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Effect of storage conditions on pollen grains viability and pollen tubes elongation of four *Cola* species (*Malvaceae*)

Emmanuel Youmbi[1*], Libert Brice Tonfack[1], Jean Bernard Donfack Mbogning[1], Bernard-Alays Nkongmeneck[2]

[1]Department of Plant Biology, University of Yaounde I, Faculty of Science, Laboratory of Biotechnology and Environment, Unit of Physiology and Plant Improvement, P.O. Box 812, (YAOUNDE-CAMEROON)

[2]Department of Plant Biology, University of Yaounde I, Faculty of Science, Laboratory of Botanic and Ecology, P.O. Box 812, (YAOUNDE-CAMEROON)

E-mail : youmbi_emmanuel@yahoo.fr

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ABSTRACT

Self incompatibility and variability in the flowering time of different species of kola trees are the major factors that deeply affect the productivity of these plants. It seems absolutely important to conserve the pollen viability in order to create gene banks for *Cola* spp. breeding programmes. In this study, 3 cultivated *Cola* species (*C. ballayi*, *C. acuminata* and *C. anomala*) and one wild species (*C. lepidota*) were used to estimate, using *in vitro* germination tests, the pollen viability of fresh pollen and dehydrated pollen grains stored during 8 weeks at −20°C and 10°C. The tube length growth of fresh and stored pollen was also evaluated. Results showed that fresh pollen grains better conserved their germination potential than dehydrated pollen grains for all the four species. Germination capacities were better preserved at −20°C for non dehydrated pollen of *C. ballayi* (8 weeks), *C. acuminata* (7 weeks) and *C. lepidota* (5 weeks), and at 10°C for *C. anomala* (7 weeks). The pollen tube lengths decrease as the period of conservation lasts, for all species. These results are basically very important and applicable in breeding programmes to create new hybrids and improve *Cola* plant species.

KEYWORDS

Cola;
Conservation;
Pollen;
Viability;
Dehydration.

INTRODUCTION

Kola tree is a tropical plant of a genus *Cola*, family *Malvaceae* that produces seeds (kola nuts) containing many stimulating and/or appetizing compounds like caffeine[12]. Commercialisation of kola nuts increases the income of many families in the forest area of Cameroon[2]. But kola nuts production is very weak, due to the irregular fructification of plant trees. Phenological studies in this genus have revealed the presence of both hermaphrodite and male flowers[15] and the yield of the kola tree is correlated to the self incompatibility of its flowers and the number of hermaphrodite flowers on the plant[12]. However, many species do not flower at the same period[14]. Pollen conservation seems then very important as the conserved pollen grain can be used at the appropriate time for the breeding programs. The main use of stored

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pollen up till now has been in plant breeding to facilitate hybridization between plants that flower at different periods; it is also of great value in crops that possess short-live pollen, and in species that flower erratically. In the case of trees or woody species which take a long time to reach flowering stage, stored pollen can sometimes be used to shorten the process and allow crossing to take place[3, 5]. The importance of studies dealing with pollen viability and longevity has been largely recognised in pollination biology as a priority for helping to understand species reproductive performance and for the successful implementation of breeding programmes[5]. However, the conservation of pollen viability depends on the dehydration state of the pollen grain and the condition of conservation[7, 13]. Also, the conservation of the germination capacity depends on the pollen tube length. No reports are available on conservation of germination capacity of stored pollen of *Cola* species. The main purpose of this work was to find the optimal conditions of pollen conservation of three cultivated and one wild species of kola tree.

