Journal of Advances in Biology & Biotechnology

24(5): 9-20, 2021; Article no.JABB.67801
ISSN: 2394-1081

Effect of Hormonal Regime and Explant Type on Cell Clusters Expression of Maize Mutants (*Zea Mays L*) Derived from Gamma Irradiated Seeds of Ev8728 variety

Kanga Ahou Nadia[1*], Ayolié Koutoua[1], Yao Kouakou François Konan[1], Kassi Amalan Angèle[1], Soro Dogniméton[1], Kouakou Tano Hilaire[2] and Kouadio Yatty Justin[1]

[1] University Jean Lorougnon Guédé, UFR (Faculty) of Agroforestry, Laboratory for Improving Agricultural production BP 150 Daloa, Côte d'Ivoire.
[2] University Nangui Abrogoua, UFR (Faculty) of Natural Sciences, Laboratory of Biology and Improvement of Plant Production, Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. Authors KYJ, AK and KAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KAA, KKF and KTH managed the analyses of the study. Author YKFK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JABB/2021/v24i530213
Editor(s):
(1) Dr. Anil Kumar, Devi Ahilya University, India.
Reviewers:
(1) Hymavathi Salava, University of Hyderabad, India.
(2) Shiferaw Teshome Woldie, Wolaita Sodo University, Ethiopia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/67801

Original Research Article

Received 02 March 2021
Accepted 09 May 2021
Published 18 June 2021

ABSTRACT

Maize (*Zea mays* L.) seeds irradiated with gamma or not from the fourth self-fertilisation cycle were selected for tissue culture. For this purpose, MS medium supplemented with 30 $g.L^{-1}$ sucrose, 100 $mg.L^{-1}$ casein hydrolysate, 100 $mg.L^{-1}$ myo-inositol and 6 $g.L^{-1}$ agar was used. For this purpose, three auxins type (2.4-dichlorophenoxyacetic acid (2.4-D), 2-methoxy-3.6-dichlorobenzoic acid (Dicamba) and Indole-3-Acetic Acid (AIA)) and explants (root, epicotyl and leaf) were tested. The results showed that 2.4-D more precisely at 3.5 $mg.L^{-1}$ was the best auxin for callus induction in the different maize mutants studied. The induction rate, dry matter weight and water content of callus varied according to the type, age, explant position and the maize mutants studied (control, 200 and

**Corresponding author: E-mail: kanganadia8@gmail.com;*
;

300 grays). Thus, the 7-day root and more precisely its basal part was better for callogenesis. In addition, mutants of the 200 grays dose were more expressive in the ability to induce callus in EV8728maize variety.

Keywords: Callus induction; maize mutants; auxins; explant; gamma irradiation.

1. INTRODUCTION

Maize, a very important tropical plant, supports major food systems in some parts of the world. Of the eight genres contained in the Maydae or Tripsaceae tribe, the genre Zea remains the most exploited for the great morphological diversity of its varieties. However, the species *Zea mays* L. is the most cultivated for its grains [1]. Initially produced for human consumption, maize is also used for feeding animals [2]. Today, world maize production is 1.06 million tons with the main producers being the United States, China and Brazil [3]. In Côte d'Ivoire, maize occupies a prominent place in agricultural activities. Annual national maize production is estimated at 654,738 tons, for a total area of 327,800 ha. Its national consumption is estimated at 28.5 kg/inhabitant/year [4].Despite this productivity, maize still faces many constraints. These include health status, pests, irregular rainfall and soil degradation [5]. For this purpose, some solutions have been proposed worldwide for a recovery of the sector. These are mainly use of adequate fertilizers, introduction of genetically modified organisms (GMOs), and the use of new improved varieties [6]. Despite these solutions, Ivorian production has remained far from sufficient to meet national and international demand. Thus, in order to contribute to find a sustainable solution to this problem, tissue culture via somatic embryogenesis coupled with seed irradiation are envisaged in this paper. This technique will not only make it possible to create and produce new, improved and healthy plant material on a large scale for increased production [7,8]. Indeed, indirect somatic embryogenesis necessarily involves callus induction. During callus induction, several factors such as culture medium, explant and genotype are involved [9]. Thus, this study aims to evaluate the effect of the hormonal regime and the explant on the callogenic expression of different maize mutants obtained after four generations of self-fertilization.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material used is composed of vitroplants obtained after *in vitro* germination of seeds from 4 cycles of maize self-fertilization irradiated (200 and 300 grays) and non-irradiated (controls) with gamma radiation of the variety EV8728 from National Centre for Agronomic Research of Côte d'Ivoire.

2.2 Methods

2.2.1 Obtaining explants by *In vitro* germination of seeds

The explants consist of different organs of vitroplants obtained by *in vitro* germination of irradiated and non-irradiated maize seeds. Thus, under a laminar flow hood, the seeds were disinfected by soaking in a fungicidal solution (31.25 $mL.L^{-1}$) for 5 minutes, then in 70 % alcohol for 3 minutes and finally in sodium hypochlorite with 3,6 % active chlorine, to which 3 drops of Tween 20 were added for 40 minutes. After rinsing (4 to 5 times) with sterile distilled water for 5 minutes, the seeds were then soaked in sterile distilled water (30 mL) and placed in the dark for 48 h. After incubation in dark, seeds with rootlets were cultured on MS basal medium [10], free of growth regulators, with 30 $g.L^{-1}$ sucrose added. The cultures were incubated in dark for 2 days and then transferred to culture roomunder 16-8 h photoperiod for 7 days. The resulting maize vitroplants were used as explants for callogenesis induction.

