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Exploring the Nigerian Date palm [Phoenix dactylifera. L] Germplasm for in vitro Callogenesis

Abstract

Date palm is believed to have been introduced into Nigeria in the early 8th century by Arab traders from north Africa. Date fruits are highly valued delicacy among many communities in Nigeria, especially during ceremonies and festivals. The national consumption of dates in 2009 is estimated at 8,958 metric tons which placed the country among the world top 10 consumers of date. Despite conducive soil and climatic conditions for date palm cultivation and the existance of local varieties with good fruits qualities, date palm cultivation is still at subsistance level and demostic production is estimated at only 1,958 metric tons. Attempts to improve the Nigerian date palm industry through the establishment of commercial date palm plantations has been hindered by lack of good planting materials. However, recent evaluation of the response of some of the Nigerian date palm cultivars to 2,4-D induced callogenesis demontrated the high propensity of the genotypes to in vitro culture, with more than 50% embryogenic callus formation on modified MS supplemented with 50µm 2,4-D in all the genotypes tested. Further research is needed to complete and optimise this protocol in order to solve the problem of date palm planting materials in the country.

Keywords: Phoenix dactylifers, Genotypes, 2,4-Dichlorophenoxy acetic acid (2,4-D), Callus Formation, Callus maintenance, morphotype.

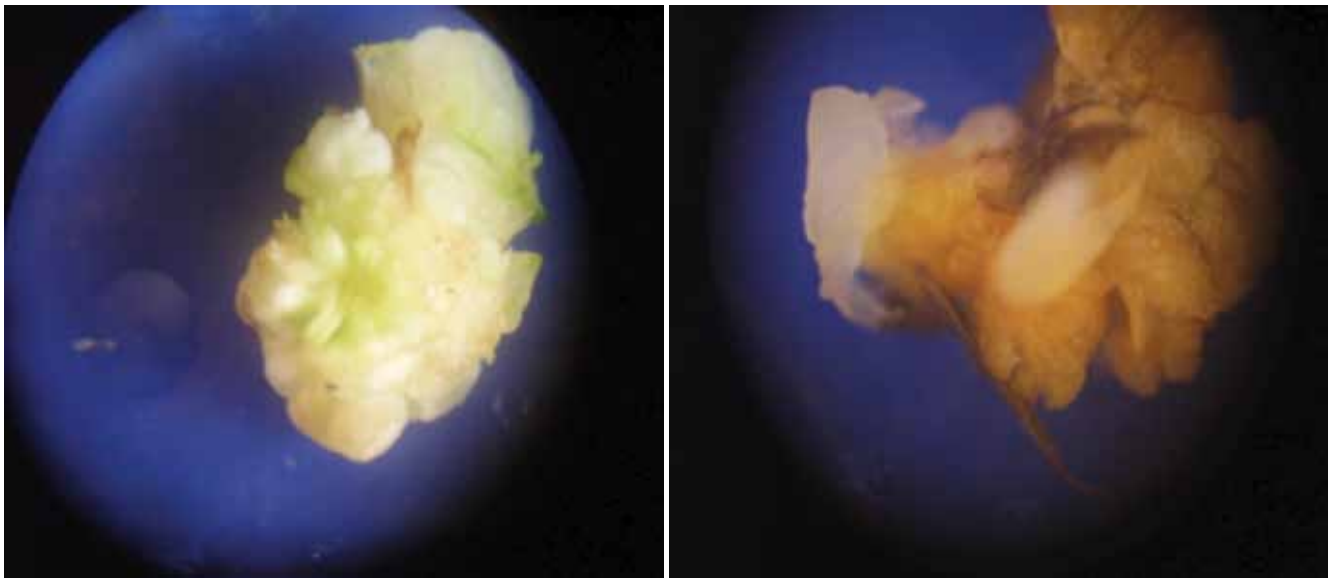

Fig1. Callus induction from (10-15mm) long juvenile leaf segments of date palm (P. dectylifera). A- white compact embryogenic callus excised from leaf segment of DPP1W after cultivation on modified MS supplement with 50µM 2,4-D for 60 days. B-White and brown compact callus from juvenile leaf segment of DPP3 after cultivation on modified MS supplemented with 50µM 2,4-D for 60 days.

