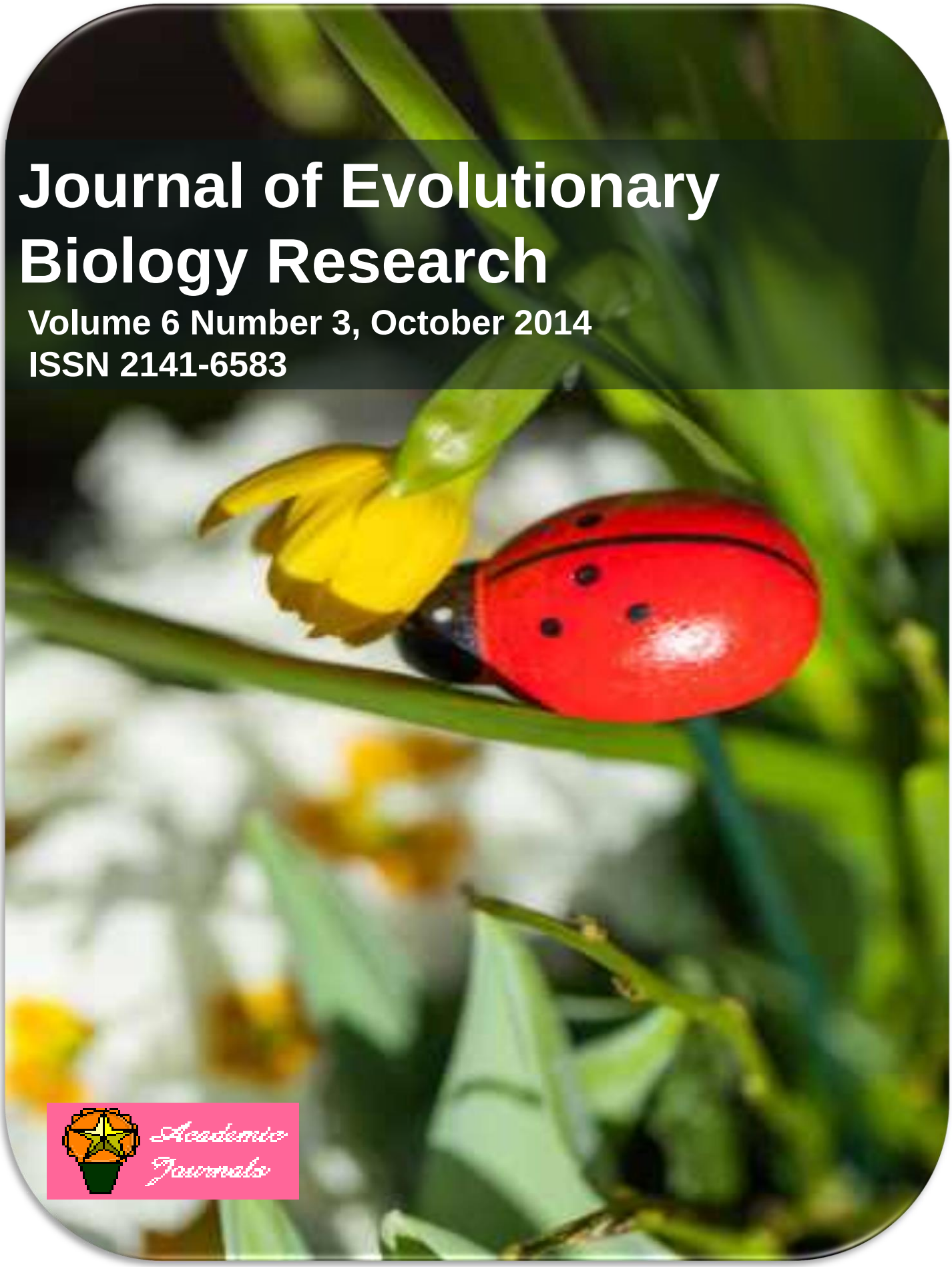

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Full Length Research Paper

Somatic embryogenesis in two Nigerian cassava cultivars (Sandpaper and TMS 60444)

