Effect Of Explant Source And Different Medium Culture On Friable Embryogenic Callus Induction Of Four Cultivars Of Cassava (Manihot Esculenta Crantz)

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Abtract: In order to obtain Friable Embryogenic Callus (FEC) for protoplast isolation, we have evaluated in this research the competance for Friable Embryogenic Callus (FEC) of four cassava cultivars M61/033, Rendre, Yalipe and Six-mois in media containing MS supplemented with 8mg/l 2,4-D; MS supplemented with 10 mg/l BAP and GD supplemented with 12mg/l picloram using apical bud (AB) and immature leaves lobes (ILL) as explants. In general, in the medium GD+12mg/l picloram, the highest efficiencies of FEC ranged from 58 % to 87 % and the highest score of FEC ranged from 4.2 to 5.4 with explants AB, however we have observed with explants ILL, the efficiencies of somatic embryos ranged form 41% to 75% and the score ranged from 4.1 to 4.4. The mediums MS2+8 mg/l 2,4-D have induced with explants AB, the efficiencies of FEC ranged from 43% to 57% and the score ranged from 3.1 to 3.8, however with ILL explants the efficiencies of FEC ranged from 39 % to 49 % and the score ranged from 2.9 to 3.7. The least FEC were observed in the medium MS2+10 mg/l BAP with BA explants, however the efficiencies ranged from 6% to 11% and the score ranged from 1.1 to 1.8. Whereas the efficiencies of FEC with ILL explants ranged from 4% to 7 % and the score ranged from 0.5 to 0.8. All of four cultivars showed capability of producing FEC although their efficiency varied according to gonotype donors explants and medium taking into acount.
Abbreviations : GD : Gressoff and Doy, MS : Murashige and Skoog, 2,4-dichloro phenoxyacetic acid, BAP : Benzylamino-purin-Acid , AB : Apical Bud, ILL : Immature Leaves lobes

Keys words: Friable Embryogenic Callus, Immature leave lobes, Apical Bourgeon, cassava, GD, MS, Picloram, BAP.

1 Introduction

Cassava (Manihot esculenta Crantz) is a staple food to nearly a billion people in about 105 countries, providing as much as a third of daily calorie intake [1]. World production was estimated at 250 million tons in 2011 [2].

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In Africa, the continent with the largest production (53% of world production), the crop plays an important role as famine-reserve crop, rural staple food, cash crop for both rural and urban households and to a lesser extent, raw material for feed and chemical industries [3]. Cassava is drought tolerant and can grow in a range of agro-ecologies including marginally fertile soils, ensuring that when other crops fail, cassava roots can still be harvested. Cassava was introduced in Central African Republic in 1850 [4]. It has become the first food crop in this country, with 600.000T /an of dry cassava, very far from maize production with 60 000T/HA [5]. The importance of cassava and the enormous potential for improvement therefore makes it a target crop for famine research to achieve the United Nations Millennium Development Goals [6]. Furthermore, cassava is vegetatively propagated via stem cuttings that areused to multiply stocks and for planting; typically five to ten cuttings can be obtained from a single plant. This propagation technic means that in times of famine the farmer does not consume the ''seed'' of cassava, unlike other staple crops (e.g. maize). Despite these advantageous traits cassava production is generally mediocre with current yields barely averaging 20% of those obtained under optimal conditions, particularly in Africa [7; 8]. Although cassava is an important food crop in the tropical regions, it has been largely ignored by agricultural scientists. The research and input into cassava breeding are minimal, considering its importance. Cassava breeding is hampered due to the high degree of genetic heterozygosity, genetic overloading, serious separation of progeny, few flowers, low pollen fertility, self-incompatibility, and low fruit set rate [9]. Therefore, cassava genetic transformation has emerged as a valuable alternative and complementary approach to improve the crop [10; 11]. For cassava, several explant types have been used in regeneration and transformation studies using various types