Introduction

Date palm (Phoenix dactylifers) is believed to have been introduced into Nigeria in the early 8th century by Arab traders from north Africa, where it is traded in exchange with the dry leaves of Henna plant (Lawsonia inermins), a plant widely used for body decoration by women in many parts of the world. Date fruits are highly valued delicacy among many communities in Nigeria and enjoys a great spiritual and cultural significance. Nigerian date consumption as at 2009 is estimated at 8,958 t out of which 78% (7,000 t) were imported from Niger republic and North Africa via the trans-Sahara trade route. This value is significant when compared with 10,000 t imported annually by the United Kingdom which is among the 4 leading importers of date (FAO, 2003). However, only 22% (1.958 t) of the date consume is produce locally and this value is far below the annual production of Mexico (3,600 t) which is the least among 18 leading producers of dates (FAO, 2003).

In Nigeria, datepalm thrives well in the semi arid region (120 to140 N and 20 to140 E). This area forms an undulating plain with general elevation ranging from 450m to 700m and is covered by ferruginous tropical siols characterised by sandy-fixed undulating topography and is characterised by wet (June to October) and dry (November to May) season (Mortimore, 1989).. The prevailing soils and climatic conditions in this region support the growth of diverse date palm ecotypes and double fruiting per season with the second fruiting terminating at Kalal stage due to high humidity (June- August). Date palm cultivation is still at subsistance stage, where diverse cultivars are grown in homestead and few ochards by local date growers. Previous attempts to improve date palm cultivation through the establisment of commercial plantations were not successfull due to limitted number of planting materials. Tissue culture remains the most efficient alternative for rapid mass propagation of selected date palm genotypes. Protocols for in vitro regeneration of date palm based on somatic embryogenesis or organogenesis were reported (Tisserat, 1979; Zaid and Tisserat, 1983; Showky and Mahmoud, 1998; Al-Khalifa, 2004; Eke et al., 2005; Asemota et al., 2007; Ahmad et al., 2009). However, genotype specificity in their response to in vitro regeneration made it necessary to develop protocol for every date palm genotype (Hesselman, 1997). Since embryogenic culture allow for cyclic recovery of more plants, regeneration via somatic embryogenesis is an attractive alternative for rapid clonal propagation of date palm. In date palm, callogenesis is a prerequisite step to achieving in vitro propagation via somatic embryogenesis.

The objective of this study was to assess the callogenic competence

Table 1. Fruit phenology and 2007 average yield/plant of the date palm genotypes used for the in vitro studies in 2008

Genotype	Fruit type	Fruit colour	seed	Yield /plant (Kg)
DDP1W	Semi dry	Dark brown	Small	79.8
DDP2W	Semi dry	Light brown	Medium	106.3
DDP3	dry	Dark red	meduim	50.8
DDP4	Soft	Light brown	small	86.1
DDP5	Semi dry	Light brown	small	63.2
DDP8	Soft	Dark brown	small	68.3
DDP9	dry	Dark brown	small	94.0

in selected date palm genotypes in response to 2,4-D treament.

MATERIALS AND METHODS

Callus induction

Two to three-year-old offshoots of female plants of 7 date palm genotypes (Table 1) were obtained from germplasm bank of National Institute for Oil palm Research, Date palm substation Dutse, Jigawa State (11.70N, 9.30 E) in August 2008. After removing the mature leaves, offshoots were trimmed down to the apical bud region. Soft tissue segments measuring about 20-40 mm were cleansed with soap solution and treated with 20% w/v Benlate (Benomyl methyl1-(butylcartamoyl)-2-benzimidazole carbamate) solution for 60 minutes. The segments were surface sterilized by dippen in 70% ethanol for 1 minute followed by immersion in 20% commercial bleach (3.5% Sodium hyphoclorite) for 15 minutes and rinsed 3 times in sterile distilled water. The surface sterilized segments were further cut into small pieces (10-15 mm) and inoculated on modified MS supplemented with 2,4-D (50. 100, 150 or 200 µM), KH_2SO_4 (170 mg/L), and Activated Charcoal (250 mg/L) was added in the medium to check the browning of the explants. The pH of the medium was adjusted to 5.8 with 1M NaOH and solidified by adding 0.8% agar-agar (BDH, England), before autoclaving at 1210C and1.04 Kg cm2 for 15 minutes. All cultures were raised in baby food jar (67x55 mm), each dispensed with 35 ml of the medium. Cultures were incubated at 29 ± 10C under continuous dark and were examined at an interval of 10 days. After 30 days of incubation, the explants were subcultured on fresh media containing the same concentations of 2,4-D and incubated for another 30 days. After 60 days of culture the number of segments forming callus were recorded and expressed as percentage callus induction.

