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Original Research Article

Response of pepper (*Capsicum* annuum) cultivars from Benin for somatic embryogenesis from callus

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The screening of resistant cultivars of pepper against environmental stress, such as drought and salinity, is a challenge to be addressed in Benin. However, it needs to be done in order to improve production and with regard to the importance of pepper in the country. This study was designed to evaluate the response of three selected pepper cultivars (Adologbo, Gbatakin, and TPS0251) to the production of calluses with a view to a large multiplication by somatic embryogenesis. The production of a large number of uniform materials is also essential for the selection of salinity-resistant varieties. Young leave explants collected from plants aged 15, 21 and 30 days after transplantation were grown on Murashige and Skoog mineral-based mediums complemented by 2,4-D and BAP at 2 mg/L and 1,86 mg/L respectively. Four weeks after cultivation, it was observed that the rate of induction of callus was not genotype-dependent and was not higher than 50 per cent on average for any cultivar of pepper. Callus was obtained only from explants collected from 30-day-old plants and all of the three cultivars were embryonic. These results will help as a basis to boost the production of plant material through somatic embryogenesis, which will help in the selection of resistant against cultivars.

Key words: Callus induction, micro-propagation, embryogenesis, salinity

INTRODUCTION

Chili pepper (*Capsicum spp*.) is a domesticated plant that originated from South and Central America. It is cultivated as vegetables and used worldwide as condiments, sweet peppers, hot peppers or as dried powder of different colors (Ravishankar et al., 2003). It is cultivated all over the world with essential importance in the human diet (Jin et al., 2009; Dias et al., 2013; Wahyuni et al., 2011). Chilli is produced in different areas of Benin, particularly in the plateau region, in the alluvial plains, in the valleys, in the lowlands, and the coast with four cropping systems namely: the pluvial system, the cultivation of recession, lowland cultivation and the irrigated system. It is one of the most cultivated and consumed vegetable crops and which undoubtedly occupies a prominent place in households.

Furthermore, it constitutes a significant source of income for potential farmers (PNUD, 2015). However, its culture faces many biotic (pests, diseases) and abiotic (drought, salinity) stresses which cause serious yield losses (Othman et al., 2010). Salinity is one of the most important environmental constraints that limit plant productivity, especially in arid and semi-arid climates (Husain et al., 2009). In Africa, nearly 40 million hectares are affected by salinization, representing 2% of the total cultivated surface. Market gardeners in southern Benin recognize the salinity of irrigation water and sea spray as a major problem for their activities (Orobiyi et al., 2013; Déguénon, 2018). Indeed, recent work conducted by laboratory of Plant physiology and abiotic stresses studies team and partners showed that the coastal zone of Benin, mainly dedicated to the production of market garden crops including chilli, is facing salinity problems due to irrigation water (Gandonou et al., 2012). Among the most effective methods of controlling salinity stress is the

Table 1. Characteristics of selected pepper cultivars

Characteristics	Adologbo	Gbatakin	TPS 0251
Fruit color	Red or orange	Red	Bright red
Cycle length	3 to 4 months	1 month	3 months and halh
Productivity	3 to 5 t/ha	7 to 10 t/ha	8 to 15 t/ha
Average fruit weight	2g	3g	6g
Fruit shape	Elongated	Ovoid	Elongated

creation of resistant or tolerant varieties using biotechnological tools. Thus, the eventual somaclonal variation generated by *in vitro* cultures through the callus stage has been used in several species for the creation of lines and plants tolerant to various environmental stresses (Lutts and Kinet, 1998; Gandonou et al., 2012). The study was therefore conducted to evaluate the aptitude of three pepper cultivars from Benin for callus production

MATERIALS AND METHODS

Plant material and experiment

Seeds of three local pepper cultivars (Adologbo, Gbatakin and TPS0251), were obtained from Market Gardening Crops Program of the Benin National Institute for Agricultural Research (INRAB). The characteristics of selected cultivars are presented in Table 1.

The seeds of the three peppers cultivars were germinated in a germinator filled with a mixture of soil and sand. The youngest leaves of 15-days, 21-days, and 30-days old seedlings of chili first rinsed with Tween 20 detergent for 30 minutes, then soaked in 70% ethanol for 1 minute and surface disinfected with 0.1 % mercuric chloride ($HgCl_2$) solution for 5 minutes. Afterwards, these were washed 3–4 times with autoclaved distilled water, 5 minutes each time, in a laminar flow hood. Leaves were then rinsed with sterile distilled water three times for three minutes. Explants of at least 1 cm^2 were cut and transferred onto semi-solid culture medium.