2.2.2 Induction medium and culture conditions

MS basal medium supplemented with 30 $g.L^{-1}$ sucrose, 100 $mg.L^{-1}$ casein hydrolysate, 100 $mg.L^{-1}$ myo-inositol and 6 $g.L^{-1}$ agar is used for callogenesis. The pH is adjusted to 5.8 and then autoclaved for 20 minutes at 120 °C under 1 bar pressure. All cultures were placed in a culture room at 25 ± 2 °C,16 /8 h photoperiodand relative humidity of 70 %. Light intensity of the order of 100 $\mu E.m^{-2}.sec^{-1}$ was provided by warm white fluorescent lamps.

2.2.3 Cell clusters Induction

2.2.3.1. Auxin type effect on callus induction

To determine the best phytohormonal for callus induction, three auxins types, 2,4-

dichlorophenoxyacetic acid (2,4-D), 2-methoxy-3,6-dichlorobenzoic acid (Dicamba) and Indole-3-Acetic Acid (AIA) at 2 mg.L^{-1} each, were added to callogenesis medium. Selected root explants were cultured on this medium. Thus, 225 explants (3 auxins x 5 explants x 5 Petri dishesx 3 doses) were used. The auxin that induced more callus was selected for further investigation.

2.2.3.2 Explant type effect on callus induction

In order to determine the type of explant favorable for callus induction, different explants (roots, epicotyls and leaves) from maize vitroplants were used. Fragments of each explant were aseptically collected and deposited on induction medium with only 2 mg.L^{-1} 2,4-D added. For each irradiation dose, 75 explants were used at a rate of 25 explants per dose. The explant that induced better callus was selected for the determination of the involvement of explants age in callus induction.

2.2.3.3 Explant age effecton callus induction

Under a laminar flow hood, the root explants of the 7- and 14-day-old vitroplants were fragmented and then transplanted to the callogenesis medium, to which 2 mg.L^{-1} 2,4-D was also added. Thus, 150 root explants (2 ages x 5 explants x 5 Petri dishes x 3 doses) were used for this test. The explant age that produced the best results was used to determine the effect of root explant position (base or apex) on callus induction.

2.2.3.4 Effect of root explant position on callus induction

Root explants of 7-day-old vitroplants were fragmented into two parts (basal and apical) and then transplanted separately to the callogenesis medium in presence of 2 mg.L^{-1} 2.4-D. 150 explants (2 explant positions x 5 explants x 5 jars x 3 doses) were used for callus induction. The position that produced the best result in callus induction was selected for further experimentation.

2.2.3.5 Effect of 2.4-D concentration on callus induction

In order to determine the auxin concentration favorable for callus induction, the culture medium was supplemented with different concentrations (1.5; 2; 2.5; 3; 3.5 mg.L-1) of 2,4-D. For this purpose, basal root explants from vitroplants were transplanted in induction medium. 375 explants (5 concentrations x 5 explants x 5 jars x 3 doses) were used.

2.2.3.6 Callogenesis parameters

During this experiment, certain quantitative and qualitative parameters of induced callus were determined after 4 weeks of cultivation. These parameters were:

- Callus induction rate (IR %) = (NbECa/NbTECu) x 100(NbECa: Number of Explants that produced Cal; TNbECu: Total Number of Explants cultivated);
- Fresh (FW) and dry (DW) callus weight: calluses, taken from the Petri dishes under fume hood, were cleaned to remove all traces of culture medium and then weighed using an electronic scale to determine the fresh weight of the calluses. These callus were then placed back in Petri dishes and placed in an oven at 65 °C for 48 hours to dry and then weighed again to determine the dry weight.
- Water content (WC %) = [(CFW-CDW)/CDW] X 100; CFW: Cal fresh weight, CDW: Cal dry weight;
- Callus texture (pasty and/or crumbly) and color: calluses Texture and color were evaluated by touching and visual inspection of three people. The average of the observations was taken as the qualitative results.

2.3 Statistical Analysis

The data were statistically analyzed with STATISTICA software, version 7.1. Analysis of variation (ANOVA) was used to test statistical significance, and significant differences between the means were calculated using Tukey's HSD test at $P < 0.05$.

3. RESULTS

3.1 Auxin Type Effect on Callus Induction

Auxin type effect on quantitative and qualitative parameters of callus are listed in Table 1. The different auxins (2.4-D, Dicamba and AIA) tested at 2 mg.L^{-1} induced calluses regardless of the irradiation dose (control, 200 and 300 grays). However, in controls, no callus induction was observed in medium at AIA. Thus, with a mean induction rate of 80%, the 2.4-D-containing

medium induced the best callogenesis rates in 200-gray mutants compared to 78% and 68% in control and 300-gray mutants, respectively. Also, the dry matter weight and water content of callus evolved in same way as the induction rate with 2.4-D as the best results. The variance analysis showed a significant effect (P <0,001) of auxins on all quantitative parameters studied except callus water content (all doses combined). Also, all calluses induced in 2.4-D presence are friable and brownish-white in contrast to those of Dicamba and AIA (pasty and whitish calluses) (Fig. 1).

