Chapter 5
Optimisation of Somatic Embryogenesis in Cassava

Kenneth E. Danso and Wilfred Elegba

Abstract Somatic embryogenesis, an efficient regeneration system that is being used successfully in genetic transformation, can be coupled with mutation induction to breed for cassava varieties with desired traits. The embryogenesis system requires the initiation of totipotent cells which can be used as targets for mutagenic treatments. However, initiation of these totipotent cells is low, highly genotypic dependent and has low plant conversion rate. Thus, for its successful application in mutation induction, the system needs to be optimised to overcome these drawbacks. To optimise the system, totipotent cells are initiated on embryo initiation medium (EIM) which consisted of Murashige and Skoog (Physiol Plant 15:473–497, 1962) basal salts supplemented with picloram and 2,4-dichlorophenoxy acetic acid (2,4-D) to produce callus. After 21 days, calli produced are cultured for embryo maturation on maturation medium (EMM) or can also be treated with mutagenic agents for mutant induction. The resulting primary embryos are recycled three consecutive times, each by fragmentation of the cotyledons followed by culture on embryo initiation medium to produce more matured embryos. Somatic embryos produced are then successfully converted into plants by abscisic acid pretreatment in embryo maturation medium or by air desiccation under the laminar flow hood. The methodology described offers optimised, reproducible procedures of somatic embryogenesis for its incorporation into mutation breeding programmes in cassava.

Keywords Somatic embryos • Cyclic embryogenesis • Picloram • 2,4-dichlorophenoxy acetic acid • Abscisic acid • Embryo desiccation

5.1 Introduction

Cassava has the potential to remain a food security crop for millions of people living in the tropics while also serving as starch substrate for many industrial applications. This duality of roles requires that cassava varieties targeting different

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end uses must be bred from existing landraces which are well adapted to the traditional farming practices. Conventionally, cassava is bred through sexual hybridisation. The crop is highly heterozygous, and cross- and self-pollination occur naturally. Consequently, there is high sexual hybridisation leading to high genetic diversity and long breeding cycle which are only useful for heritable traits. For traits lacking sources of heritable variation, recombinant DNA techniques or induced mutations might provide alternate means for introgression of desired traits into existing varieties.

Both recombinant DNA and mutation induction require the generation of totipotent cells or tissues that could act as targets for insertion of genes of interest or exposure to mutagenic agents. Of the several regeneration methods, only somatic embryogenesis (SE) has been identified as an efficient regeneration system for the development of such totipotent tissues in cassava (Feitosa et al. 2007; Puontis-Kaerlas 1997; Raemakers et al. 1997; Zainudin et al. 2012). These totipotent cells can be used for large-scale clonal propagation of genetically uniform elite clones (Verdreil Jean-Luc et al. 2007) or treated with mutagens (physical and chemical) to produce mutants with traits of interest (Joseph et al. 2004). The production of an intermediate embryogenic calli in indirect somatic embryogenesis can also serve as targets which can also be treated with mutagens (physical or chemical) to obtain mutants with useful traits.

Of the over 3000 mutant varieties in the IAEA database, only two cassava mutants have been documented (Forster and Shu 2012). These are *Tekbankye* and *Fuxuan* which were released in 1997 and 2005 in Ghana and China, respectively. Both mutants were obtained through the irradiation of stem cuttings using gamma rays (Asare and Safo-Kantanka 1995). The choice of gamma rays might be due to the bulky nature of the stem cuttings used for the irradiation. The alternate use of chemical mutagens may be limited by the bulky stem cuttings that do not allow easy absorption of the mutagens (Leitão 2012). Somatic embryos or friable embryogenic calli may therefore provide ideal propagules for mutation induction in the crop.

In spite of its usefulness, the somatic embryogenic system is highly genotype dependent often making protocols for embryo production non-reproducible and also has low plant conversion rate. Therefore, for its application in mutation induction, the procedures for embryo production needs to be optimised, made more efficient and reproducible.

Somatic embryos can be induced either directly or indirectly through a callus-phase intervention. The production of embryos via callogenesis involves three distinct steps. These are initiation of embryogenic competent cells from a suitable explant on embryo initiation medium amended with auxin (**Step 1**), maturation of somatic embryos on embryo maturation medium amended with (low auxin) or without auxin (**Step 2**) (Stamp and Henshaw 1987) (Fig. 5.1) and embryo conversion into plants on a standard cassava culture media for shoot development (**Step 3**).