Murashige and Skoog (MS) mineral-based culture medium supplemented with 2,4-D and BAP at the rate of 2 mg/l and 1.86 mg/l respectively. Prepared culture media were autoclaved for 20 minutes at 121°C and allowed to cool in the culture room for later use. The work was carried out under the laminar flow hood, previously sterilized with UV and 70% ethanol and instruments were properly sterilized for this purpose.

The experiments were arranged in Completely Randomized Design (CRD) with six replications. Cultured explants were placed in the dark at a constant temperature of 26°C and 60% relative humidity for 4 weeks.

At callus stage, difference between embryogenic and non-embryogenic callus was made on the basis of the external appearance (texture, color and shape) of callus. Embryogenic calli were compact and characterized by their white color and globular structure while non-embryogenic calli were moist, translucent and brown in color.

Data collection and analysis

After four weeks, number of explants that produced callus and the quality of callus were determined. The percentage of induced callus (PC) was determined according to Kumar et al. (2010) formula.

$$PC = \frac{Number\ of\ explant\ with\ cal}{Number\ of\ explant\ cultivated}\ X\ 100$$

Capacity of embryogenic calli was expressed as a percentage of embryogenic calli production (PCE) given by the formula

$$PCE = \frac{Number\ \ of\ \ embryogenic\ calli}{Total\ number\ of\ obtained\ callus}\ X\ 100$$

The descriptive statistics (mean, frequency and standard deviation) on the rate of calli produced and the rate of embryogenic callus was carried out for the three cultivars. The test of two means was performed to assess the ability of the cultivars to produce calli using MINITAB 16 software.

RESULTS

Callus induction

After four weeks of culture, the explants of the cultivars Adologbo, Gbatakin and TPS0251 induced whitish calli The callus induction rate (50 %) was observed in Adologbo and Gbatakin while cultivar TPS0251 showed 33.33% of callus. Percentage of induced callus was statistically similar (P=0.677). Figure 1 showed Callus induction rate of three pepper cultivars

Subculturing on same media was favorable to the callus growth as they had visible growth after four weeks (Figure 2).

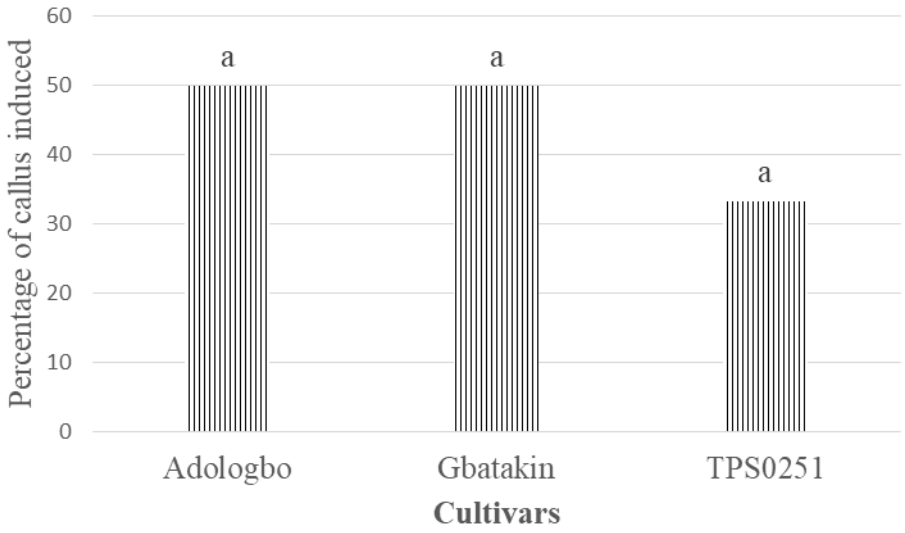

Figure 1: Callus induction rate of three pepper cultivars

Values with same letter are not significantly different (P = 0.677)