3.2 Effect of Explants Type on Callus Induction

Relative results on the effect of explants type on callogenesis are shown in Table 2. The explant type significantly influenced (P <0,001) all quantitative parameters studied. indeed, the different explants, except the leaves, irradiated or not, all induced callus. However, the roots induced more callus (70%) than the epicotyls (28.66%) for all irradiation doses combined (mutant combined). However, the synthesis of cell clusters was more stimulated with 200-gray mutants compared to 300-gray mutants regardless of explants type. This induction rate was 78% with root explants compared to 34% in epicotyl explants at 200 grays dose. In addition, dry weight growth also varied from 13.78 to 21.87 mg with root explants and from 8.06 to 17.50 mg with epicotyl, i.e. an average of 19.07 mg and 11.52 mg respectively for all irradiation doses (mutant combined). This weight was also greater at the 200 grays dose and even greater with the root explants. On the other hand, the callus water content did not vary between root and epicotyl regardless of the irradiation dose. The resulting calluses had a friable or pasty appearance with whitish, brown-white, yellow-green or brown-green colors.

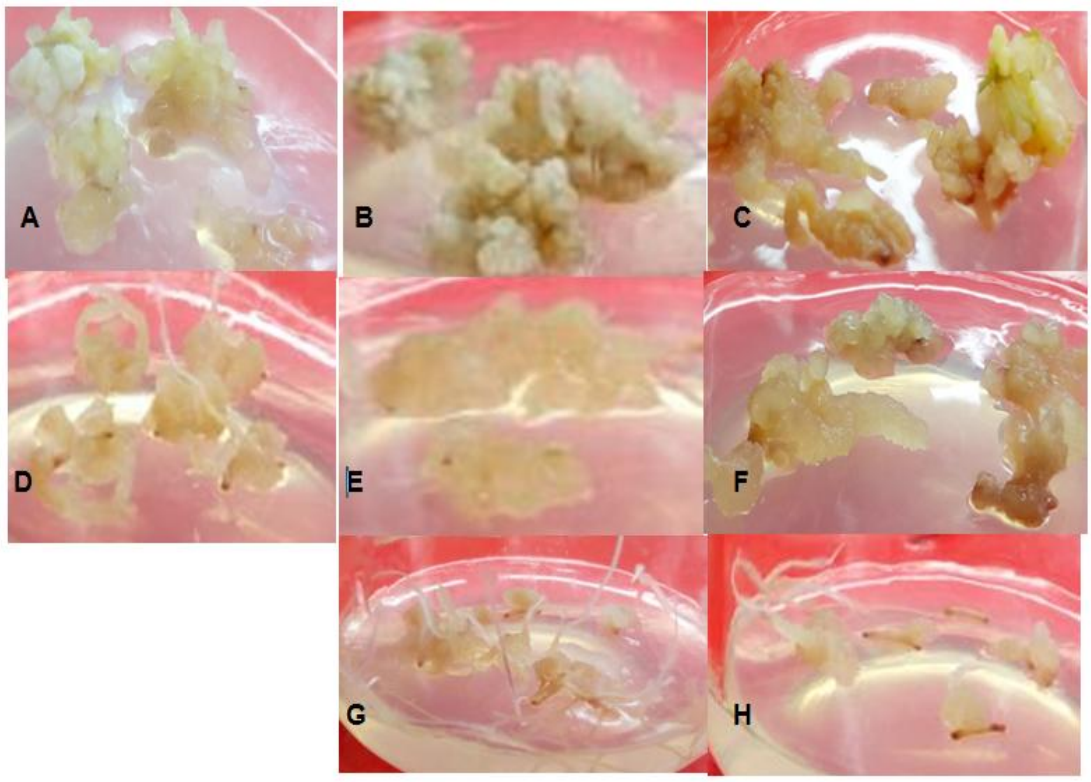

Fig. 1. Maize callus obtained on MS medium with 2.4-D, dicamba and AIA added

A: 2 mg.L^{-1} 2.4-D - non-irradiated control (friable and whitish callus); B: 2 mg.L^{-1} 2.4-D - 200 grays (friable and brownish-white callus); C: 2 mg.L^{-1} 2.4-D - 300 grays (friable and brownish callus); D: 2 mg.L^{-1} Dicamba - non-irradiated control (pasty and whitish callus); E: 2 mg.L^{-1} Dicamba - 200 grays (pasty and whitish callus); F: 2 mg.L^{-1} Dicamba - 300 grays (pasty and brownish callus); G: 2 mg.L^{-1} AIA - 200 grays (pasty and whitish callus); H: 2 mg.l-1 AIA - 300 grays (pasty and whitish callus) . MS: Murashige & Skoog; 2.4-D: 2,4-dichlorophenoxyacetic acid; AIA: 3-indole acetic acid.

Table 1. Auxin type effect on callus induction and callus characteristics

Mutant	Auxin (mg.L^{-1})	IR (%)	DMW (mg ±s)	WC (%)	Texture/Color
	AIA	0,0 ± 0,0^{c}	0,0 ± 0,0^{c}	0,0 ± 0,0 ^{b}	-
Control	Dicamba	52,0 ± 9,04^{b}	14,32 ± 0,74^{b}	91,53 ± 0,40^{a}	Pa/ Bl
	2,4-D	78,0 ± 4,66^{a}	17,14 ± 0,89^{a}	90,28 ± 0,31^{a}	Fr/ Bw
F		45,69	186,69	31510,30	
P		***	***	***	
	AIA	44,0 ± 3,05^{b}	16,25 ± 1,91^{b}	89,13 ± 1,72^{a}	Pa/ Bl
200 grays	Dicamba	54,0 ± 5,2^{b}	21,57 ± 2,17^{a}	91,92 ± 0,30^{a}	Pa / Bl
	2,4-D	80,0 ± 4,21^{a}	22,35 ± 4,01^{a}	92,02 ± 0.83^{a}	Fr / Bw
F		21,31	6,01	2,23	
P		***	**	ns	
	AIA	26,0 ± 3,05^{b}	10,77 ±1,29^{b}	90,5 ± 0,62^{a}	Pa / Bl
300 grays	Dicamba	46,0 ± 5,2^{a}	9,54 ±1,39^{b}	90,66 ± 1,27^{a}	Pa /Br
	2,4-D	68,0 ± 6,11^{a}	18,07 ± 2,11^{a}	92,15 ± 0,50^{a}	Fr /Br
F		18,59	5,57	0,86	
P		***	**	ns	