Several authors have used different explants for primary somatic embryo induction. The most commonly used explants include cotyledons and embryonic axis from zygotic embryos (Konan et al. 1994; Stamp and Henshaw 1987), immature leaf lobes (Li et al. 1998), meristem and shoot tips (Puontis-Kaerlas 1997) and

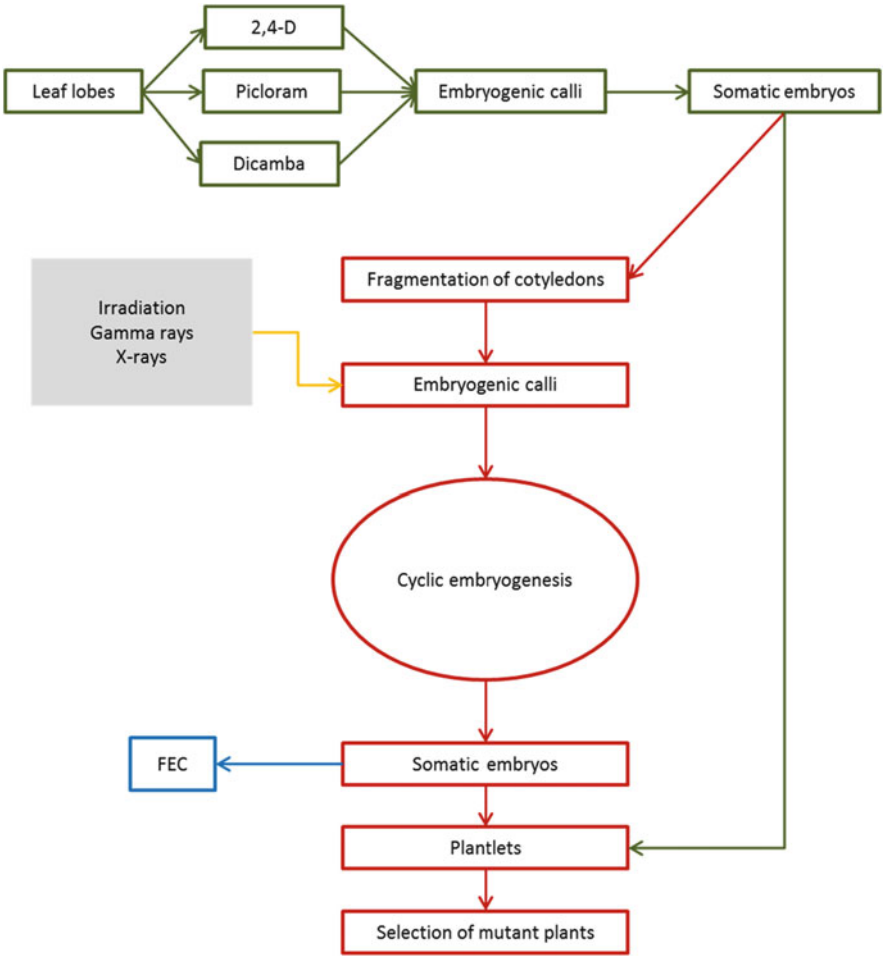

Fig. 5.1 Flow diagram illustrating production of primary, cyclic embryos and intervening calli in cassava which can be incorporated into mutation breeding programmes

BAP-enlarged axillary buds (Nyaboga et al. 2013). High embryogenic competence is, however, restricted to meristematic and embryonic tissues (Puontis-Kaerlas 1997). In cassava, the most commonly used explant is immature leaf lobes (Raemakers et al. 1997). Other factors including genotype, culture medium as well as growth regulators have been found to influence primary embryo production (Taylor et al. 2001).

5.1.1 Production of Cyclic Embryos

The frequency of primary somatic embryos production in cassava is very low (Raemakers et al. 1997). It is also a genotype-dependent process thereby making efficiency of the somatic embryogenesis system especially for mutation induction limited. In most embryogenic systems, primary embryos are often used as source of explant to scale up embryo production through recycling (Danso and Ford-Llyod 2004; Raemakers et al. 1993). In cyclic embryogenesis, cotyledons of matured primary embryos are fragmented, cultured on embryo initiation medium (solid or liquid MS medium supplemented with auxin) to induce embryogenic calli. The embryogenic calli induced contain globular embryos, which develop to cotyledonary stage on embryo maturation medium usually containing a cytokinin (0.1 mg/L BAP). Repetitive subculture of mature embryos every 21 and 30 days, respectively, leads to cyclic embryogenesis (Sofiari et al. 1997). The frequency of embryogenic competence decreases after three or four repetitive subcultures, and induction of new primary embryos is recommended to maintain competence.

