* and *ketano*) respectively.

The mean shoot number recorded in the current study was much lower than the values (23 and 16) from shoot tip explants of *mazia* and *digomerza* enset landraces respectively, but comparable to the shoot number (7) from *arkiya* landrace on MS medium containing 4.5 mg l^{-1} BAP and 1.5 mg l^{-1} NAA (Gezahegn and Mekbib 2016). Regeneration of very high number of shoots from shoot tip culture on formulated macronutrients medium with double-standard concentration of MS micronutrients, with a wide range of variation among the landraces (14.7 to 74.7 shoots) was also reported (Birmeta and

Welander 2004). Since the authors used different basal media composition, and data recording was made lately, 9 to 10 weeks after initiation, making direct comparison is difficult. However, the wider variations even in the same experiments reported indicate much of the variation among the results seems to be due to the differences in the types of enset landraces used. The variation in multiplication response among enset landraces was also observed in the current study; from the two enset landraces, significantly higher number of shoot was regenerated from *guarye*.

The increase in concentration of BAP from 0.0 to 5.0 mg l^{-1} significantly increased the number of shoots regenerated per shoot. However, the increase in concentration of BAP from 5 mg l^{-1} to 7.5 mg l^{-1} didn't result in significant increase in shoot numbers. Diro (2003) also reported that, higher concentration of other forms of auxins, benzyl adenine, didn't show a significant increase in the number of shoots per shoot both with intact and halved shoot tips. From the successive culturing of shoots on the best performed media (MS medium supplemented with mg l^{-1} BAP and 1.0 mg l^{-1} IBA), an average 8.0 new shoots per shoot tip could be regenerated in one month.

Rooting of micro shoots was primarily initiated on full and half-strength MS medium containing different concentrations of IBA after 12 days of culturing. Similarly, Negash et al. (2000) reported the development of primary root in approximately ten days and the occurrence of secondary roots within the next four days. However, some of the explants showed the tendency of rooting while they were in multiplication and elongation medium, with or without PGRs. Birmeta and Welander (2004) also reported the development of roots in the elongation medium without a need of rooting stage. The highest mean number of roots per shoot (3.0) regenerated on both full and half-strength MS medium supplemented with 3.0 and 2.0 mg l^{-1} IBA, respectively, was slightly lower than the root number (3.77) reported by Gezahegn and Mekbib (2016) on MS medium supplemented with 2.0 mg l^{-1} IBA, but higher than the values (0.0 to 1.5) reported by Diro and Staden (2004) on MS medium supplemented with 12 different concentrations of auxins and cytokinins. The highest mean root lengths recorded in the current study was also by far lower than the value (5.96 cm) reported by Gezahegn and Mekbib (2016). The variation of the results could be due to the difference in the types of landraces used.

The results also indicated that there was an increasing trend of root number per shoot and mean root length with increased concentration of IBA up to 3.0 mg l^{-1} on full strength MS and, up to 2.0 mg l^{-1} and 3.0 mg l^{-1} on half-strength MS medium respectively. On the other hand, the highest root length was recorded from lowest concentration (0.5 mg l^{-1}) of IBA (Gezahegn and Mekbib 2016), the variation could be due to the difference in the types of landraces used.

Some level of shoot-tip necrosis was observed during rooting of shoots, of which, few (11%) of the shoots were died. Mathew and Philip (1996) reported that, shoot- tip necrosis is one of the constraints during *in vitro* regeneration of *Ensete superbum*. According to the authors, the cause for that was explained to be shortage of calcium in the growth medium. Birmeta and Welander (2004) could minimize enset shoot-tip necrosis by replacing calcium chloride with calcium gluconate monohydrate in the growth medium. On the other hand, Martin et al. (2007) reported that, shoot-tip necrosis in micropropagation of

banana was influenced by cultivar type, and from several methods tested to reduce the necrosis, only addition of calcium chloride was effective.