*AIA (3-indole acetic acid); 2.4-D (2,4-dichlorophenoxyacetic acid); Dicamba (2-methoxy-3,6-dichlorobenzoic acid). IR: induction rate of callus; DMW: dry matter weight; WC: water content. Pa: pasty; Fr: friable; Bl: whitish; Br: brownish; Bw: brownish-white. The mean values are followed by their standard error (±). Values with the same letters are not significantly different (Tukey's test at 5%). P: Approximate probability of the Tests; F: Fischer's constancy. The effects are significant at α <0.05 (***: very highly significant, **: highly significant, ns: not significant)*

Table 2. Effect of explant type from irradiated and non-irradiated seeds on maize callogenesis

Dose or Mutant	Explants	IR (%)	DMW (mg ±s)	WC (%)	Texture/Color
	Root	70,0 ± 3,33[a]	13,78 ± 1,18[a]	91,63 ± 0,38[a]	Fr/ Bw
Control	Epicotyle	28,0± 3,26[b]	8,06 ± 0.77[b]	90,28 ±0,31[a]	Pa / Bw
	leaf	0,0 ± 0,0[c]	0,0 ± 0,0[c]	0,0 ± 0,0[b]	--
F		171,0	71,74	24310,25	
P		***	***	**	
	Root	78,0 ± 4,66[a]	21,57 ± 2,72[a]	91,69 ±0,22[a]	Fr/ W
200 grays	Epicotyle	34,0± 5,2[b]	17,50 ± 2,05[b]	92,33 ± 0,38[a]	Pa/Yg
	leaf	0,0 ± 0,0[c]	0,0 ± 0,0[c]	0,0 ± 0,0[b]	--
F		93,84	33,54	42879,10	
P		***	***	**	
	Root	62,0 ± 5,53[a]	21,87 ± 4,02[a]	91,97 ± 0,37[a]	Fr/Bw
300 grays	Epicotyle	24,0± 2,66[b]	9,0 ± 0.9[b]	91,89 ±0,41[a]	Pa/Bg
	leaf	0,0 ± 0,0[c]	0,0 ± 0,0[c]	0,0 ± 0,0[b]	--
F		77,61	21,30	26790,90	
P		***	***	**	

*IR: Induction rate of callus; DMW: dry matter weight; WC: water content. Pa: pasty; Fr: Friable; W: whitish; Bw: brown-white; Yg: yellow-green; Bg: brown-green. The mean values are followed by their standard error (±). Values with the same letters are not significantly different (Tukey's test at 5%). P: Approximate probability of the Tests; F: Fischer's constancy. The effects are significant at α <0.05 (***: highly significant, **: highly significant)*

3.3 Explant age Effect on Callus Induction

7- and 14-day old roots grown on MS medium were supplemented with 2 mg.L^{-1} 2.4-D, it was observed that all the root explants induced callus (Table 3). However, 7-day roots induced on average more callus (67.5%) than 14-day roots (43%) in all mutants (control, 200 and 300 grays). Thus, with 7-day explants, induction rates were highest (64%; 78.6% and 60% control, 200 and 300 grays, respectively) compared to 14-day explants (41.3%; 49.3% and 40%, respectively). The growth of cell clusters is also higher with 7-day explants compared to 14-day explants. The dry weight of the roots in 7-day old root explants is higher (15.9 mg and 19.18 mg) with calluses from irradiated explants (200 and 300 grays, respectively) than in control calluses (12.37 mg). Moreover, the water status of calluses is insignificant whatever the explant age and the irradiation dose (mutant). All calluses from 7-day-old roots are friable and whitish or brownish in color, as opposed to 14-day-old calluses which are pasty and whitish.

3.4 Effect of Explant Position on Callus Induction

Basal and apical positions of 7-day old seedlings roots grown on MS medium supplemented with 2 mg.L^{-1} 2.4-D significantly influenced the maize callus induction rate. This rate ranged from 52.0 to 73.33% (Table 4). Indeed, basal roots induced the maximum callus (68.88%) than apical roots (56.44%) at all doses combined. In addition, basal roots from the 300 grays dose induced the highest rate of callogenesis (73.33%). The explant position had a significant effect ($P < 0.001$) on dry matter weight of callus from the control and 300-gray mutants. However, no effect was observed on calluses from 200-gray mutants. However, calluses from the 200 and 300 grays induced from basal roots had the highest dry matter weights (16.19 and 20.54 mg respectively) compared to 12.2 mg for the control. On the other hand, the callus water content in 300 grays was only influenced ($P < 0.001$) by the explant position. The calluses obtained have friable (basal root) and pasty (apical root) aspects with different colors such as brownish-white, brownish and whitish.