In micropropagation, cyclic embryogenesis can be used to scale up the frequency of embryo multiplication through repeated subculture of matured embryos which are allowed to develop into organised somatic embryos (OSE) for conversion into plants (Fig. 5.1). The cyclic embryogenic technique is also useful in plant breeding as the intervening friable embryogenic calli (FEC) can be treated with physical (gamma or X-rays) or chemical mutagens (e.g. ethyl methanesulphonate, EMS) for production of valuable mutants (Joseph et al. 2004). The system can also be coupled with all cassava genotypes that are amenable to transformation. Successful production of somatic embryos via cyclic embryogenic system depends on the type of growth regulator in the culture medium and the number of repetitive cycles of subculture (Sofiari et al. 1997).

5.1.2 Influence of Growth Regulators on Primary Embryo Induction

A critical factor influencing somatic embryogenesis is the type of auxin used for embryo initiation. For several years, indirect somatic embryo production in cassava has relied on the use of synthetic auxin 2,4-dichlorophenoxy acetic acid (2,4-D) for embryogenic calli initiation prior to transfer to embryo maturation medium (Stamp and Henshaw 1987; Taylor et al. 2001). The use of 2,4-D for embryo initiation often results in low frequency of primary embryo production as well as poor conversion into plants due to lack of root primordium especially when duration of calli production on the initiation medium is prolonged (Raemakers 1993). More recently, many authors have reported of the successful use of other synthetic auxins such as picloram (4-amino-3,5,6-tricloropiconic acid) and dicamba (3,6-dichloroanisic acid) (Mongomake et al. 2015). The application of new

generation of growth regulators such as oligosaccharides, jasmonate, polyamines, brassinosteroids (George et al. 2008) and phloroglucinol (Teixeira da Silva et al. 2013) has also proved to be useful for primary somatic embryos induction in many plant species. Therefore to improve the embryogenic system for mutation induction, the use of synthetic auxins such as picloram or dicamba as well as jasmonates and brassinosteroids is worth investigating particularly in cassava landraces whose embryogenic competence is not known.

High frequency of somatic embryo production can also be achieved through the manipulation of the culture medium. It has been reported that a switch from Murashige and Skoog (1962) basal medium (MS) to DKW or DCR medium increased the production of somatic embryos even from older leaf explants which hitherto were considered non-embryogenic (Driver and Kuniyuki 1984). It has also been reported that additional copper sulphate or silver nitrate in the culture medium can also have positive effect on embryo production (Danso and Ford-Llyod 2004).

5.1.3 Somatic Embryo Conversion into Plants

In vitro culture of cassava via organogenesis or somatic embryogenesis can be deployed in mutation breeding programmes if the efficiency of plant conversion is optimised. It has been shown that the efficiency of somatic embryo maturation and plant conversion can be improved via the use of abscisic acid (ABA) which is known to synchronise embryo maturation (Groll et al. 2002; Quainoo and Dwomon 2012), confer desiccation tolerance (Torres et al. 2001) and enhance plant conversion (Quainoo and Dwomon 2012). Also, desiccation of somatic embryos prior to culture on standard cassava medium has been used to enhance plant conversion (Matthews et al. 1993). The protocol described below aims to optimise cassava somatic embryo induction at both the primary and the cyclic stage using the synthetic auxins 2,4-D and picloram. The effect of these two auxins on cyclic embryogenesis as well as the influence of ABA on embryo maturation and plant conversion of cassava accession is also worth investigating.

The somatic embryogenesis technique will be more beneficial in mutation induction if the duration and frequency of embryo production as well as plant conversion from somatic embryos are optimised to make it more efficient and applicable to all cassava genotypes. In the following protocols, we describe an optimised reproducible embryogenic system for Ghanaian cassava landraces for mass production of somatic embryos which can be treated with physical or chemical mutagens in mutation breeding programmes. The protocols can be adapted for other farmer- or industry-preferred cassava cultivars.

5.2 Materials

5.2.1 Chemicals and Equipment

1. 1 M HCl.
2. 1 M NaOH.
3. Laminar flow hood (NuAire Biological Safety Cabinet, UK).
4. Stereomicroscope (Leica Zoom 2000, China).
5. Magnetic hotplate stirrer.
6. Autoclave (Astell Scientific, UK).
7. Oven (Gallenkamp Hotbox, Sanyo).