Callus maintenance

White compact callus was selected and subcultured on MS media supplemented with 50, 100, 150, 200 µM 2,4-D. For each treatment, callus with an average fresh weight of 500 mg was equally divided into 5 masses and incubated in baby food jar containing 35 ml of the media. Each treatment had three replications laid in completely randomised design. All treatments were cultured in dark for 30 days. Increase in the fresh weight of the callus was recorded and the proliferation coeffecient was culculated. Data obtained were subjected to analysis of varience (ANOVA) (SAS, 1998) and maens were seperated using Duncans multiple range test.

RESULTS

Callus induction

Preliminary experiment showed that fungal contermination in leaf segments obtained from offshoots was as high as 90% and can be reduce to < 10% by treating the explant with 20% benlate for 60 minutes. Explant cultured in the dark on 2,4-D free medium did not produce callus. Majority of the explant turned brown and showed intense oxidation and die after 4 weeks of culture. Explant culture on the induction media containin 2,4-D enlarge in size and innitiated white-brown colored callus at the cut edges after 2 months of culture in the dark (Fig. 1). All the datepalm genotypes tested in this study demonstrated optimum response to callus induction when cultured on induction medium containing 50µM 2,4-D (Table 2). In general, response of the genotypes to callogenesis increased when the concentration of 2,4-D was increased from 50 µM to 200 µM except in DPP4 and DPP5 for which increase in the concentration of 2,4-D from 150 µM to 200 µM does not result in

Table 2. Response of datepalm genotypes to in vitro callogenesis from leaf segments obtained from offshoots in 2008 under different 2,4-D regimes

Genotype	50 µM (2,4-D)	100 µM (2,4-D)	150 µM (2,4-D)	200µM (2,4-D)	Mt
	% CI means±sd	%CI means±sd	%CI means±sd	%CI means±sd	
DPP1W	50±7.29	77±8.77	79±10.87	92±6.07	wc
DPP2W	62±9.64	69±9.47	78±11.52	85±9.47	wc
DPP3	54±10.50	75±12.66	69±12.18	84±7.81	wc,b
DPP4	57±9.64	66±11.12	85±11.05	85±9.40	wc
DPP5	59±12.12	66±6.01	79±7.11	79±7.24	wc,b
DPP8	59±11.20	75±9.79	72±7.95	91±5.93	wc
DPP9	55±9.22	69±4.81	76±4.97	98±4.82	wc
Genotype	PC means±sd	PC means±sd	PC means±sd	PC means±sd	-
DPP1W	2.67±0.30	3.06±0.35	3.09±0.48	4.36±0.48	wc
DPP2W	2.14±0.42	2.78±0.50	3.07±0.15	3.13±0.94	wc
DPP3	2.50±0.28	2.49±0.16	2.95±0.60	3.27±0.46	wc
DPP4	2.45±0.18	2.80±0.18	4.09±0.42	4.0±0.38	wc
DPP5	2.35±0.06	3.07±0.24	3.45±0.34	3.96±0.56	wc
DPP8	2.38±0.09	2.45±0.15	3.53±0.33	5.16±0.56	wc
DPP9	2.34±0.12	3.62±0.38	3.94±0.49	4.45±0.38	wc

Key: CI-callus induction, PC- Proliferation coefficient, sd- standard deviation, Mt-callus morphotype

$$\text{Proliferation Coefficient} = \frac{\text{Fresh Weight (30 days after subculture)}}{\text{Fresh Weight (before subculture)}}$$

any increase in response to in vitro callogenesis. There were signicant differences among the genotypes in their response to callogenesis (Fig. 2). DPP1W (74.88%) and DPP8 (74.22%) produce the highest percentage callus induction and were different ($P < 0.0001$) from DPP9 (69.91%). Differences among the four 2, 4-D concentrations tested in this study were also significant (Fig. 4). Among the 2,4-D concentrations tested, 200 µM (85.25%) produce the highest percentage callus induction and the number of responding leaf segments significantly decreases with reduction in the concentration of 2,4-D.

Callus proliferation

In order to asses the effect of 2,4-D on the proliferation of embryogenic callus in datepalm, white compact callus masses of average weight 100mg were subculture on media enriched with 2,4-D at the same concentrations used for callus induction for 30 days. On the basis of increase in callus fresh wieght the treatments were grouped into two; the first group (50 µM -100 µM 2,4-D) increase the callus fresh weight by 1.0 to 2.5 fold and the second group (150 µM-200 µM) resulted in the increase in the callus weight by 3.0 to 4.5 (Table 2). Significant differences were observed among the 2,4-D concentrations (Fig 5) indicating that, increase in callus fresh weight was influenced by the concentration of 2,4-D. The highest proliferation was recorded with 200 µM and proliferation correspondingly decreases when the 2,4-D concentration was reduced. Significant differences were also observed among the genotypes in their response to in vitro callus proliferation (Fig 3) providing an insight that callus proliferation was in one way or the other influenced by the date palm genotype.