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The embryogenic ability of two cassava cultivars (Sandpaper and TMS 60444) was investigated by culturing their young leaf lobes on an induction medium (DKW2 50P). The explants formed organized embryogenic structures regardless of the concentration of picloram in the medium. Organized embryogenic structures induction was enhanced by increasing the concentration of picloram in the induction medium for both cultivars. The optimum level of picloram at which maximum stable frequencies (53.1±17.6 and 51.5±14.6 for Sandpaper and TMS 60444 respectively) of organized embryogenic structures were obtained was 5 mg/l. Friable embryogenic callus were further produced in GD2 50P medium supplemented with 500 µM tyrosine. The produced friable embryogenic calluses are prime target tissues for genetic transformation and plantlet regeneration.

Key words: Cassava cultivars, explants, organized embryogenic structures, friable embryogenic callus, picloram, tyrosine.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most pervasive, highly valued and profiled crop in Nigeria. Grown in almost every field and frequently intercropped with okra (*Hibiscus esculenta*), maize (*Zea mays*), beans (*Phaseolus vulgaris*) and other crops, it has successfully gained acceptance and dominance over yams and cocoyam (*Colocasia esculenta*) as staple food in the country. Cassava's vegetative propagation is a mixed blessing (Thro et al., 1999). Its cultivation has been severely frustrated due to its high seed dormancy and

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Abbreviations: OES, Organized embryogenic structures; **FEC,** friable embrygenic callus; **GD, 50P,** Gresshoff and Doy medium + 5 ml of 10 mM picloram.

Table 1. Effect of picloram concentration on the induction of OES from *in vitro* young leaf lobes of Sandpaper and TMS 60444 cassava cultivars.

Picloram (mg/l)	Sandpaper	TMS 60444
	% positive	% positive
1	12.3±4.2	9.4±2.6
3	39.2±13.8	34.1±9.4
5	53.1±17.6	51.5±14.6
7	46.4±11.2	44.6±12.7
9	43.5±12.9	41.2±21.4
12	44.3±15.8	40.8±9.2

slow germination rate. Similarly, reduction in productivity and loss of superior genotypes has been attributed to accumulation of viral and bacterial diseases through vegetative propagation by stem cuttings. Another challenge to farmers is that cassava stakes in most environments cannot be stored but must be replanted soon after harvesting.

Conversely, somatic embryogenesis and plantlet regeneration via tissue culture is a prerequisite for developing new biotechnology applications for cassava and most crops. These approach and the compelling advantages of genetic transformation are opening up new possibilities in generating improved cassava genotypes by integrating desired agronomic traits into farmer-preferred cultivars such as yield increases, increase in nutritive quality, and reduced postharvest deterioration and cyanide content etc. (Ubalua and Mbanaso, 2013).

Other advantages derivable from these methods are the occurrence of somaclonal variation which offers the possibility of uncovering the natural variability in plants and the opportunity to use this genetic variability for development of new varieties (Evans and Sharp, 1986). We now report a method for inducing somatic embryogenesis in sandpaper (a farmer-preferred cultivar) and TMS 60444 (a model cassava cultivar) cultivated in the Southern States of Nigeria.

MATERIALS AND METHODS

Young leaf lobes of the *in vitro*-grown cassava plantlets (Sandpaper and TMS 60444) from National Root Crops Research Institute (NRCRI), Umudike, Umuahia, Nigeria were excised and used for the induction of organized embryogenic structures (OES) on DKW2 50P for three weeks.

The medium (DKW2 50P) pH was adjusted to 6.12 before autoclaving at 121°C for 15 min. Filter sterilized picloram was added to the medium when the medium temperature was 42°C before dispensing in sterile petri dishes. Ten leaf lobes were aseptically placed on the surface of petri dishes containing 25 ml of solid DKW2 50P solidified with 8 g/l of agar and supplemented with 20 g/l of sucrose.

A stereo dissecting microscope, sterile hypodermic needle and forceps were used for the exercise. The Petri dishes were sealed with parafilm and after three weeks of incubation under dimmed light conditions at 26±2°C, the cultures were scored for the presence of organized embryogenic structures. The promising yellowish structures were pooled together and the whitish mucus discarded. The pooled yellowish structures were meshed with a sterile spatula on a sterile mesh.