5.2.2 Culture Media

Culture media was prepared from Murashige and Skoog (1962) basal salts powder (Sigma Aldrich). Gamborg B5 vitamins were prepared from stock solutions (*see* **Note 1**). The composition of the basal medium and Gamborg B5 solutions are shown below.

*5.2.3 Shoot Initiation Medium (***see** *Notes 2–4)*

Shoot initiation medium was prepared using Murashige and Skoog (1962) basal salts powder and Gamborg B5 vitamins (Table 5.3).

5.2.4 Embryo Initiation Medium

Embryo initiation medium was prepared using Murashige and Skoog basal salts and vitamins and Gamborg B5 vitamins. The composition of the medium is shown below. Sucrose and phytagel were added (*see* **Notes 2–5**).

5.2.5 Embryo Maturation Medium

Embryo initiation medium was prepared using Murashige and Skoog basal salts and vitamins and Gamborg B5 vitamins. The composition of the medium is shown below. Sucrose and phytagel were added (*see* **Notes 2–5**).

5.2.6 Somatic Embryo Conversion Medium

Embryo initiation medium was prepared using Murashige and Skoog basal salts and vitamins and Gamborg B5 vitamins. The composition of the medium is shown below. Sucrose and phytagel were added (*see* **Notes 2–6**).

5.3 Methods

5.3.1 Preparation of Shoot Initiation Medium

1. For the shoot initiation medium (SIM), mix together MS basal medium (MS) powder and Gamborg B5 vitamins (100 mg/L myo-inositol, 1.0 mg/L nicotinic acid, 1.0 mg/L pyridoxine-HCl, 10 mg/L thiamine-HCl and 2 mg/L glycine) modified with 30 g/L sucrose, 0.5 mg/L benzylaminopurine (BAP), 0.02 mg/L naphthalene acetic acid (NAA) and 0.5 mg/L GA_3 (Tables 5.1, 5.2 and 5.3).
2. Adjust the medium to pH 5.8 using 1 M NaOH or 1 M HCl.
3. Add 3.5 g/L of Phytagel for solidification (*see* **Note 3**).
4. Sterilise the medium in an autoclave at 121 °C for 15 min and a pressure of 15 psi.
5. Keep sterilised media at room temperature prior to usage or store in a fridge at 4 °C.

5.3.2 Collection and Sterilisation of Donor Plants

1. Collect cassava stakes from the field grown plants and transfer into the laboratory (*see* **Note 7**).
2. Prepare cuttings of 10–15 cm long each bearing two to three nodes.
3. Plant stakes in black polyethylene pots filled with loamy soil in a heat chamber at a temperature of 35–45 °C provided by six bulbs each of 200 W to enhance virus elimination.
4. Water planted stakes every other day using watering can in order to monitor water requirements and thereby provide sufficient water without water logging.

Table 5.1 Composition of Murashige and Skoog (1962) basal salts

Compound	Concentration (mg/L)
Ammonium nitrate (NH_4NO_3)	1650
Potassium nitrate (KNO_3)	1900
Calcium chloride ($CaCl_2 \cdot 2H_2O$)	440
Potassium hydrogen phosphate (KH_2PO_4)	170
Magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$)	370
Boric acid (H_3BO_3)	6.2
Sodium molybdate ($NaMoO_4 \cdot 2H_2O$)	0.25
Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$)	0.025
Copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$)	0.025
Zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$)	8.6
Manganese sulphate ($MnSO_4 \cdot 4H_2O$)	22.3
Potassium iodide (Kl)	0.83
Na_2EDTA	37.25
$FeSO_4$	27.85

Table 5.2 Composition of Gamborg B5 vitamins and growth regulators (*see* **Note 2**)

Myo-inositol	100 mg/L
Nicotinic acid	1.0 mg/L (×1000)
Pyridoxine-HCl	1.0 mg/L (×1000)
Thiamine-HCl	1.0 mg/L (×1000)
Glycine	2.0 mg/L (×1000)
Growth regulators	
BAP	0.5 mg/L (×1000)
NAA	0.02 mg/L (×1000)
GA_3	1.0 mg/L (×1000)

Table 5.3 Composition of shoot initiation medium

MS medium	4.4 g/L
Myo-inositol	100 mg/L
Nicotinic acid	1.0 mg/L
Pyridoxine-HCL	1.0 mg/L
Thiamine-HCL	1.0 mg/L
Glycine	1.0 mg/L
Benzylaminopurine	0.05 mg/L
Naphthalene acetic acid	0.02 mg/L
Sucrose	30 g/L
Phytagel	3.5 g/L

5.3.3 Sterilisation and Culture for Shoot Initiation

1. Harvest 14–20 days old sprouted shoots into a honey jar bottle and wash under running tap water for 1 h in the laboratory.