During acclimatization of plantlets in a simple greenhouse, which is not automated 75% survival rate was recorded for both *guarye* and *kibnar* after 21 days. A comparable survival rate (80 to 90%) of in vitro regenerated enset after 15 days of acclimatization was reported (Gezahegn and Mekbib 2016). Whereas, Birmeta and Welander (2004) also reported (100%) success in acclimatization of in vitro regenerated enset plantlets in a glass house. The variation of the results could be probably due to the difference in type of landrace, the condition of the greenhouse, or the season of acclimatization.

Conclusions

Most of enset landraces used in traditional medicine are more susceptible to devastating diseases like bacterial wilt and other various constraints. The conventional mode of enset propagation results in poor propagation rates and vulnerable to transmission of diseases. Efficient micro-propagation protocol has been developed using soot tip explants. MS medium supplemented with 5 mg l^{-1} BAP + 1.0 mgl^{-1} IBA was suitable for shoot multiplication of *Ensete ventricosum* landraces; *guarye* and *kibnar*, from which a mean shoot number (8.8) per shoot tip was regenerated. The shoots were better rooted on full and half strength-MS media containing 3.0 and 2.0 mgl^{-1} IBA respectively, and the plantlets were acclimatized in a greenhouse with 75% survival rate. As conventional method is associated with a number of drawbacks, this micropropagation protocol will have a great importance in conservation and rapid propagation of the vulnerable medicinal enset landraces.

Authors' Contributions

All authors designed the study. GWN carried out the laboratory work, data mining and analysis, and drafted the manuscript. All Authors were major contributors in interpreting the data and critical revision.

Declarations

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Competing Interests

The Authors have *no relevant financial or non-financial interests to disclose.*

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Figures

Figure 1

Ex vitro multiplication of *guarye* and *kibnar* enset landraces in a greenhouse as explant source for micropropagation: a) emerging shoots after 2 months of planting, b) suckers 2 months after emergence and c) sucker transplanted in to a pot individually, d) suckers grown for 2 months after transplanting

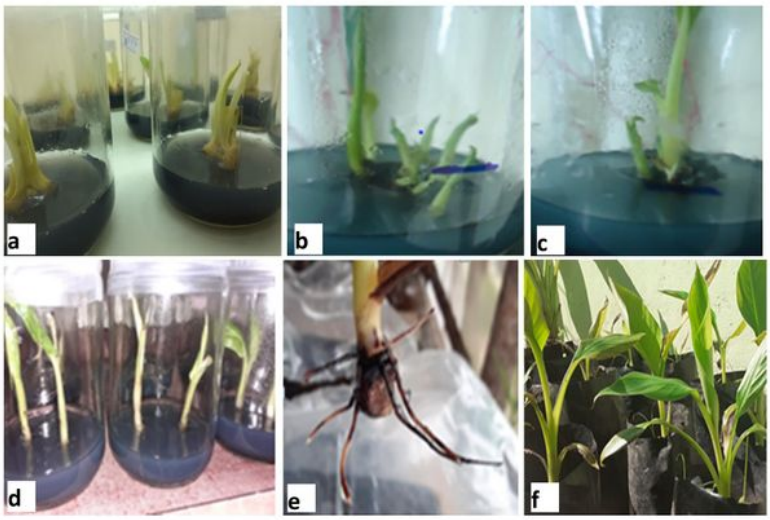

Figure 2

Micropropagation of *E. ventricosum, guarye* landrace from shoot tip on MS medium containing different concentration of BAP and IBA (a) shoot initiated from half shoot tip on MS medium containing1.5 mg l^{-1} BAP and 500mgl^{-1} cefotaxime after 2 weeks of culture. (b) and (c) Multiplication of shoots on MS medium containing 5 BAP+1.0 IBA and free of PGRs respectively after 10 weeks of culture. (d) Micro-

shoots cultured on rooting medium. (e) *In vitro* rooted shoots on MS medium containing 3 mgl^{-1} IBA after 2 weeks in rooting media. (f) Acclimatized plantlets in a soil media after 2 month of planting. Bars = 1cm