3.5 Effect of 2.4-D Concentration on Callus Induction

All 2.4-D concentrations tested allowed the callus expression in all maize mutants studied (Table 5). However, the results showed that only the 3.5 mg.L^{-1} concentration induced high callogenesis rates in different non-irradiated (86% with a mean dry weight of 12.66 mg) and irradiated (86.66% and 80% with a mean dry weight of 17.51 and 13.47 mg, at 200 and 300 grays, respectively) mutants as opposed to the other concentrations. Thus, the lowest induction rate was obtained in the medium with low concentration of 2.4-D. In addition, the callus water content varied only with the different concentrations of 2.4-D and not with the mutants (control, 200 and 300 grays). This content averaged 91.65% for all mutants combined.The majority of induced calluses are friable except for those in the medium at 1 mg.L^{-1} of 2.4-D. The coloring of these calluses varied from white, brown to yellow (Fig. 2).

4. DISCUSSION

The influence of hormonal regime on callogenesis in the maize mutants (control, 200 and 300 grays) studied was demonstrated using three types of auxins (Dicamba, AIA and 2,4-D). Indeed, during tissue culture, the hormonalaction is essential to induce calluses and somatic embryos [11,12]. Thus, in this study, Dicamba, AIA and 2,4-D alone were tested for callus induction. The use of these hormones, alone and/or combined, for callus induction has been reported by several researchers [11,13,12,14]. This callogenesis dependence to the hormone could beexplained by the differential reactivity or sensitivity of explants to auxins [15,13,12]. Our work revealed that 2.4-D is the best auxin in callogenic expression. The work of Konaté [16] reported similar results. Indeed, this author mentioned that inauxin absence, no callus induction is observed in voandzou. Auxins are therefore essential in callus induction. However, callus induction rates are a function of type and concentration of auxin. This would explain the absence or low presence of calluses in AIA media. This result would indicate that the auxin type influences the callogenesis responses of root explants differently. Similar results have been reported in Indian elite maize [17].In this study, explants (roots, hypocotyls and cotyledons) from maize mutants irradiated (200 and 300 grays) and non-irradiated (control) with gamma radiation reacted differently to callogenesis in 2.4-D presence. Other researchers have reported the use of these explants in callus induction [18,19]. Our results showed that regardless of the dose used, the root explant responded better to callogenesis.

Table 3. Effect of explant age on maize callogenesis

Dose	Explant age (day)	IR (%)	DMW (mg ±s)	WC (%)	Texture/Color
	7	64,0 ± 3,49[a]	12,37 ± 0,94[a]	91,99 ± 0,30[a]	Fr/ W
Control	14	41,3± 3,06[b]	6,83 ± 0.36[b]	92,82 ±0,32[a]	Pa/ W
F		23,80	29,40	3,50	
P		***	***	ns	
	7	78,66 ± 3,06[a]	15,90 ± 1,41[a]	92,57 ± 0,26[a]	Fr/Br
200 grays	14	49,33± 3,30[b]	9,54 ± 0,80[b]	92,80 ± 0,10[a]	Pa/ W
F		42,35	15,38	4,80	
P		***	***	ns	
	7	60,0 ± 3,90[a]	19.18 ± 2,90[a]	92,07 ± 0,26[a]	Fr/ W
300 grays	14	40,0 ± 2,76[b]	11,92 ± 1,21[b]	92,86 ± 0,26[a]	Pa/ W
F		17,50	5,32	4,40	
P		***	*	ns	

*IR: Induction rate of callus; DMW: Dry matter weight; WC: Water content. Pa: Pasty; Fr: Friable; W: whitish; Br: brownish. The mean values are followed by their standard error (±). Values with the same letters are not significantly different (Tukey's test at 5%). P: Approximate probability of the Tests; F: Fischer's constancy. The effects are significant at α<0.05 (***: highly significant, *: significant, ns: not significant)*

Table 4 . Explant position effect on maize callogenesis

Mutant	Explant position	IR (%)	DMW (mg ±s)	WC (%)	Texture/Color
	BR	68,0 ± 4,70[a]	12,20 ± 1,19[a]	92,63 ± 0,35[a]	Fr/ BW
Control	AR	58,66 ± 4,12[b]	7,65 ± 0.57[b]	92,46 ± 0,46[a]	Pa/ W
F		9,22	11,75	0,1	
P		**	***	ns	
	BR	65,33 ± 3,06[a]	16,19 ± 1,25[a]	92,91 ± 0,33[a]	Fr/ Br
200 grays	AR	58,66 ± 4,56[b]	14,94 ± 1,02[a]	91,78 ± 0,27[a]	Pa/ Br
F		8,07	0,468	4,70	
P		**	ns	ns	
	BR	73,33 ± 2,51[a]	20,54 ± 3,20[a]	92,29 ± 0,25[b]	Fr/ W
300 grays	AR	52,0 ± 3,80[b]	11,16 ± 0,70[b]	90,85 ± 0,27[a]	Pa/ W
F		21,85	8,17	14,50	
P		***	**	***	

*IR: induction rate of callus; DMW: dry matter weight; WC: water content. BR: basal root; AR: apical root. Pa: pasty; Fr: Friable; W: whitish; Br: brownish; BW: brown-white. The mean values are followed by their standard error (±). Values with the same letters are not significantly different (Tukey's test at 5%). P: Approximate probability of the Tests; F: Fischer's constancy. Effects are significant at α <0.05 (***: highly significant, **: highly significant, ns: no significant)*