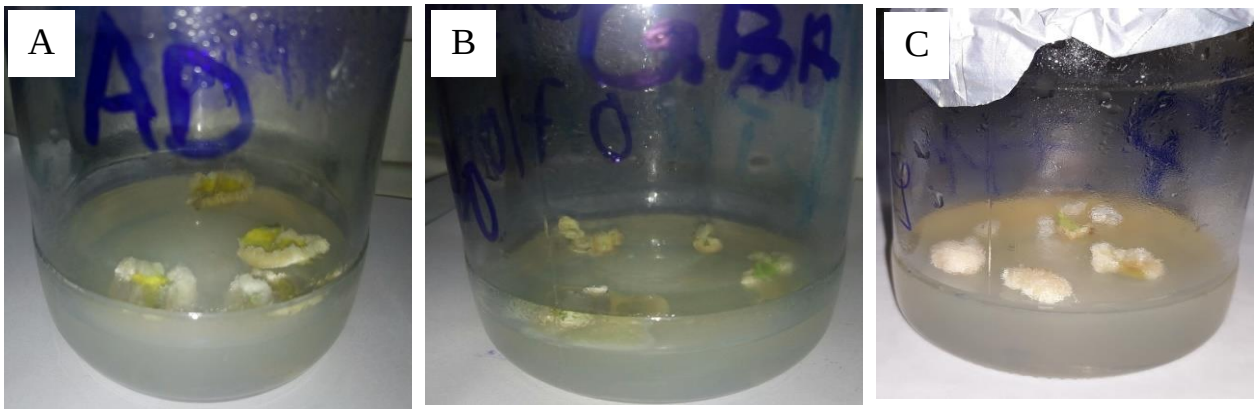

Figure 2: Callus induced in chilli pepper cultivars

A: Callus induced for cultivar Adologbo, B: Callus induced for cultivar Gbatakin, C: Callus induced for cultivar TPS 0251.

Formation of embryogenic calli

All calli produced by the 3 pepper cultivars evolved into embryogenic calli. Thus, there was no significant difference (P > 0.05) between the rate of embryogenic calli produced by the different cultivars of peppers. Cultivars Adologbo, Gbatakin and TPS0251 showed 100% embryogenic calli produced (Figure 3).

In terms of texture, all the induced calli of the different cultivars developed into detachable grain, after two weeks of subcultures of the calli. Therefore, all induced calli were embryogenic (Figure 4).

DISCUSSION

The present study indicated that chilli cultivars grown in Benin can be used for calli induction in. Callus induction (callogenesis) is an essential step during varieties creation and genetic improvement of plants using *in vitro* tissues culture. Plant tissue is an important source of genetic variability and can be viewed as a stock of genetic material but it is difficult to obtain callus from certain genotypes (Gueye et al., 2009). The embryogenic capacity of leaf cells explants depends on tissue age and on the duration and the formation of callus is genotype-dependent (Gueye et al., 2009).This callogenesis was initiated with three cultivars of chilli pepper during our research.

Our study aimed to obtain calli from leaf explants of three different pepper cultivars (Adologbo, Gbatakin and TPS0251) to determine the quality of these calli. In order to obtain these calli only one induction medium was used. This medium was a combination of two growth regulators: 2 mg / l of 2,4-D + 1.86 mg / l of BAP. Plant hormones play a crucial role in the development and