The method as described by Taylor et al. (1996) was adopted for the incubation, maturation and subsequent generation of friable embryogenic callus (FEC). The meshed OES were placed in small dots on freshly prepared GD2 50P + 500 µM tyrosine plates and incubated in a closed paper box. These embryos were recycled 3 times for a period of nine weeks for callus proliferation. The experiments were conducted using completely randomized design. The treatments were repeated three times (40 explants per treatment) and data were taken three weeks after each treatment.

RESULTS AND DISCUSSION

Induction of somatic embryos and subsequent regeneration of plants represents one of the most exciting models for studies of plant morphogenesis (May and Trigiano, 1991; Samaj et al., 1999).

The present study presents interesting aspects of callus initiation and somatic embryo induction from young leaf lobes of Sandpaper and TMS 60444 cassava cultivars. Young leaf lobes from *in vitro* mother plants of the cassava cultivars Sandpaper and TMS 60444 were used to induce organized embryogenic structures in the induction medium (DKW2 50P). The two cultivars produced OES at varying frequencies, although some of the explants did not respond to the treatment. A colour change of the lobes from greenish to pale yellow within ten days of incubation in the induction medium was observed. Organized embryogenic structures were observed between 14 and 21 days of incubation (Figure 1). Table 1 presents the developmental trend of OES in the induction medium containing increasing concentrations of picloram. The explants formed OES regardless of the concentration of picloram (Table 1) in the medium, although induction was promoted by continuous incubation. However, there are reports of some species that forms embryos in light as well as in