Table 5. Callogenesis parameters under2.4-D concentrations effect

Mutant	2,4-D (mg.L^{-1})	IR (%)	DMW (mg ±s)	WC (%)	Texture/Color
	1,5	50,0 ± 4,47^{c}	6,12 ± 0,21^{b}	94,24 ± 0,20^{a}	Pa /Br
Control	2	66,66± 4,21^{b}	11,20± 0,69^{ab}	90,70± 0,31^{c}	Fr /Br
	2,5	50,0± 4,47^{c}	12,55 ± 1,23^{a}	90,71± 0,34^{c}	Fr /Br
	3	60,0± 5,16^{b}	7,91± 0,68^{ab}	90,35± 0,22^{c}	Pa /Br
	3,5	86,0 ± 4,21^{a}	12,62 ± 2,42^{a}	92,23 ± 0,31^{b}	Fr / Br
F		11,25	5,07	31,20	
P		***	**	***	
	1,5	56,66 ± 8,02^{b}	17,02 ± 3,21^{a}	92,38 ± 0,27^{b}	Pa /Br
200 grays	2	66,66± 4,21^{ab}	15,95± 1,36^{ab}	91,93± 0,60^{b}	Fr /Br
	2,5	60,00± 5,16^{b}	9,86± 0.68^{b}	92,33± 0,56^{b}	Fr / W
	3	73,33± 9,88^{ab}	13,77± 2,75^{ab}	91,57± 0,58^{b}	Fr /Br
	3,5	86,66 ± 4,21^{a}	17,51 ± 1,39^{a}	93,34 ± 0,39^{a}	Fr / Y
F		13,16	6,19	4,80	
P		***	**	*	
	1,5	56,66 ± 6,14^{b}	7,37± 0,54^{ab}	91,61± 0,63^{a}	Pa / W
300 grays	2	60,0± 5,16^{ab}	13,37 ± 3,22^{a}	92,02 ± 0,70^{a}	Fr /Br
	2,5	66,66 ± 4,21^{ab}	7,69± 0,99^{ab}	88,42± 0,72^{b}	Fr /Br
	3	60,0± 7,30^{ab}	5,70± 0,51^{b}	90,64± 0,63^{ab}	Fr /Br
	3,5	80,0 ± 7,30^{a}	13,47 ± 2,37^{a}	92,28 ± 0,42^{a}	Fr / W
F		12,29	5,76	6,10	
P		***	**	**	

*IR: Induction rate of callus; DMW: dry matter weight; WC: water content. Pa: pasty; Fr: friable; W: whitish; Br: brownish; Y: yellowish. 2.4-D: 2.4-dichlorophenoxyacetic acid. Mean values are followed by their standard error (±). Values with the same letters are not significantly different (Tukey's test at 5%). P: Approximate probability of the Tests; F: Fischer's constancy. The effects are significant at α <0.05 (***: very highly significant, **: highly significant, *: significant)*

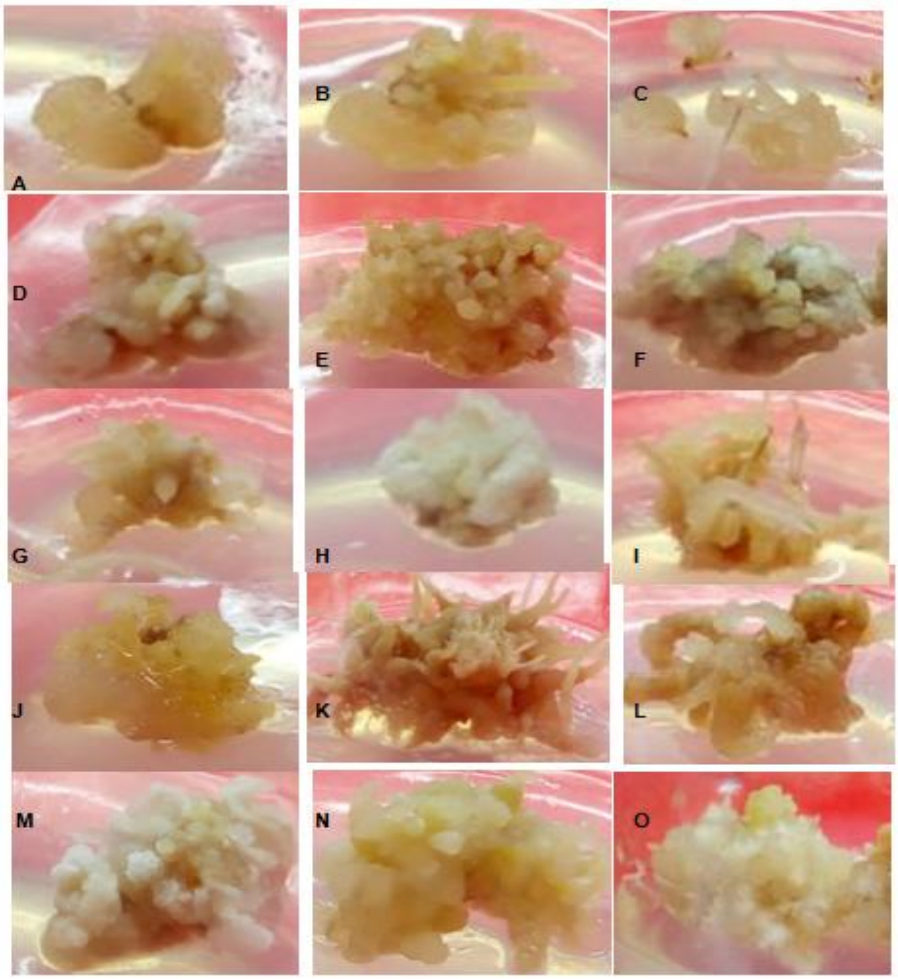

Fig. 2. Callus obtained on MS culture medium supplemented of 2.4-D (1.5; 2; 2.5; 3; 3.5 mg.L^{-1}) after 2 months