2. Sterilise shoot tips under the laminar airflow hood (NuAire Biological Safety Cabinet, UK).
3. Wash them with sterilised distilled water mixed with Tween 20 for 30 min (*see* **Note 8**).
4. Wash shoot tips again with three changes of sterilised distilled water.
5. Shoot tips are then first immersed in 70 % (v/v) aqueous ethanol for 5 min.
6. Washed shoot tips again with sterilised distilled water. This procedure is repeated three times to complete the sterilisation regime.
7. Dissect the shoot tips under stereomicroscope by gradual removal of leaves using sterilised forceps and scalpel blades which have previously been sterilised in an oven at temperature of 110 °C for 2 h.
8. Excise meristematic dome/tissues (0.2 mm in diameter) with one or two leaf primordia from the dissected shoot tips (*see* **Note 9**).
9. Culture meristematic tissue on the shoot initiation medium (Table 5.3).

5.3.4 Culture Incubation

Maintain the cultures under growth room conditions at a temperature of 26 ± 1 °C, 16/8-h light/dark photoperiod with light provided by white fluorescent tubes at intensity of 3500 lux.

5.3.5 Initiation of Primary Somatic Embryos

1. Excise young leaf lobes (1–3 mm) from 8-week-old in vitro-grown plantlets under a stereomicroscope using forceps and scalpel (*see* **Note 10**).
2. Culture excised leaf lobe on embryo initiation medium which consisted of modified MS basal salts and Gamborg B5 vitamins supplemented with 8 mg/L 2,4-D or 16 mg/L picloram (*see* Table 5.3 and **Notes 2, 4 and 11**).
3. Place the leaf lobes with their adaxial surfaces in contact with the medium (*see* **Note 12**).
4. Incubate cultures in a dark box (or wrap in aluminium foil) for 21 days to enhance calli formation.
5. Examine leaf lobe explants for embryogenic calli formation after 21 days (*see* **Note 13**).
6. Transfer into embryo maturation medium (Table 5.4).
7. Transfer Petri dishes containing the embryogenic calli into growth room lighting conditions for embryo maturation.
8. Count the number of embryos with well-distinct cotyledons 14 days after culture on embryo maturation medium. Mature embryos have well-developed cotyledons with distinct hypocotyl (Fig. 5.2).

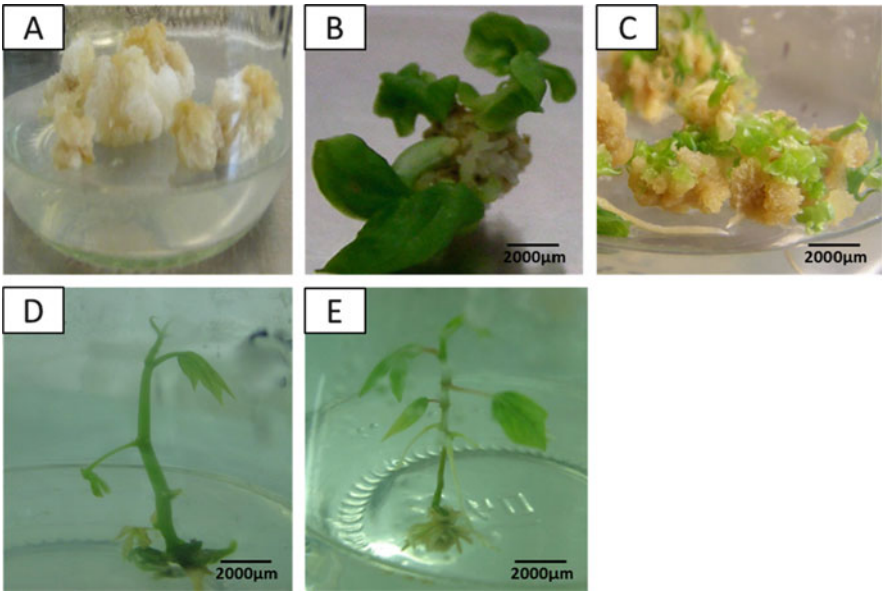

Fig. 5.2 (**a**) Callus initiated on EIM supplemented with picloram. (**b**) Primary somatic embryos obtained from leaf lobes. (**c**) Cyclic embryos obtained from fragmented cotyledons. (**d**) Plantlet obtained from desiccated embryo. (**e**) Plantlets obtained from ABA pretreated somatic embryos