of starting materials which include friable embryogenic callus (FEC), somatic embryos (SE), cotyledons, and protoplasts. FEC and cotyledons remain the most widely used explants for transformation and regeneration due to their success in these procedures [12]. FEC is induced from organised embryogenic structures (OES), derived from primary SE, on Gresshoff and Doy[13] salts and vitamins supplemented with picloram (GD medium) [14]. FEC is of a single cell origin and proliferates rapidly thus the tissue has a reduced risk of chimerism. However the long span of tissue culture in vitro may cause somaclonal variation. Friables embryogenics callus have been used as the starting material for all transformation methods. Somatics cotyledons and FEC are induced from somatic embryos and it is these explants that are used for transformation. Somatic embryogenesis of cassava was first described by Stamp and Henshaw [15]. Explants are initiated from sterile pieces of a whole plant and may consist of pieces of organs such as leaves or may be specific cell types such as pollen. Many explant features are known to affect the efficiency of culture initiation and transformation. Younger, more rapidly growing tissue or tissue at an early stage of development is the most effective. Somatic embryos have been induced from various cassava explants including immature leaf lobe [16], [17], [18], [19], [20], [21], [14], [22], [11], meristems [16 ; 20 ;21] zygotic embryos or floral tissue [12], on several media containing various plant growth regulators. The generation of embryogenic structures needs to be optimised for each cassava cultivar as not all cultivars are amenable to somatic embryogenesis, and regeneration or transformation, and efficiency of SE is highly genotype-dependent. This research reports on the capabilities of selected cassava cultivars to produce FEC. These cultivars were chosen because of their potential and for somatic hybridization.

2 Materials and Methods

2 1 Plant material

Plantlets of four cassava cultivars M61/033, Six-mois, Rendre and Yalipe, were maintained as shoot cultures on MS (Murashige and Skoog, 1962) medium supplemented with 20 g/l sucrose, solidified with 0.3 g/l gelrite, pH 5.8 (MS2), at 25 °C under a 16/8 h photoperiod (3500 lx). Explants were subcultured every 4–6 weeks.

2.2 Embryogenic tissue induction

2.2.1 Treatments

Six(6) treatments were used for callus induction

Six weeks old in vitro immature leaf lobes (ILL) and apical bud (AB) were excised from each mother plants using fine forceps and a scalpel and placed in contact with the media on GD vitamin supplemented with 12 mg/l picloram, 2 % sucrose, 0.3 % gelrite according to Peng Zhang et al, [11], on MS2 supplemented with 8mg/l 2, 4-D, 2 % sucrose, 0.3 % gelrite according to Guohua et Qiusheng [18], on MS2, 2 % sucrose, 0.3 % gelrite supplemented with 10 mg/l BAP according to Zhang et Puonti kaerlas [23], each medium supplemented with 2 µM $CuSO_4$ and cultivated in the dark 25°C. Ten Petri dishes per cultivar, per treatment were used with each Petri dish containing ten immature leaves lobes explants and ten apical bud explants (100 explants per cultivar). Explants were transferred to fresh media after 2 weeks in culture and production of embryogenic tissues from leaf lobe explants were scored after 6 weeks in culture. The percentage of explants producing Friable Embryogenic Callus (FEC) (frequency %) was recorded from each treatment, while a 0-10 range scale system was used for scoring FEC production (amount of FEC per explant) efficiency where 0 indicates the absence of embryogenic structures and 10 indicates that the entire leaf margin contained Friable embryogenic structures.

2.1.3 Statistical analysis

The analysis of the FEC induction was based on one way analysis of variance (ANOVA), all statistical analyses were performed by R3.2.4. Tukey test was used to classify the means.

3 Results

3.1 FEC scores

Both of the explants immature leaves lobes (ILL) and apical bud (AB) have reached succefully friable embryogenic callus either in the medium GD+12 mg/l picloram or MS2+8mg/l 2,4-D and MS2+10 mg/l BAP with cultivars M61/033, Six-mois, Rendre and Yalipe.