DISCUSSION

Mass propagation and genetic improvement of date palm requires the development of reliable in vitro regeneration system. Since callogenesis is a prerequisite step to achieving in vitro propagation via somatic embryogenesis, development of protocol for efficient callogenesis is a critical stage in achieving rapid propagation of genotypes of interrest.

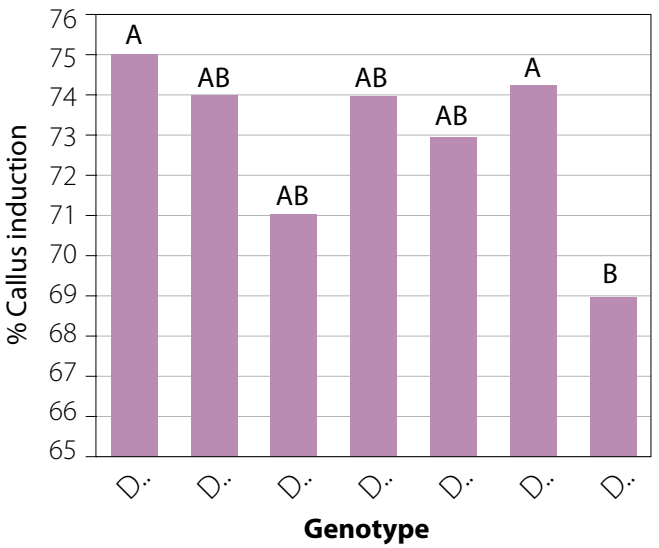

Fig. 2. Effect of genotype on the in vitro callogenesis in date palm

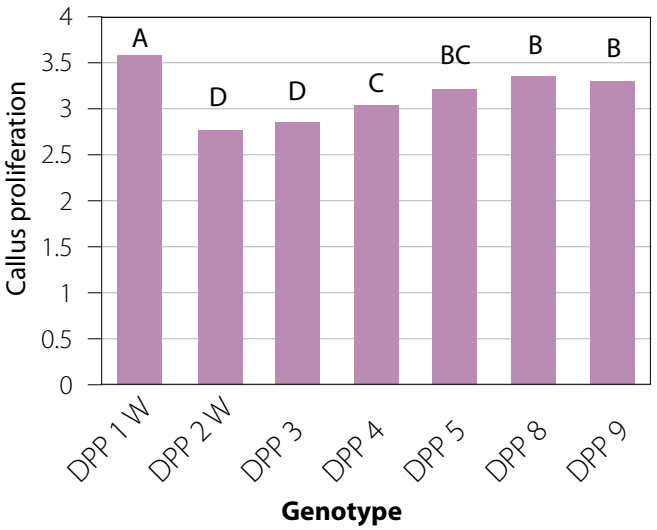

Fig. 3. Effect of genotype on callus proliferation 30 days after subculture

In this study, the seven date palm genotypes effectively produced embryogenic callus from immature leaf segments in media containing 2,4-D. In the case of date palm, leaf segments were reported to be the most competent tissue to form callus (Gueye et al., 2009). Callogenesis in date palm leaf segments in the presence of 2,4-D is characterized by two key events; The activation of fascicular paranchyma (FP) and reinitiation of cell cycle by callogenic perivalcular sheath cells (PSCs) leading to dedifferenciation and callus formation (Gueye et al., 2009).

Due to its stability 2,4-D has been used in the induction of callogenesis in wide range of species (Gaj, 2004; Ali et al., 2008). All the 2, 4-D concentrations used in this study produced callogenesis > 50%, demonstrating the efficacy of 2,4-D in the induction of callogenesis in date palm. This finding coincide with the report of Sane' et al. (2006) and Othmani et al. (2009) on callogenesis in leaf segment of P. dectylifera. Auxin mediated callus induction has been linked to certain factors which may trigger the complete chain of events that influence the ability of cultured cells to grow in an organized fashion. The presence of specific receptor, that reside either on cell membrane or within the cytoplasm (Mockeviciute and Anisimoviene, 1999). Specific binding site for both auxin has been identified (Kim et al., 2001). A class of proteins called expansins mediates the proton ability to cause cell wall loosening by breaking the hydrogen bonds between the polysaccharide components of the wall (Cosgrove, 2001). Proton (H+) pumping and lowering of cytosolic pH result in an elevation of intracellular calcium level (Shishova et al., 1999). Both cytosolic pH and calcium ions have been associated with early auxin action (Zhang, 2003). Calcium ions, either themselves and or along with calcium binding proteins e.g., calmodulin activate the protein kinase cascade which in turn activates other proteins, including the transcription factors (Wagner, 2001). These factors presumably interact with the auxin-response elements and regulate the expression of auxin-inducible or auxin-responsive genes and exert its effect on cell cycle and stimulate cell division (Johri and Mitra, 2001).