5.3.6 Cyclic Embryo Initiation and Production

1. Isolate matured primary somatic embryos (*see* Figs. 5.1 and 5.2 and **Note 14**).
2. Fragment cotyledons in Petri dishes with a scalpel blade under the laminar flow hood (*see* **Note 15**).
3. Transfer 0.2 g of somatic embryos to embryo initiation medium (Table 5.5).
4. Incubate the cultures in dark box or wrap in aluminium or an incubator without light for 21 days to enhance calli development.
5. Examine cultured fragmented embryos for embryogenic calli formation.
6. Transfer cultures into embryo maturation medium (Table 5.5)
7. Transfer into growth room lighting conditions (16/8 h day/dark photoperiod, 26 °C light intensity of 3500 lux) for maturation of somatic embryos.
8. Isolate matured somatic embryos after 14 days.
9. Fragment isolated embryos again and culture on primary embryo initiation medium (Table 5.5) for continuation of cyclic embryogenesis (Fig. 5.1).
10. Repeat the procedure for three subcultures (*see* **Note 16**).

Table 5.4 Composition of embryo maturation medium

MS powder	4.4 g/L (Table 5.1)
Gamborg B5 vitamins	(Table 5.2)
Sucrose	30 g/L
Phytagel	3.5 g/L
$CuSO_4$	2 µM
BAP	0.1 mg/L

Table 5.5 Composition of embryo initiation medium

MS powder	4.4 g/L (Table 5.1)
Gamborg 5 vitamins	(Table 5.1)
Phytagel	3.5 g/L
Sucrose	30 g/L
2,4-dichlorophenoxyacetic acid (2,4-D)	8 mg/L
Picloram (4-amino-3,5,6-tricloropicolinic acid)	16 mg/L
Copper sulphate ($CuSO_4$)	2 µM

Table 5.6 Composition of plant conversion medium

MS powder	4.4 g/L (see Table 5.1)
Gamborg B5 vitamins	(see Table 5.2)
Sucrose	30 g/L
Phytagel	3.5 g/L
$CuSO_4$	2 µM
BAP	0.1 mg/L
ABA	1.0 mg/L

5.3.7 Abscisic Acid Effect on Conversion of Somatic Embryos into Plant

1. Weigh 0.2 g of embryogenic calli induced on embryo initiation medium (Table 5.5).
2. Transfer cultures to embryo maturation medium (Table 5.6) and add 1 mg/L ABA. The concentration of ABA has influence on plant conversion from somatic embryos (*see* Fig. 5.3 and **Note 6**).
3. Transfer cultures to the growth room conditions (16/8 h day/light photoperiod, temperature of 26 °C) for incubation for 30 days.
4. Isolate matured embryos from the embryogenic calli.
5. Transfer isolated embryos to standard cassava culture medium for plant conversion (Table 5.3).