A: MS + 1.5 mg.L^{-1} 2.4- D (Root - Unirradiated Control) pasty brownish callus; B: MS + 1.5 mg.L^{-1} 2.4- D (Root - 200 grays) pasty brownish callus; C: MS + 1.5 mg.L^{-1} 2.4- D (Root - 300 grays) pasty whitish callus; D: MS + 2 mg. L^{-1} 2,4- D (Root - Non-irradiated control) friable and brownish; E: MS + 2 mg.L^{-1} 2.4- D (Root - 200 grays) friable and brownish callus; F: MS + 2 mg.L^{-1} 2,4- D (Root - 300 grays) friable and brownish callus. G: MS + 2.5 mg.L^{-1} 2.4- D (Root - Unirradiated control) friable and brownish callus; H: MS + 2.5 mg.L^{-1} 2.4- D (Root - 200 grays) friable and whitish callus; I: MS + 2.5 mg.L^{-1} 2.4- D (Root - 300 grays) friable and brownish callus; J: MS + 3 mg. L^{-1} 2,4- D (Root - Non-irradiated control) pasty and brownish; K: MS + 3 mg.L^{-1} 2,4- D (Root - 200 grays) friable and brownish callus; L: MS + 3 mg.L^{-1} 2,4- D (Root - 300 grays) friable and brownish callus. M: MS + 3.5 mg.L^{-1} 2.4- D (Root - Unirradiated control) friable and whitish callus; N: MS + 3.5 mg.L^{-1} 2.4- D (Root - 200 grays) friable and yellowish callus; O: MS + 3.5 mg.L^{-1} 2.4- D (Root - 300 grays) friable and whitish callus. MS [10]. 2.4-D: 2,4-dichlorophenoxyacetic acid

Our results are contrary to those of Kouakou et al. [19] who showed that callogenesis is better with hypocotyl than with root followed by cotyledon in cotton. The work of Kadiri et al. [20] also reported opposite results. Indeed, these authors mentioned that 45% of leaflets grown produced calluses compared to other explants. Arora and Chawla [21] indicate that the explant type and possibly its anatomical structure play a significant role in the initiation of callogenesis. The specificity of the response in different organs or tissues depends on their reactivity to the components of culture medium [13]. In addition, our results also revealed that the explant age has a significant influence on callogenesis. Thus, the youngest (7 days old) explants gave the best results compared to those 14 days old. According to Haggman et al. [22] and Ayolié et

al. [15], generally in tissue culture, the youngest explants are more favourable to callogenesis (immature embryos, young leaves, meristems, etc.). According to Runa and Maheshwar [12], the variation in induction rate is due to the differences in culture conditions and the age of explants.Our results corroborate those of Tautorus et al. [23] who showed that with the species *Picea mariana* whatever the explant cultured, the callus formation rate is 65 % with immature explants and 8 % with immature explants. Thus, Laine and David [24] and Ayolié et al. [15] have shown that immature explants have better competence than older explants for somatic embryogenesis. Our results also showed that in addition to having a young explant, the explant position contributes effectively to the induction of callogenic clusters. The basal root was more reactive than the apical part. This result could be explained by the fact that the root apex is the seat of auxin synthesis. The accumulation of internal and external 2.4-D would be too much (toxic effect) for callus induction. Contrary results were reported by Haouala et al. [25] in *Gerbera jamesonii* Bolus. Indeed, these authors mentioned that whatever the medium used, the rate of callogenesis increases from the basal position towards the apex.The variation in its concentration showed that 3.5 $mg.L^{-1}$ of 2.4-D is the best concentration for the expression of callogenicity regardless of mutant used. Our work is in agreement with that of Ayolié et al. [15] who showed that with wheat the best concentration of 2.4-D for callus induction is 3.5 $mg.L^{-1}$. Below this concentration, the explants tend to produce roots instead of callus. In other work in cereals, callogenesis has been obtained at lower concentrations of 2.4-D: 1 $mg.L^{-1}$in Soft Wheat [26], and 2 mg.l-1 in Durum and Soft Wheat [27] or much higher: 6 and 8 $mg.L^{-1}$ [28]. However, the rate of callogenesis depends on the auxin concentration used.

5. CONCLUSION

Since indirect somatic embryogenesis is dependent on the ability of explants to produce good callogenesis, the study of callogenesis optimization in the maize mutants studied showed that 2.4-D at 3.5 $mg.L^{-1}$ concentration was the best auxin for better callus induction. This callogenic ability was enhanced by the use of basal part of the younger root explants. Thus, mutants reacted differently to callogenesis. Thus, mutants resulting from gamma irradiation at 200 grays dose were more prompt to callogenesis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fukunaga K, Hill J, Vigouroux Y, Matsuoka Y, Sanchez GJ, Liu K, Buckler SE, Doebley J. Genetic diversity and population structure of Teosinte. 2005 ;45.
2. Dronne Y. Les matières premières agricoles pour l'alimentation humaine et animale : le monde. Ressources Alimentaires Pour Les Animaux D'élevage. 2018 ;31(3):165-180.
3. FAO. Food and agricultural organization of the United Nations ; 2018. Available:http://www.fao.org/faostat/fr/#data/QC/visualize. Site consulté le 15/07/2019.
4. Countrystat., 2013. http://www.countrystat.org/civ/cont/pxwebquery/ma/107spd080/fr. Consulté le 12 décembre 2019.
5. Lobell DB, Bänziger M, Magorokosho C, Vivek B. Nonlinear heat effects on African Maize as evidenced by historical yield trials. Nature Climate Change. 2011;1:42-45.
6. Weyens N, Van der Lelie D, Taghavi S, Vangronsveld J. Phytoremédiation: les partenariats plante-endophyte relèvent le défi. Opinion Actuelle en Biotechnologie. 2009;20(2):248-254.
7. Pereira JES, Mattos MLT, De Luces-Fortes GR..- Identificação e controle com antibióticos de bactérias endofíticas contaminantes em explantes de batata micropropagados. *Pesquisa Agropecuária Brasileira.* 2003;38(7):827-834.
8. Pavan KG, Sreenu P, Sridevi M, Reddy MK, Sateesh KP. Coleoptilar node - A season-independent explant source for in vitro culture in maize (*Zea mays L.*). Journal of Applied Biology & Biotechnology. 2018;6(3):20-28.
9. Kouakou TH, Kouadio YJ, Koné M, Zouzou M, Anno AP. Evolution des composés phénoliques au cours de la callogenèse et de la culture de suspensions cellulaires chez Gossypium hirsutum L. Bioterre. 2004;4(1):143-151.
10. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissues culture. Physiologia Plantarum. 1962;15:473-497.