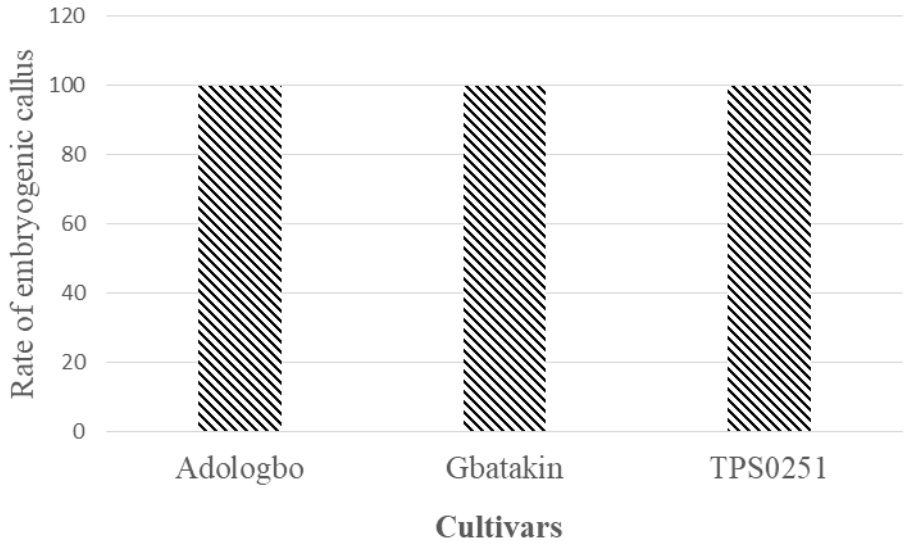

Figure 3: Differentiation rate of induced callus into embryogenic callus

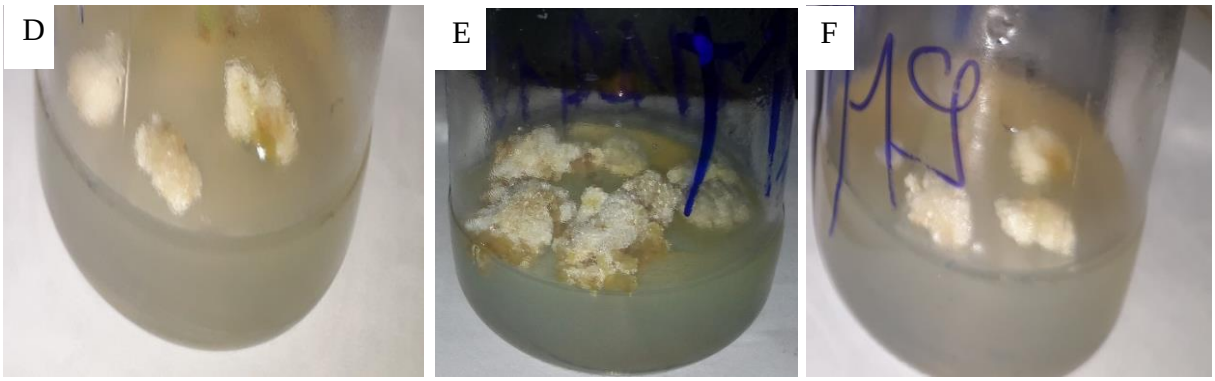

Figure 4: Formation of embryogeniccallithe 3 different cultivars.

D: Embryogeniccallifrom explant of cultivar Adologbo, E: Embryogeniccalli induced from explant of cultivar Gbatakin, F: Embryogeniccalli induced from explant of cultivar TPS0251.

growth of plants. Both auxins and cytokinins are needed synergistically to induce cell division and growth in plant tissue cultures (Kothari et al., 2009). The presence of 2,4-D and BAP in our induction medium was sufficient for the induction of callogenesis in young leaves of chilli peppers of three cultivars. The interaction between these two growth regulators was positive for callus induction, which was expected, since in general adequate balance between auxins and cytokinins is required for the differentiation of specialized cells in leaf explants of *Capsicum annuum*. Another significant observation was made in sweet potato by Bett et al. (2015) confirming the highest percentage of callus produced with leaf explants was using 2 mg/L of 2,4-D while stem explants produced the best results with higher range of 5 mg/L. The response of plants cultivated *in vitro* with plant hormones depends to the type of explants and the genotype of the plant. Other studies reveal that callus induction from leaf explants, hypocotyls or cotyledons of *Capsicum* spp. respond better under the influence of 2,4-D than any other form of auxin (Umameshwari and Lalitha, 2007; Rao and Sangapure, 2014) or even with 2,4-D alone (Filho, 2006; Kittipongpatana et al., 2007, Gueye et al., 2009). Leaf explants from plants aged for 4 week were used for the cultivars of Adologbo, Gbatakin and TPS0251. Our approach is supported by the work that uses leaf explants of two pepper cultivars, "Edino" and “Brujo", 3 to 4 weeks old for callus induction. Furthermore, Kumar (2010) has used foliar explants of chilli pepper plants of 14 to 15 days old to obtain callus. Chilli pepper is one of the recalcitrant plants for *in vitro* dedifferentiation and regeneration (Pishbin et al., 2014).

The responses of tissue cultures are greatly influenced by three main factors such as: physiology of mother plant,

in vitro manipulation and *in vitro* stress physiology (Benson, 2000). The concentrations used for 2,4- D (2mg/L) and BAP (1.86 mg/L) was favorable to obtain high percentage of callus from the the four-weeks leaf explants of Adologbo, Gbatakin, and TPS0251 cultivars.

CONCLUSION

The present study presents the advantage of achieving production of callus in three pepper cultivars (Adologbo, Gbatakin and TPS0251) produced in Benin. The use of these two growth regulators 2,4-D and BAP only encouraged calli induction in all three cultivars from four week old explants. The percentage of callus inductions obtained indicates that the medium used is suitable for the three cultivars of pepper. It is also important to note that all the calli obtained from the three cultivars are embryonic calli. These results are the basis for any genetic improvement of the somatic embryogenesis in the pepper cultivars study.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper.

REFERENCES

Benson EE (2000). Special symposium: *in vitro* plant recalcitrance do free radicals have a role in plant tissue culture recalcitrance? *In vitro* Cell. Dev. Biol. Plant. 86: 163-170.

Bett D, Murdoch LH, Wood ER, Dudchenko PA (2015). Hippocampus, delay discounting, and vicarious trial-and-error. Hippocampus 25(5): 643–654.

Déguénon E (2018). Communication présentée le mercredi 30 mai 2018 lors du Forum des jeunes chercheurs sur la Physiologie Végétale et ses applications au service du développement de l'agriculture : Cas de l'igname et des cultures maraîchères.