MATERIAL AND METHODS

Plant materials

All the kola trees in Cameroon are located in the South of the 8th parallel[14]. The pollen grains used in this study were freshly collected from four *Cola* species. Three of them were cultivated at Yaounde (mean temperature 27°C during the flowering period) in the Centre Region (*C. ballayi* and *C. acuminata*); and at Dschang (mean temperature 18°C during the flowering period) in the West Region (*C. anomala*). *C. lepidota*, a wild species was collected at Mutenguene (mean temperature 25°C during the flowering period) in South-West Region. Anthers were collected on at least three flowers per flowering plant and three plants (at least five years old) per specie from the field and kept in labelled and sealed plastic bags to prevent pollen dehydration during transportation to the laboratory. Anthers from the same plant were mix together. Pollen grains from each plant were then collected with scalpel, divided into seven groups (0, 1, 2, 3, 4, 5 and 6) corresponding to 0-6 weeks of dehydration periods.

In vitro culture and pollen germination

The standard medium of[1] supplemented with 1% agarose was used to evaluate the pollen germination. The prepared medium was mounted on slides, and pollen grains were spread using a magnifying glass. These slides were stored in Petri dishes under saturated atmosphere as described by[22]. The Petri dishes were incubated at constant temperature of 30°C for 24 hours. A photon microscope was used to count the number of germinated pollen. Pollen grains were considered as germinated when the tube length was longer than half the pollen diameter[20]. Germination data were collected on about 400 pollen grains on three replications (plants).

Pollen dehydration, storage and viability testing

Pollen grains were spread on Whatman paper, placed in Petri dishes, introduced into desiccators and the 6 different durations of dehydration were tested and compared to non dehydrated pollens. After that, pollens of groups 0, 1 and 2 were each divided in two subgroups and each subgroup were separated in at least 10 samples before introduction in labelled small glass bottles and storage. Two conditions of pollen storage were investigated: in the refrigerator at 10°C and in the deep freezer at –20°C. Germination capacity of stored pollen was tested every week until the total loss of viability. The frozen pollen grains were kept to stand at room temperature for 10 min and not rehydrated before shedding on the germination medium. Germination tests were conducted every 7 days on one sample per subgroup, with 3 replications. The pollen tube lengths were measured on an average of 60 germinated fresh pollen grains. A mathematical model was derived and used to describe the elongation of the pollen tube.

Statistical analysis

A split plot experimental design was used to evaluate the effect of dehydration and storage conditions on germination ability and the effect of storage on pollen tube elongation. The results were analysed using SAS software. A regression analysis of the pollen tube length was done with Microsoft Excel software.

RESULTS

Dehydration affects the germination of *Cola* spp. pollen grains

The rates of germination of the fresh pollen grains of *C. ballayi, C. acuminata, C. anomala* and *C. lepidota* were 64.65, 58.08, 49.74 and 35.78% respectively before desiccation. Germination of pollen grains from the four *Cola* species significantly decreased ($p<0.05$) with desiccation. After 1 week of desiccation, the rate of germination significantly decreased ($p<0.05$) by 89.62%, 87.48, 51.21 and 87.67% for *C. ballayi, C. acuminata, C. anomala* and *C. lepidota* respectively. Only pollen grains of *C. anomala* conserve about 15 % of it germination capacities (7.45 ± 1.13%) after 3 weeks of desiccation. After 6 weeks of desiccation, only the pollen grains of *C. ballayi* still germinate, but conserving only 0.05% of its germination rate (Figure 1).

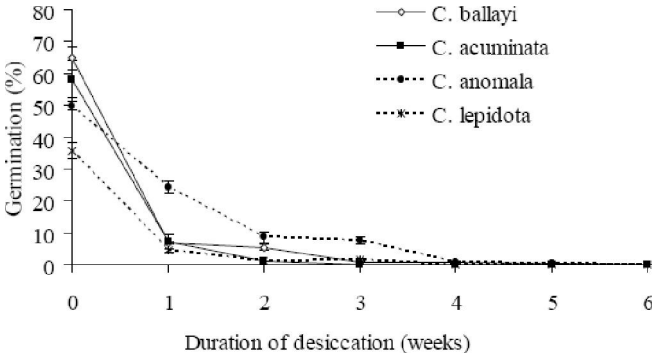

Figure 1 : Effects of dehydration on in vitro germination of pollen grains of *Cola* spp.