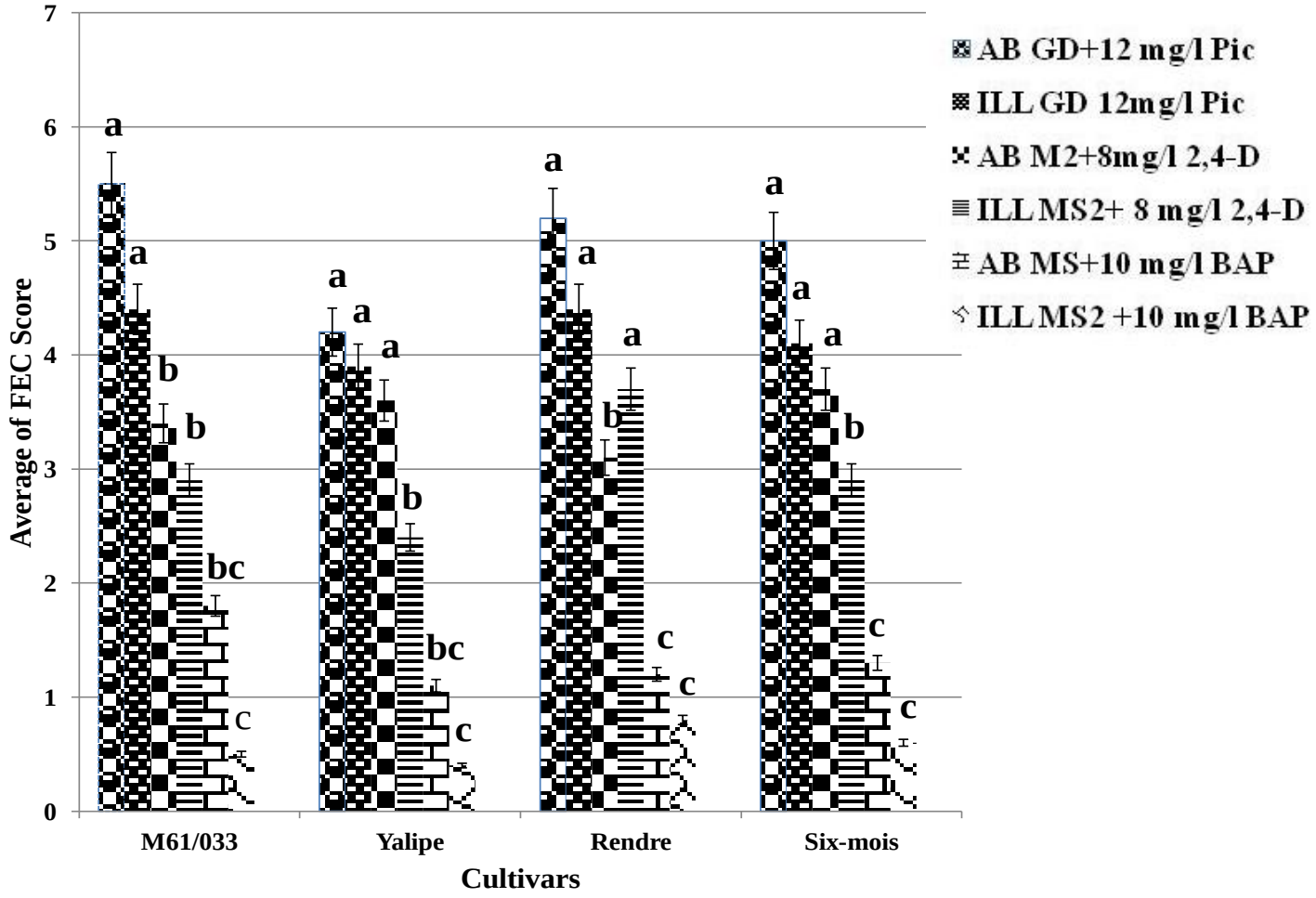

Figure 1 : *Effect of different medium induction with donors explants (AB) and (ILL) on FEC. Means with different letters for each cultivar are significantly different (P<0.05)**.** Vertical bars are standard errors.*