The current study has shown that callogenesis in date palm increase with corresponding increase in the concentration of 2,4-D from 50-200µM. This phenomenon was also reported to be more vigorous in date palm leaf segments cultured on media enriched with 50-200µM 2,4-D (Othmani et al., 2009) and 54–270 µM NAA (Gueye et al., 2009).

Callogenesis was to great extent influenced by the date palm genotypes. For example, callus induction and proliferation were >74% and > 3.3 fold respectively in DPP1W and DPP8, these values were sinificantly higher (P>0.001) than results obtained in the other five genotypes tested in this study.The type of callus tissue developed was also found to be genotype dependent. While white and compact callus was common to DPP1W, DPP2W, DPP4 DPP8 and DPP9, a mixture of white

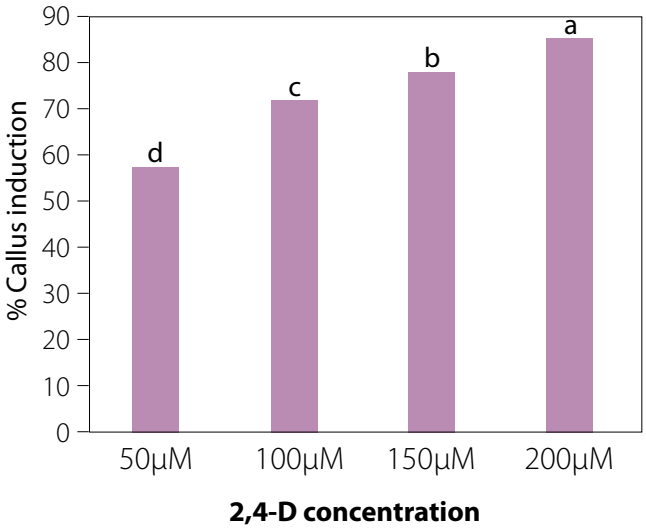

Fig.4. Effect of different concentrations of 2,4-D on callogenesis in date palm

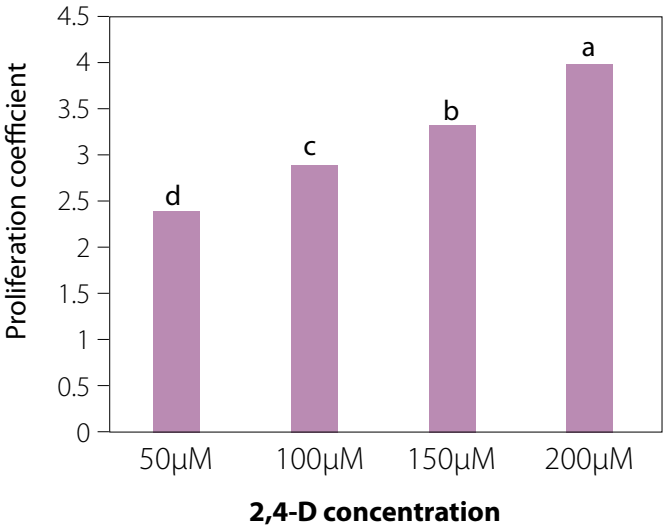

Fig. 5. Effect of different concentrations of 2,4-D on callus proliferation 30 days after subculture

and brown compact callus was common feature in DPP3 and DPP5. Similar observations were reported in African cassava by Atehnkeng et al., 2006. Variation in the response of the date palm genotypes to in vitro callogenesis could probably due to their physiological differences, particularly the endogenous IAA levels. Endogenous IAA levels were demontrated to be the main difference between leaf segments with various grades of callogenic competence in date palm (Gueye et al., 2009).

In this study all the date palm genotypes demonstrated a high success for callogenesis in leaf segments obtained from 2-3 year old offshoots. These genotypes exhibited optimum callogenesis when culture on modified MS supplented with 50 µM 2,4-D and their response increases with an increase in the concentration of 2,4-D, demonstrating their high propensity to in vitro callogenesis. Further research is needed to complete and optimise this protocol in order to solve the problem of date palm planting materials in the country.

Acknowlegement

The authors thanks Jigawa Research Institute, kazaure, Jigawa State, Nigeria for financing the research project.

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