Storages in refrigerator and in deep freezer differently affect the germination of *Cola* spp. pollen grains

Non-dehydrated and one week dehydrated *Cola* pollen grains stored in refrigerator and in deep freezer better conserved their germination capacities after storage (TABLE 1). In general, non dehydrated pollen grains showed better germination rate after storage in refrigerator and in deep freezer. The statistical analysis showed that the effect of desiccation was highly significant

After one week of storage, non-dehydrated and frozen pollen grains of *C. ballayi* and *C. acuminata* still germinated at 44.41% and 35%; conserving 69 and 61% of their germination capacities respectively. In addition, frozen pollen grains of *C. ballayi* germinated at 20.39% after 5 weeks and at 1.57% after 8 weeks of conservation in deep freezer. The statistical analysis showed that the storage duration and desiccation were highly significant ($p= 0.001$) for *C. ballayi* and *C. acuminata* while the condition of storage was not significant. But the germination of non-dehydrated pollen grains of *C. ballayi* was remarkably higher than that of dehydrated pollen grain of the same specie (TABLE 2). The interaction between desiccation and storage duration was also highly significant for *C. ballayi*.

After two weeks of conservation in refrigerator, the non-dehydrated pollen grains of *C. anomala* germinated at 27.26%, conserving 55% of their germination capacity. When being previously submitted to one week desiccation, refrigerated pollen grains of *C. anomala* still germinate at 5.18% and 0.13% after 6 and 7 weeks of conservation in the refrigerator respectively. Statistical analysis showed that the effect of desiccation, the condition of storage, the storage duration as well as the interaction between desiccation and storage duration were highly significant ($p= 0.001$) for *C. anomala*. One week dehydrated pollen grains of *C. acuminata* stored during 6 weeks in the refrigerator germinated at 0.40% while non-dehydrated pollen grains lost their germination capacity after the

TABLE 2 : Statistical analysis on the effect of desiccation, storage conditions and storage duration on germination capacity of pollen grains of four *Cola* species.

SV	df	Calculated Fisher number of *Cola* spp.			
		C. ballayi	C. acuminata	C. anomala	C. lepidota
Replications	2	00 ns	0,2 ns	0,07 ns	0,04 ns
A	2	37.29***	14.67***	6.79***	12.13***
B	1	1.31 ns	1.66 ns	15.35***	0.17 ns
C	8	7.72***	7.6***	24.63***	11.29***
Interaction AxB	2	1.2 ns	0.97 ns	2.08 ns	0.05 ns
Interaction AxC	16	4.89***	34.47***	4.91***	7.28 ns
Interaction BxC	8	0.17 ns	0.46 ns	0.93 ns	0.08 ns
Error	156	-	-	-	-
Total	161	-	-	-	-

SV: Source of variation; df: degree of freedom; A: desiccation; B: storage condition; C: storage duration; ns: not significant; *: significant at p = 0.001**

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TABLE 1 : Variation of *in vitro* germination (%) capacity of pollen grains of four *Cola* species non-dehydrated and dehydrated stored at 10°C and -20°C.