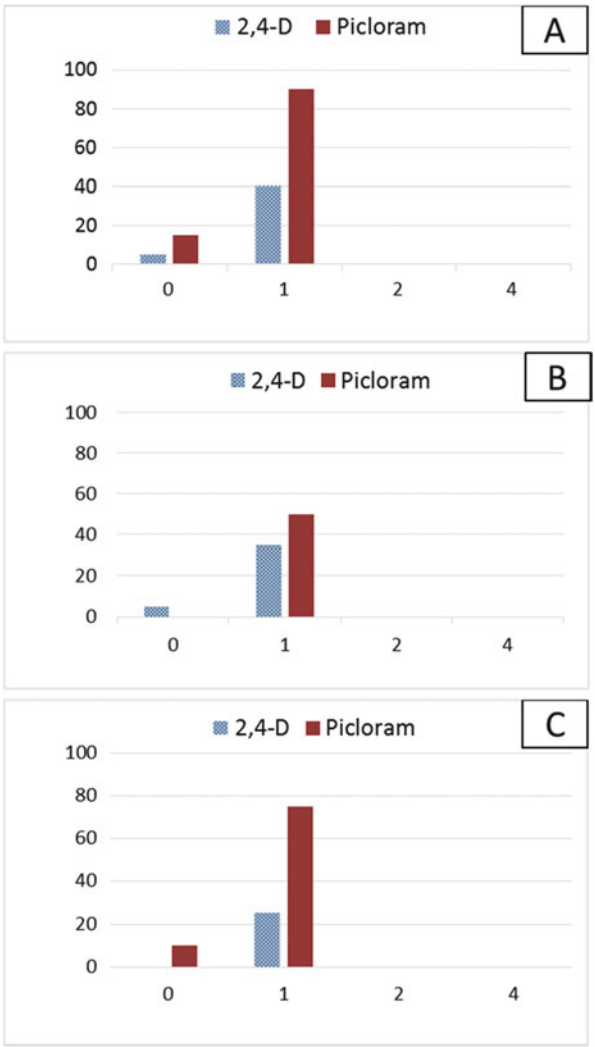

Fig. 5.3 Effect of abscisic acid on 2,4-D and picloram-induced somatic embryos from (**a**) Nkabom, (**b**) Wenchi and (**c**) ADI 001 cassava landraces

5.3.8 Desiccation of Embryos for Plant Conversion

1. Transfer matured somatic embryos (14 days old) induced on either 8 mg/L 2,4-D or 16 mg/L picloram to sterile Petri dishes.
2. Weigh 0.6 g embryos and desiccate by keeping them in a sealed Petri dish under growth room conditions for 4 days. Desiccation reduces the moisture loss which results in high frequency of plant regeneration. When desiccated embryos are cultured on picloram amended medium plant regeneration is improved (*see* Fig. 5.4 and **Note 17**).

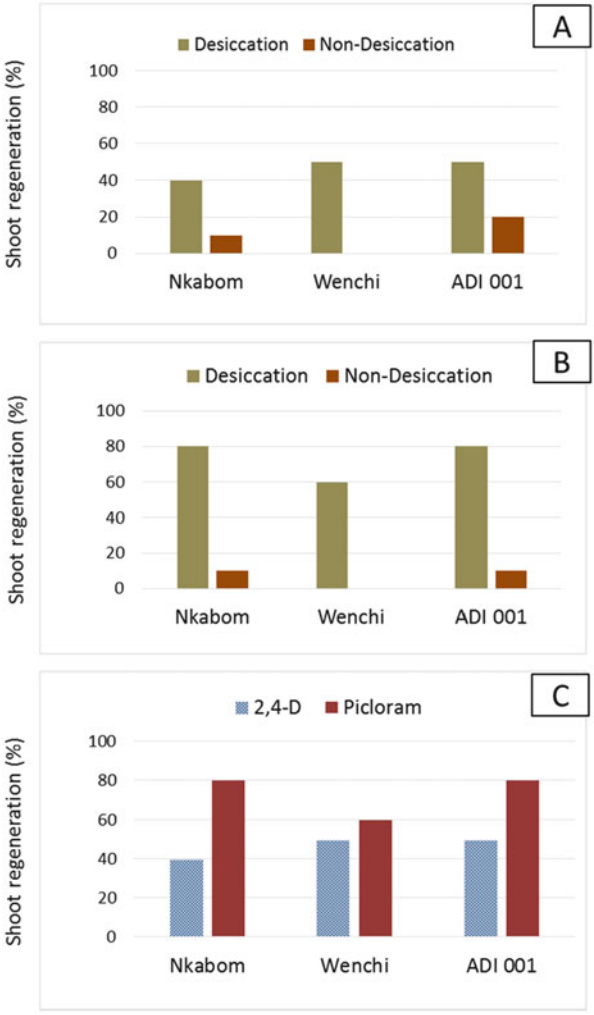

Fig. 5.4 The effect of desiccation on shoot regeneration from (**a**) 2,4-D, (**b**) picloram-induced somatic embryos and (**c**) comparison of 2,4-D and picloram-induced somatic embryos in three cassava landraces (Nkabom, Wenchi, ADI 001) 8 weeks after culture