The donors explants apical bud (AB) with all of four cultivars M61/033, Six-mois, Rendre and Yalipe in the medium GD+12 mg/l picloram have produced the highest efficiencies and score of FEC. The highest score of Friable Embryogenic Callus ranged from (5.5 to 4.2) with explants donor (AB) respectively with cultivar M61/033(5.5), cultivar Rendre (5.3), cultivar Six-mois (5.2) and cultivar Yalipe (4.2). We have observed with donors explants Immatures leaves lobes (ILL) in the medium GD+12mg/l picloram, the score of Friable Embryogenic Callus ranged from (4.4-3.9). The highest FEC with ILL explant was recorded with cultivar M61/033 (4.4) and the least score with cultivar Yalipe (3.9). We have recorded with cultivar Rendre (4.3) and cultivar Six-mois (4.1). In the medium MS2+8mg/l 2,4-D, the highest score of FEC with AB donor explant, were 3.7 with cultivar Six-mois and the least score was 3.1 with cultivar Rendre. The cultivars Yalipe and M61/033 have respectively 3.6 and 3.4. The highest score of FEC in the medium MS+8 mg/l 2, 4-D with ILL explants were 3.7 with cultivar Rendre, the least score were observed with cultivar Yalipe 2.4. We have recorded 2.9 scores for cultivars M61/033 and Six-mois. In the medium MS2+10 mg/l BAP, we have recorded with cultivars M61/033, Six-mois, Rendre and Yalipe, score of FEC with BA explants, ranged from 1.8 with cultivar M61/033 to 1.1 with cultivar Yalipe. The score of FEC were respectively, 1.3 and 1.2 with cultivars Six-mois and Rendre. We have observed in the same medium MS2+8 mg/l 2, 4-D, with donors explants ILL, the FEC score ranged from 0.8 with cultivar Rendre to 0.4 with cultivar Yalipe. The cultivars Six-mois and M61/033 have respectively 0.6 and 0.5.

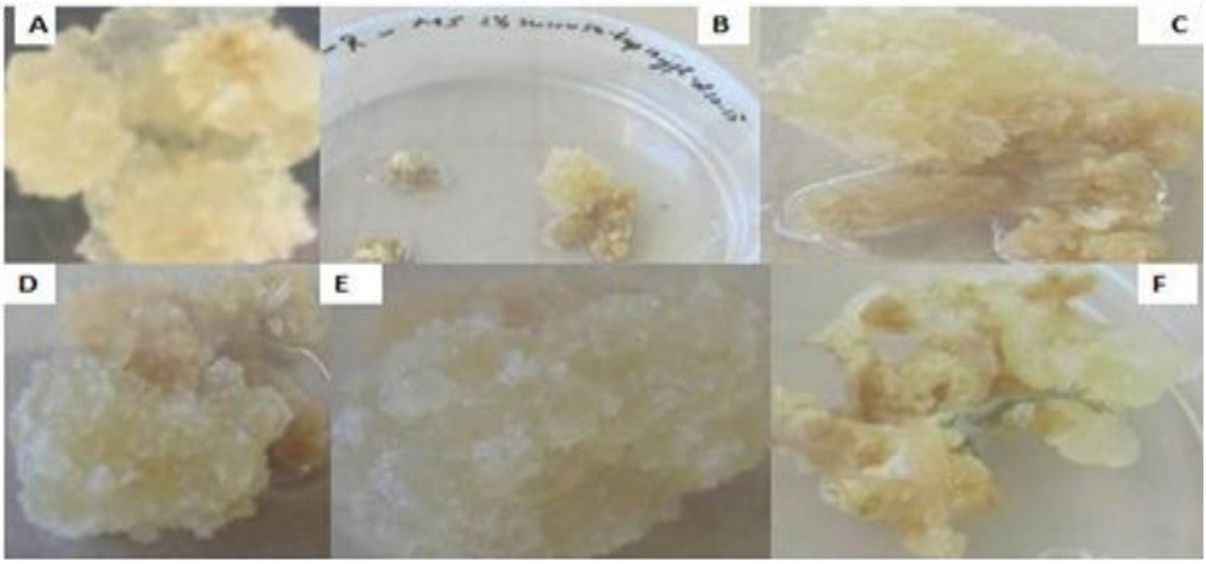

Figure 2 : *Capacity of (ILL) and (AB) explants for FEC induction, 2A) FEC of Cultivar M61/033 with (AB) explant on GD+12 mg/l picloram, 2B) Somatic embryos of cultivar Rendre with explant (ILL) on MS2+10 mg/l of BAP, 2C) Somatic Embryos cultivar Yalipe with (ILL) on MS2+ 8mg/l 2,4D, 2D) Somatic Embryogenic with Six-mois on MS2+8 mg/l 2,4-D, 2E) FEC of Rendre on the GD +12 mg/l picloram, 2F) Somatic Embryon of cultivar M61/033 (ILL) on MS2 + 10 mg/l Bap.*

