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Research note

Influence of meristem-tip size and location on morphological development in *Dioscorea cayenensis - D. rotundata* complex 'Grosse Caille' and one genotype of *D. praehensilis*

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Abstract

In vitro morphological development of meristem-tips, excised from microshoots, is reported for one clone 'Grosse Caille' of *D. cayenensis-D. rotundata* complex and for one clone of *D. praehensilis*. The effects of size and location of meristem-tip on viability and morphological development after 3, 8 and 11 months *in vitro* culture were studied. The larger size of meristem-tip gave the best rooting and plantlet formation while the smaller size gave the best bud production. Axillary buds were more reactive than apical meristem-tips in callusing and rooting when small-sized meristems of the 'Grosse Caille' clone were used; the use of larger meristem-tips did not induce more differences between apical and axillary buds. The production of plantlets was successful, after 11 months of culture, with a score of 82 and 39 % survivors, for 'Grosse Caille' clone and *D. praehensilis* genotype, respectively.

Abbreviations: Ap–apical meristem-tips, ApLS–large-sized apical meristem-tips, Ax–axillary meristem-tips, AxLS–large-sized axillary meristem-tips, BA– 6-benzyladenine, cv-cultivar, LS–large-sized meristem-tip, NAA–naphthalenacetic acid, SS–small-sized meristem-tip

Yams are monocots and dioecious plants propagated vegetatively by tuber sets, a practice which increases the risk of spread of disease. Yam mosaic disease is a major problem and the *D. cayenensis* Lamk.- *D. rotundata* Poir. complex is particularly susceptible (Thouvenel & Dumont 1990). Mantell *et al.* (1980) tried to eradicate flexous rod viruses in *D. alata* L. by apical shoot-tip (0.6–2.5 mm long) and meristem-tip culture (0.2–0.5 mm long). Saleil *et al.* (1990) reported yam mosaic virus eradication in *D. trifida* L. by apical meristem-tip culture (0.2–0.5 mm long).

In order to produce microplants free from virus, information on morphological development of excised meristems and their ability to develop into microplants is required. To date, there has been no report on *in vitro* meristem-tip culture of edible and wild yam species of African origin. In this paper, the effects of explants size and location on the *in vitro* morphological development of meristem-tips excised from microshoots of one *D. cayenensis-D. rotundata* complex cultivar and of a *D. praehensilis* Benth. genotype, are described. These two species belong to the *Enantiophyllum* section.

Experiments were carried out with two clones belonging to the yam *in vitro* germplasm collection (Malaurie *et al.* 1993). Their identification numbers were clone PB04 for the 'Grosse Caille' cultivar of *D. cayenensis-D. rotundata* complex, and clone EA08 for the genotype of *D. praehensilis*. Meristem-tips were excised as previously described by Malaurie *et al.* (1995). Two meristem-tip sizes were chosen: SS -small- (0.3 mm to 0.5 mm) and LS -large- (0.6 to 0.8 mm). Meristem-tips were excised from either the

apical buds or the axillary buds on the stem. The number of meristem-tips excised from microplants differed in the treatments of the two clones. For PB04 clone, 208 small-sized meristem-tips (from the 208,138 were excised from apical and 70 from axillary buds) and 73 large-sized meristem-tips were used. For the EA08 clone, 113 small-sized (34 Ap and 79 Ax) and 46 large-sized meristem-tips were used. In order to study bud location effect, a separate experiment was performed with only large-sized meristem-tips excised from apical (ApLS) -11 meristem-tips- and axillary (AxLS) buds -35 meristem-tips- from the *D. cayenensis-D. rotundata* complex 'Grosse Caille'. Meristem-tips were cultured as previously described by Malaurie *et al.* (1995). The top layer was supplemented with 5.4 μM NAA and 0.9 μM BA. Data were recorded as previously reported (Malaurie *et al.* 1995). For the contingency analysis, the frequencies were compared with the Pearson's χ^2.

Survival rate of excised *D. cayenensis-D. rotundata* complex clone PB04 meristem-tips was affected by their initial size. LS meristem-tips had significantly better survival after 3 and 11 months of culture. In morphological development, rooting, shoot elongation were strongly and significantly affected by meristem-tip size. In all cases at 3, 8 and 11 months, LS meristem-tips gave the best results in rooting (34-82-93 %, versus 11-62-78 % SS) and shoot elongation (34-81-99 % versus 4-59-89 % SS). Budding was better with SS (12 %) versus LS (2 %) at 8 months of culture (Table 1).

Survival rate in excised meristem-tips of the *D. praehensilis* clone was affected by their initial size only at the third month of culture with a survival significantly better from SS explants (60%) compared with LS explants (37%). For rooting and shoot elongation, significant differences between SS and LS explants appeared only after 8 months of culture: LS gave better results (73 and 60%, respectively) than SS (39 and 18%, respectively). By contrast, small-sized meristem-tips allowed significantly better bud production (41% vs. 7%). Eleven months after meristem-tip inoculation, significant differences between the two explant types were observed for all aspects of morphological development. So for callusing, and as noted above for rooting and shoot elongation, LS gave better results (75, 83 and 83%) than SS (40, 43, 29%), whereas SS once again gave better results in budding (40% versus 0%) (Table 1).