3. Change Petri dishes every other day to remove condensed moisture on the lid of the Petri dish.
4. Reweigh the embryos after 4 days of partial desiccation to determine the final weight.
5. Calculate the moisture loss in somatic embryos in percentage using the following formula:

$$\%\text{Moisture loss} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

6. Transfer desiccated embryos to shoot initiation medium (Table 5.2).

5.4 Notes

1. All culture media described in this protocol consisted of Murashige and Skoog (Murashighe and Skoog 1962) basal salts powder (Sigma Aldrich) and Gamborg B5 vitamins. Growth regulators were added to suit particular experiments.
2. Stock solutions of vitamins and growth regulators were prepared and aliquoted into 1.5 mL Eppendorf tubes and stored in the refrigerator at 4 °C. They were used as and when required but not more than 8 weeks. Vitamins and growth regulators were filter sterilised and added to the basal salts. In the absence of filter steriliser, they may be added to the medium and autoclaved together.
3. Thirty grammes (30 g) of sucrose was added to the media and stirred to dissolve on a hotplate. pH was adjusted to 5.8 using 0.1 M sulphuric acid and 0.1 M sodium hydroxide.
4. Phytagel (3.5 g) was dissolved in 200 mL of sterile distilled water and was heated to melt in a microwave and then added to the culture media and the solution made up to 1 L prior to pH adjustment.
5. $CuSO_4$ was added to enhance somatic embryo initiation from leaf lobe explants. Silver nitrate may also be added. Both compounds suppress ethylene suppression in culture medium and improve somatic embryo production.
6. ABA enhances embryo maturation and shoot conversion from somatic embryos. It also makes somatic embryos tolerant to desiccation which also improves plant regeneration.
7. Cassava accessions ADI 001, Nkabom and Wenchi used for development of this protocol are landraces originally grown by farmers in the Brong Ahafo Region of Ghana. They have very good poundability; thus they are good for making *fufu*, a traditional meal. All the accessions mature after 12 months of planting. The stakes were collected from the Crop Science Department of the Kwame Nkrumah University of Science and Technology (KNUST) farms and were transported to Biotechnology and Nuclear Agriculture Research Institute (BNARI). Stakes were collected from plants with visually no symptoms of cassava mosaic virus diseases (CMVD). To proof for the absence of viral diseases, the plants should be screened using molecular techniques.
8. Tween 20 was added to improve efficiency of sterilisation with the sterilants.
9. The size of the meristem should not be more than 0.2 mm to avoid transmission of cassava mosaic viruses. It must also not be too small to make shoot regeneration very difficult.
10. In cassava somatic embryogenesis, young leaf lobes are ideal for primary somatic embryo induction. Older leaves are seldom used because they may only dedifferentiate into callus on culture medium without producing somatic embryos.
11. 2,4-D can initiate primary embryos even if the embryogenic competence of the explant is low. However, the frequency of matured embryo production is low on a 2,4-D-amended medium. Contrarily, picloram enhances embryogenic

competence in explants but has low frequency of primary embryo production. It also induces somatic embryos earlier with high frequency of embryo production if the explant is embryogenically competent. Both auxins are however removed at the maturation stage of somatic embryo induction (**Step 2**).

12. The orientation of the leaf lobes has influence on somatic embryo induction. When the adaxial surface is in contact with the medium, somatic embryo development is enhanced compared to when the abaxial surface is in contact.
13. Somatic embryos mature with well-developed hypocotyl and cotyledons within 14–21 days.
14. Of all the explants used for embryo induction, primary embryos have high embryogenic competence.
15. Fragmentation increases the frequency of embryo production because each fragment is highly embryogenically competent and can develop into full mature embryo.
16. Serial subculture in cyclic embryogenesis should end at three or four cultures as long subcultures reduce embryogenic competence in the subsequent cycle. Longer serial subculture may also result in somaclonal variation among plants regenerated.
17. Desiccation reduces the moisture content of the somatic embryos and thus mimics natural process of seed maturation in planta. The reduction in moisture content enhances germination. A combination of desiccation and ABA pretreatment improves somatic embryo germination.

5.5 Conclusion

Somatic embryogenesis can be coupled with mutation induction to overcome the limitations associated with conventional breeding of cassava. The embryogenetic system must therefore be optimised to reduce genotypic dependence. This can be achieved through indirect somatic embryogenesis which can provide both friable embryogenic calli and organised somatic embryos which are totipotent tissues that can be used as targets for mutagenic treatment (Fig. 5.2). Picloram-amended culture medium resulted in early embryogenic calli development, higher primary embryo production and more plant recovery from leaf lobe explants compared to 2,4-D. Recycling of the primary embryos on picloram-amended medium also led to higher embryo production. The presence of abscisic acid as well as air desiccation also resulted in high plant recovery. The optimised somatic embryogenesis protocol described can be coupled with mutation induction for production of useful traits in cassava breeding programmes.

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