3.2 FEC frequencies

Tableau I*: Frequencies of FEC obtained with explants (ILL) and (AB) in different mediums cultures*

Cultivars	Culture medium + 2µM CuSO4	frequencies of FEC induction /explant	
		A B induction (%)	ILL induction (%)
M61/033	GD+ 12 mg/l Pic	87	75
	MS2 + 8 mg/l 2,4-D	57	45
	MS2+ 10 mg/l BAP	7	5
Rendre	GD+ 12 mg/l Pic	77	71
	MS2+8mg/l 2,4-D	54	49
	MS2+10 mg/l BAP	6	4
Six-mois	GD + 12 mg/l Pic	81	64
	MS + 8 mg/l 2,4-D	43	39
	MS + 10 mg/l BAP	9	4
Yalipé	GD+ 12 mg/l Pic	58	45
	MS+ 8 mg/l 2,4 D	45	41
	MS+ 10 mg/l BAP	11	7

The highest frequencies of FEC with AB donors explants were obtained in the medium GD+12 mg/l picloram with cultivar M61/033 (87%) and the lowest with cultivar Yalipe (58 %). The cultivars Six-mois and Rendre have respectively 71% and 64%. Whereas the frequencies of FEC induction with donor explant ILL in the same medium GD+12 mg/l picloram ranged from 75% with cultivar M61/033 to 45% with cultivar Yalipe. The cultivars Rendre and Six-mois have respectively 71% and 64 %. The frequencies of FEC with donors explants ILL in the medium MS2+8mg/l 2,4-D varied from 57% with cultivar M61/033 to 43% with cultivar Six-mois and the cultivars Rendre and Yalipe have respectively 54 % and 45% . Whereas these frequencies of FEC with donors explants AB varied from 49 % with cultivar Rendre to 39 % with cultivar Six-mois and we observed respectively 45 % and 41 % with cultivars M61/033 and Yalipe. The medium MS2+10 mg/l BAP has induced with AB, the percentage of FEC varied from 11% with cultivar Yalipe to 6 % with cultivar Rendre and the cultivars Six-mois and M61/033 have respectively 9 % and 7 %. Whereas we have observed in the medium MS+10 mg/l BAP, the FEC frequencies with donors explants ranged from 7% with cultivar Yalipe to 4 % with cultivar Rendre and cultivars M61/033 and Six-mois have respectively 6% and 5%.

3.3 Correlation between FEC frequencies and efficiency scores (FEC potential)

The score (0–10 scale of amount of FEC per explant) correlated with the frequency (number of AB/ILL producing FEC). For all cultivars in the medium GD+12 mg/l picloram the frequencies of FEC induced with donors explants AB varied (87% - 58%) and the scores of FEC varied (5.5-4.2). Whatever with donors explants ILL in the same medium, the frequencies of FEC ranged (75%-45%) and the scores of FEC (4.4-3.9). In the medium MS2+8 mg/l 2,4-D, the frequenties of FEC induced with donors explants AB varied (57% - 43 %) and the scores of FEC varied (3.7-3.1). however with donors explants ILL, the frequencies of FEC (49%-41%) and the scores of FEC (3.7-2.4) for all of cultivars. In the medium MS+10 mg/l BAP, the frequenties of FEC induced with donors explants AB varied (11% - 6 %)

and the scores of FEC varied (1.1-1.2). However with donors explants ILL , the frequencies of FEC varied (7% - 4%) and the scores of FEC (0.4-0.8) for all of cultivars. These results have shown correlation between FEC frequencies and FEC scores, more the frequencies have increased more were the FEC scores whereas less the frequencies were, less the scores decreased.