11. Kouakou TH. Contribution à l'étude de l'embryogenèse somatique chez le cotonnier (*Gossypium hirsutum L.*) : Evolution de quelques paramètres biochimiques au cours de la callogénèse et de cultures de suspensions cellulaires. Thèse de doctorat 3è cycle, Université de Cocody, Abidjan, Côte d'Ivoire. 2003;144.
12. Runa R, Maheshwar PT. Callus induction and callogenic response of *Rauvolfia serpentina* and *Catharanthus roseus* by using various growth hormone concentrations singly and in combination. International Journal of Scientific & Engineering Research. 2014;5(1):300-308.
13. Zouzou M, Kouakou TH, Koné M, Amani NG, Kouadio YJ. Effect of genotype, explants growth regulators and sugars on callus induction in cotton (*Gossypium hirsutum L.*). Australian Journal of Crop Science. 2008;2(1):1-9.
14. Saja JSB. *In vitro* study of the callus induction of two varieties of wheat seeds by plant growth regulators. Agricultural Journal. 2018;13(3):67-71.
15. Ayolié K, Yacoubi EH, Rochdi A. Influence du 2,4-d et de l'explant embryonnaire sur la callogenèse du blé dur. Bulletin de la Société de Pharmacie de Bordeaux. 2007;146:97-112.
16. Konaté S, Koné M, Koffi KE, Zouzou M, Kouadio YJ. Induction et prolifération de cals à partir de l'axe embryonnaire du Voandzou (*Vigna subterranea (L.*) Verdc. Fabaceae) : effet de la segmentation de l'explant, des phytohormones, de la source de carbone et du génotype. Afrique science. 2013;9(1):140-150.
17. Manivannan A, Kaul J, Singode A, Dass S. Callus induction and regeneration of elite Indian maize inbreds. African Journal of Biotechnology. 2010;9(44):7446-7452.
18. Karl JR. Le maïs n'est pas neutre au jour; durée et floraison. Le bulletin de coopération sur la génétique du maïs. 2005;89(7):267-368.
19. Kouakou TH, Kouakou KL, Kouassi KM, Koffi KE, Koné M. Influence de la taille des vitroplants et du type d'explant sur la réponse à la callogenèse chez le cotonnier (*Gossypium hirsutum L.*) cultivé en Côte d'Ivoire. International Journal of Biological and. Chemical Sciences. 2014;8(2): 588-595.
20. Kadiri A, Ighilhariz Z, Bouabdallah L, Halfaoui Y..-Influence du génotype, du type d'explant et de la balance hormonale sur la callogenèse chez le pois chiche (*Cicer arietinum L.*). Revue « Nature & Technologie ». B-*Sciences* Agronomiques et Biologiques. 2014;11:59-66.
21. Arora A, Chawla SH. Organogenic plant regeneration via callus induction on chickpea (Cicer arietinum) role of genotypes, growth regulators and explants. Indian Journal of Biotechnology. 2005;4:251-256.
22. Haggman H, Jokela A, Krajnakova J, Kauppi, Niemi K. Aronen T. Somatic embryogenesis of scots pine: cold treatment and characteristics of explants affecting induction. Journal of experimental Botany. 1999;50(341):1769 - 1778.
23. Tautorus TE, Attree SM, Fowke LC, Dunstan DI. Somatic embryogenesis from immature and mature zygotic embryos and embryo regeneration from protoplasts in black spruce Picea-mariana mill. Plant science (Shannon). 1990;67(1):115-124.
24. Laine E, David A. Somatic embryogenesis in immature embryos and protoplasts of Pinus-caribaea. Plant science (Limerick). 1990;69(2):215-224.
25. Haouala F, Farhat N, Chabchoub L. Effets du type et de la position de l'explant sur l'induction de cals chez le gerbera (Gerbera jamesonii Bolus). Tropicultura. 2010;28(1):57-60.
26. Haliloglu K. Wheat immature embryo culture for embryogenic callus induction. Online Journal. Biological. Science. 2002;2(8):520-521.
27. Oudija F, Ismaili M, Amsa M. Effet de la concentration en NaCl sur l'embryogenèse somatique et sur les capacités de régénération chez le blé. African Crop Science. Journal. 2002 ;10(3): 211-219.
28. Bartok T, Sagi F. A new endosperm-supported callus induction method for wheat (*Triticum aestivum L.*). Plant Cell, Tissue Organ Culture. 1990;22(1):37-41.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67801