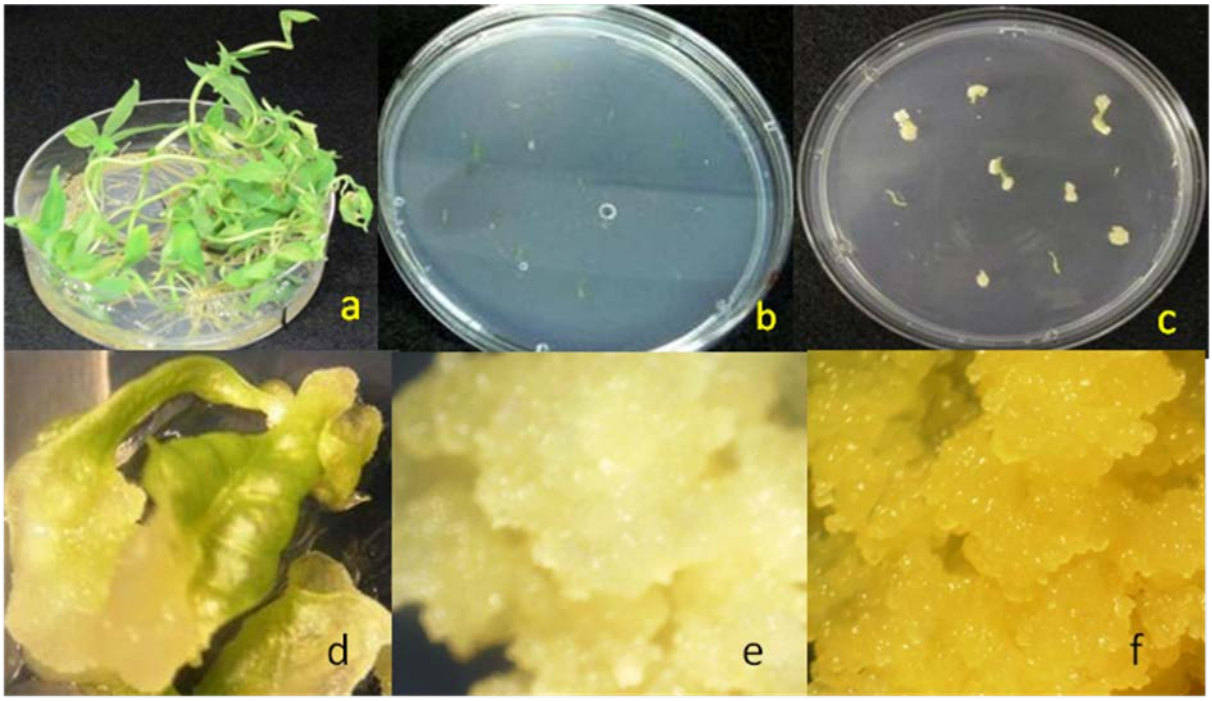

Figure 1. Induction and production of OES and FEC in cassava (*Manihot esculenta*). a, mother plant; b, OES induction on DKW2 50P; C, developing OES; d, magnified developing OES; e, FEC from sandpaper; f, FEC from TMS 60444.

darkness (Gingas and Lineberger, 1989). Data as shown in Table 1 reveals that OES induction was enhanced by increasing the concentration of picloram in the induction medium for both cultivars. The optimum level of picloram at which maximum stable frequencies (53.1±17.6; 51.5±14.6) of OES induction was obtained was 5 mg/l (Table 1). Percentage decline was observed at a concentration of 7 mg/l picloram which is higher than 5 mg/l while sandpaper cultivar produced a relatively higher positive response of 53.1±17.6% compared to 51.5±14.6 from TMS 60444 at 5 mg/l, and 44.3±15.8 and 40.8±9.2% were produced at 12 mg/l concentration respectively (Table 1). Similar results were obtained by Takahashi et al. (1999) and Li et al. (1998) on solid medium supplemented with 36 µM 2,4-D, where approximately 50% of the explants were able to form embryos.

A number of factors including choice of growth regulators and explants have been reported to be important for successful somatic embryogenesis (Luo et al., 1999). In this study, induction of OES was favourable by increasing picloram concentration to 5 mg/l (Table 1). The optimum concentration of picloram observed for OES induction did not vary from the previous reports by Taylor et al. (2001) and Ubalua et al. (2010), although 8 and 12 mg/l of picloram was reported by Rossin (2008) for optimum OES induction. Furthermore, there are reports in literature of the use of other regulators like 2,4-dichlorophenoxy acetic acid and copper sulphate in the induction medium, suggesting that different regulators could be amenable for the production of OES in the medium (Henry et al., 1994; Duncan, 1997).

Upon transfer of the OES to Gresshoff and Doy (GD) basal medium supplemented with 20 g/l sucrose, 50 mg/l picloram and 500 µM tyrosine, FEC was produced simultaneously following 4 weeks of culture. Friable embryogenic callus (Figure 1e and f) was successfully generated from both cultivars within the 4 weeks of culture and were maintained and multiplied by serial subculture every 3 weeks on solid Gresshoff and Doy (GD) basal medium supplemented with 20 g/l sucrose, 50 mg/l picloram and 500 µM tyrosine.

Comparatively, TMS 60444 produced more quality proliferating FECs (Fig. 1f) than the sandpaper cultivar (Figure 1e). Although various explants from many plants have been observed to produce somatic embryos the choice of explants is still a determinant factor (Williams and Maheswaran, 1986). Reports also abound that embryo development is dependent on reduction or absence of auxin in the induction medium (Carman, 1990). However, in the present study, production of FEC were significantly improved following subculture on GD medium supplemented with 500 µM tyrosine, suggesting that the OES from the two cassava cultivars responded optimally to tyrosine. The obtained result is consistent

with the earlier reports on the beneficial effect of tyrosine on FEC production by Taylor et al. (2001) and Ubalua et al. (2010). The variations in quality and amount of FEC produced may be dependent on the cultivar genotype, although the ability of the genotypes to produce somatic embryos is influenced by the type of explants, type of auxin and concentration (Rossin, 2008). This genotypic dependent variation in somatic embryogenesis has also been described in other cassava cultivars from different countries (Hankoua et al., 2006; Atehnkeng et al., 2006). In conclusion, despite the potential of the produced FEC as a prime target for genetic transformation and plantlet regeneration, the aspect of somaclonal variation in crop improvement programmes is compelling. Currently, somatic embryogenesis is an emerging path way for plant disease elimination which makes it also a desirable and an important technique.

Conflict of Interests

The author(s) have not declared any conflict of interest.

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