Dias GB, Gomes M, Moraes MT, Zottich UP, Rabelo GR, Carvalho AO, Moulin M, Gonçalves LSA, Rodrigues R, Da cunha M (2013). Charaterization of *Capsicum* species using anatomical and molecular data Gentet molecular data. Genetic Mol. Res. 12(4): 6488-6501.

Filho LDPF (2006). Calogênese em anteras e diversidade genética de acessos de pimenta (*Capsicum* spp.) do banco de germoplasma da Universidade Federal de Roraima combase em marcador RAPD. Dissertation(Master's degree in Natural Resources)– Universidade Federal de Roraima, Boa Vista. 54p.

Gandonou CB, Houmba NR, Ahoton LE, Desquilbet S, Fakambi K, Datinon B, Marshall E, (2012). Evaluation de la levée et de la croissance chez douze accessions de pourghère (*Jatropha curcas*) au Bénin. Bulletin de la Recherche Agronomique du Bénin (BRAB), 72 : 1840-7099.

Gueye B, Morcillo F, Collin M, Gargani D, Overvoorde P, Aberlenc-Bertossi F, Tranbarger TJ, Sane D, Tregear JW, Borgel A, Verdeil JL (2009). Acquisition of callogenic capacity in date palm leaf tissues in response to 2,4-D treatment. Plant Cell., Tissue and Organ Culture 99(1): 35–45.

Husain T, Hussain A, Ahmed M (2009). Studies of vegetative behavior and climatic effects on some pasture grasses growing wild in Pakistan. Pakistan J. Bot., 41 (5): 2379-2386.

Jin R, Pan J, Xie H, Zhou B, Xia X (2009). Separation and quantitative analysis of capaicinid in chilli pepper by received phase argentative LC. Chromatographia 70(5-6):1011- 1013.

Kittipongpatana N, Maneerat P, Pattanakitkosol P, Kittipongpatana OS (2007). Effect of some factors on the growth of *Capsicum annuum* L. cell suspension culture and biotransformation of hydroquinone to arbutin. CMU J. Natural Sciences 6(2): 207-218.

Kothari SP, S. Shu S, Wysocki PD (2009). Do managers withhold bad news? Journal of Accounting Research 47(1): 241–276.

Kumar A, Lingadurai S, Jain A, Barman NR (2010). Erythrina variegata Linn: A review on morphology, phytochemistry, and pharmacological aspects. Pharmacognosy Review 4 (8): 147-152.

Lutts S, Kinet JM (1998). Les effets des stress sur la croissance et le développement : Vue synoptique. Bull. Soc. roy. Sci. liège 67: 117-136.

Orobiyi A, Dansi A, Assogba P, Loko LY, Sanni A (2013). Chili (*Capsicum annuum* L.) in southern Bénin : production contraints, Varietal diversity, preference criteria and participatory evaluation. Int. Res. J. Agric. Sci. Soil Sci. 3(4):107-120.

Othman A, Jalil AMM, Weng KK, Ismail A, Ghani NA, Adenan I (2010). Epicatechin content and antioxidant capacity of cocoa beans from four different countries. African Journal of Biotechnol. 9(7):1052-1059.

Pishbin N, Mousavi A, Kalatejari S, Shariatpanahi M, Jahromi BB (2014). The effect of plant growth regulators and different types of explants on *in vitro* regeneration of sweet pepper (*Capsicum annuum* L.). International J. Biosciences 5(5) : 139-146.

PNUD (2015). Renforcement des capacités des maraîchers des communes de Adjohoun, de Bopa, et de Ouaké sur l'amélioration des techniques de production et de réduction des impacts des risques climatiques sur la culture du piment. Dépôt légal n° 7752 du 03/02/2015 , 1er trimestre, Bibliothèque Nationale du Bénin, 64p.

Rao S, Sangapure P (2014). Callus induction and organogenesis in *Capsicum annuum* L. cv pusajwala and g4. World J. Pharma. Res. 4(1): 644-657.

Ravishankar GA, Suresh GP, Raos J (2003). Biotechnologycal studies on *Capsicum* metabolites production and plant improvement In: DeAk, Editor, *Capsicum* : the genus *Capsicum* london : CRC Press. pp. 96 - 128.

Umameshwari A, Lalitha V (2007). *In vitro* effect of various growth hormones in *Capsicum annuum* L. on the callus induction and production of capsaicin. Journal of Plant Sciences 2(5): 545-551.

Wahyuni Y, Ballester AR, Sudarmonowati E, Bino RJ, Bovy AG (2011). Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. Phytochemistry 72(11-12): 1358-1370.