Bud location (apical, Ap, or axillary, Ax) of SS meristem-tips did not affect the survival rate or morphological development of the excised *D. praehensilis* clone EA08 meristem-tips. A significant difference at 3 months was only seen in callusing, where Ap meristem-tips gave better results than Ax meristem-tips (50% versus 30%). Unlike the previous clone, the survival rate of the PB04 clone was affected by the bud location at 3 and 8 months after excision, with significantly superior results for the apical position. Callusing increased significantly at 8 and 11 months for the meristem-tips excised from axillary buds, with 55 and 45% compared to 34 and 26%, respectively, for the apical origin. For rooting too, the best result was obtained from axillary buds with 75% versus 58% from apical buds, after 8 months of culture (Table 1).

Bud location of excised large-sized meristem-tips affected survival only after 11 months of culture, where axillary buds (AxLS) had 77% surviving compared to 36% ApLS. Rooting (after 8 and 11 months of culture) and budding (after 3 months of culture) seemed better from the axillary meristem-tips displaying 78, 89 and 23% success, respectively; but insufficient population sizes did not allow statistical comparison. From 3 to 11 months of culture, no more buds appeared (Table 1).

Results presented here indicated that we succeeded in the production of plantlets from excised meristem-tips, with a score of 82% and 39% on the survivors at the 11 month, for *D. cayenensis-D. rotundata* complex 'Grosse Caille' and *D. praehensilis* genotype. These levels were far better than those obtained by Mantell *et al.* (1980) with a best yield of 19% plantlets from meristem-tips of *D. alata* cv. 'White Lisbon'. By contrast, Saleil *et al.* (1990) reported 80% plantlets regenerated from meristem-tips of *D. trifida* after only 94 days of culture. In addition to the genotypic origin, these important differences of behavior when compared with our results, may be due to the culture method involving a 3 steps subculture used by these authors.

The present study has demonstrated that, for the size effect and for the two clones tested, larger size of the meristem-tip gave the best rooting and shoot elongation while the small size gave the best bud production. The use of apical or axillary buds did not influence morphological development of the small-excised meristem-tips for *D. praehensilis* clone EA08. In the cv 'Grosse Caille' of the *D. cayenensis-D. rotundata* complex, this influence of bud location was modest, and appeared after long-term culture for callusing and rooting. The increase in size of the meristem-tips did

Table 1. Effect of meristem-tip size for PB04 clone of *D. cayenensis- D. rotundata* complex and *D. praehensilis* (clone EA08), and bud location for the PB04 clone, on morphological development at 3, 8 and 11 months of excision.[1]

Length of culture	Treatment	Number observed	Survival (%)	Percentage survivors exhibiting				Number observed	Survival (%)	Percentage survivors exhibiting			
				Callusing	Rooting	Shoot elongation	Budding			Callusing	Rooting	Shoot elongation	Budding
		PB04 clone (*D. cayenensis-D. rotundata* complex)						EA08 clone (*D. praehensilis*)					
3 months													
	SS	208	78	26	11	4	18	113	60	36	24	2	18
	LS	73	92	33	34	34	25	46	37	33	20	11	9
	df=1		**	NS	***	***	NS		***	NS	NS	<3[2]	NS
	Ap	138	85	23	11	4	21	34	65	50	21	0	24
	Ax	70	63	33	10	4	13	79	58	30	25	3	15
	df=1		***	NS	NS	<3[2]	NS		NS	*	NS	<3[2]	NS
	ApLS	11	91	27	36	18	9						
	AxLS	35	91	31	20	34	23						
	df=1		NS	NS	<3[2]	NS	<3[2]						
8 months													
	SS	162	72	40	62	59	12	56	41	48	39	18	41
	LS	67	89	40	82	81	2	15	33	60	73	60	7
	df=1		*	NS	**	**	*		NS	NS	*	***	*
	Ap	118	78	34	58	57	12	20	47	50	35	20	35
	Ax	44	61	55	75	66	11	36	38	47	42	17	44
	df=1		**	*	*	NS	NS		NS	NS	NS	NS	NS
	ApLS	10	91	30	50	50	0						
	AxLS	32	86	34	78	84	0						
	df=1		NS	NS	<3[2]	<3[2]	<3[2]						
11 months													
	SS	114	52	32	78	89	5	35	21	40	43	29	40
	LS	55	75	38	93	99	2	12	22	75	83	83	0
	df=1		***	NS	*	NS	<3[2]		NS	*	*	***	**
	Ap	81	55	26	74	88	6	14	23	36	36	29	29
	Ax	33	46	45	88	91	3	21	20	43	48	29	48
	df=1		NS	*	NS	NS	<3[2]		NS	NS	NS	NS	NS
	ApLS	4	36	25	25	100	0						
	AxLS	28	77	32	89	100	0						
	df=1		**	<3[2]	<3[2]	<3[2]	<3[2]						

[1]Meristem-tip size: small size-SS or large size-LS; Bud location: ApLS-apical large-size or AxLS-axillary large-size. [2]Insufficient theoretical sample size (<3). ***, **, *respectively significantly different for the 0.001, 0.01 and 0.05 levels, after comparison with the Pearson's χ^2 test. NS, not significant.

not induce differences in development between the apical and axillary meristems.

The lack of bud location effect observed in this experiment with large as well as small explants, should allow an important yield in micropropagating such material from excised meristem-tips. A similar response in shoot elongation is observed for the two species studied. Therefore, the results of this experiment could be valid for other ones of yams belonging to the *Enantiophyllum* section. Before initiating a program on the production of virus-free microplants, the

size and location effect of the meristem-tip should be reconsidered for the eradication aspect.

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