4 Discussion

All of cassava cultivars (M61/033, Rendre, Six-mois and Yalipe), cultured in different media have reacted differently and these cultivars have shown significantly difference for frequencies and scores from one cultivar to another with donors explant (ILLL) and (AB). The aim of this research is to identify the best medium of FEC, for protoplast isolation of cassava in culture in the following research. FEC have been obtained succefully, although some of media (e.g MS2 +10 mg/l BAP), have induced less of FEC with explants ILL or AB, after six week culture. The highest score induction (>2,7) and the highest frequencies (>50%) of FEC were recorded with all of cultivars in the medium GD+12 mg/l picloram with donors explants AB and ILL. However in contrary the least score induction(<2) and the least frequencies (<12 %) of FEC were recorded with all of cultivars in the medium MS+10 mg/l BAP with donors explants AB and ILL. Although SE induction can be induced over a longer period of 3 months, it is not advisable to use these SE for FEC or cotyledon production, because of the time factor, increased risk of somatic mutation or genetic instability and lower FEC production potential. Atehnkeng et al.[16] evaluated proembryo formation between 27 and 35 days, but some cultivars e.g. TME 596, TME 594 took 56–58 days to form torpedo-shaped embryos from leaf lobes. Proembryo formation was also not adequate as an indicator of SE potential. In general, SE or FEC potential should be evaluated from 4 to 6 weeks after induction, as was carried out in this study. Differences between cultivars in the efficiency and frequency of SE production, and the type of developmental stages occurring (such as globular, heart and torpedo embryos), were observed and may be explained by the genotype-explant combination. The ability of cassava genotypes to produce somatic embryos or Friable Embrigenic Callus is influenced by the explant type (such as ILL or AB) as well as the type of auxin (for example picloram, BAP and 2,4-D) and concentration. The results of this study, on the genotypic variation in SE or FEC, concur with previous reports of other cassava cultivars from Africa, South America and Asia[16 ; 20 ; 24]. The most commonly used explants type to induce SE is ILL[16 ; 17 ; 18 ; 21] followed by shoot apical meristems (AM) [16 ; 20 ; 21]. In this study, AB and ILL explants, plant growth regulators (BAP, picloram and 2,4-D) and media culture (GD and MS) were tested for the induction of FEC. Friable Embriogenic Callus could be induced from both AB and ILL explants in GD and MS media with picloram or 2,4-D and BAP for all cultivars with varying frequencies and scores. The media GD+12 mg/l picloram showed the highest FEC potential overall compared with the medium MS2+8mg/l 2,4-D and MS2+ 10 mg/l BAP. It was demonstrated that AB placed on GD supplemented with 12mg/l picloram and 2 µMCuSO4 induced higher SE or FEC efficiencies compared with ILL on the same media for all cultivars. For all cultivars tested, MS2 + 10 mg/l BAP gave lower FEC efficiencies and score, compared to GD+12 mg/l picloram. Regeneration of cotyledons from SE in cultivar M61/033 was successfully achieved by transferring FEC to MS medium supplemented with 0.1 mg/l BAP (data not shown). A comparison between AB and ILL explants in our study, demonstrated a higher SE or FEC potential (frequency and efficiency) for AB compared with ILL in most cultivars. Hankoua et al.[20] showed a higher success rate with leaf lobes (21.7%) than axillary meristems (13.8%). Similar variation of SE potential from different explant sources have been reported for cultivars from South America, Asia and other African cultivars[16 ; 25]. High FEC production and regeneration capacity favour these cultivars as a target for genetic modification and somatic hybridization for important traits such as CMD resistance and high interested traits.

5 Conclusion

These results indicated that the response of the four cultivars explants ILL and AB to Friable Embryogenic Callus (FEC) depended on genotypes donors explants, on the medium used and the growth parameter taking into account. These results can be used for producing FEC in order to isolate protoplast for plant hybridization. Therefore, important traits such as resistance to cassava mosaic diseases, reduced toxic cyanogene content in tuberous roots, high protein content and drought tolerance can be introduced to these cultivars.

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