Storage period (weeks)		Refrigerator (10°C)				Deep freezer (-20°C)			
		C. ballayi	*C. acuminata*	*C. anomala*	*C. lepidota*	*C. ballayi*	*C.acuminata*	*C. anomala*	*C. lepidota*
ND	0	64.65±3.76	58.08±5.87	49.74±1.39	35.78±2.39	64.65±3.76	58.08±5.87	49.74±1.39	35.78±2.39
	1	42.61±2.16	13.32±2.23	20.80±3.48	6.02±0.90	44.41±4.57	35.18±4.10	6.55±2.00	7.46±1.09
	2	6.94±1.46	3.96±0.40	27.26±6.50	3.04±0.49	35.81±4.95	23.04±3.11	0.59±0.28	1.58±0.56
	3	4.40±0.25	1.74±0.59	9.38±1.75	2.15±0.11	21.90±2.78	4.84±1.51	0.15±0.15	2.91±1.13
	4	1.34±0.49	0	1.95±0.80	0.08±0.08	14.35±1.33	6.95±2.15	0	0.85±0.52
	5	1.54±0.15	0	1.28±3.97	0	20.39±2.77	1.40±0.39	0	0.43±0.40
	6	0.30±0.10	0	0.73±0.73	0	9.82±1.98	0	0	0
	7	0	0	0.15±0.13	0	1.17±0.48	0	0	0
	8	0	0	0	0	1.57±0.15	0	0	0
1w	0	6.71±0.37	7.27±2.34	24.27±1.75	4.41±0.70	6.71±0.37	7.27±2.34	24.27±1.75	4.41±0.70
	1	2.55±0.64	2.00±0.51	23.89±2.35	1.02±0.47	2.6±0.35	10.18±1.67	2.00±2.00	5.30±1.16
	2	2.50±1.07	1.33±0.66	19.40±2.47	0.66±0.42	1.06±0.17	1.66±0.31	5.26±0.92	3.44±0.66
	3	3.57±1.10	1.33±0.43	22.04±2.35	0.53±0.43	0.67±0.21	4.00±1.21	2.19±0.74	0.57±0.24
	4	0.28±0.09	0.75±0.24	11.57±2.34	0.25±0.28	1.40±0.34	0.79±0.18	0.51±0.42	0.22±0.13
	5	0.46±0.23	0.7±0.66	8.89±1.57	0	0.49±0.49	0.44±0.24	0	0
	6	0	0.40±0.09	5.18±1.06	0	0.07±0.13	0	0	0
	7	0	0	0.13±0.37	0	0	0	0	0
	8	0	0	0	0	0	0	0	0
2w	0	5,47±0,93	1.08±0.09	8.68±1.67	1.12±0.11	5.47±0.93	1.08±0.09	8.68±1.67	1.12±0.11
	1	1,83±0,50	1.72±0.24	5.78±0.56	1.03±0.29	1.99±0.74	2.04±0.18	7.77±2.57	2.12±0.10
	2	0,50±0,34	0.86±0.13	2.69±0.41	0.50±0.44	0.72±0.32	0.83±0.23	2.52±0.54	0.41±0.09
	3	0	0	1.56±0.39	0	0	0.57±0.37	0.63±0.44	0.05±0.09
	4	0	0	0.45±0.48	0	0	0	0	0
	5	0	0	0	0	0	0	0	0
	6	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0

ND: Non dehydrated pollen grains, 1w: one week dehydrated pollen grains; 2w: two weeks dehydrated pollen grains.

same period of storage.

Non-dehydrated and frozen pollen grains of *C. lepidota* germinated up to 5 weeks after storage; but the germination rate highly decreased from 35.78% to 7.46% after one week of storage (TABLE 1). The effects of desiccation and storage duration were highly significant (p= 0.001) for *C. lepidot*a but interactions were not significant.

Effect of the storage on pollen tube elongation

Regarding pollen tube elongation of non-dehydrated pollen grains stored in refrigerator or in deep freezer, polynomial regression analyses were performed (Figure 2 and 3). The results showed that pollen tube length decreased with the storage duration in deep freezer (-20°C) and in the refrigerator (10°C).

The highest pollen tube lengths for *C. ballayi, C. anomala and C. lepidota* (1010.61, 713.11 and 693.48µm respectively) were obtained before storage in deep freezer. The regression equations obtained from the behaviour of pollen tube length after storage in deep freezer (-20°C) for *C. acuminata*, *C. anomala* and *C. lepidota* gave the higher and fairly similar amplitudes of 25.12, 19.72 and 23.13 respectively (Figure 2). The pollen tube lengths were

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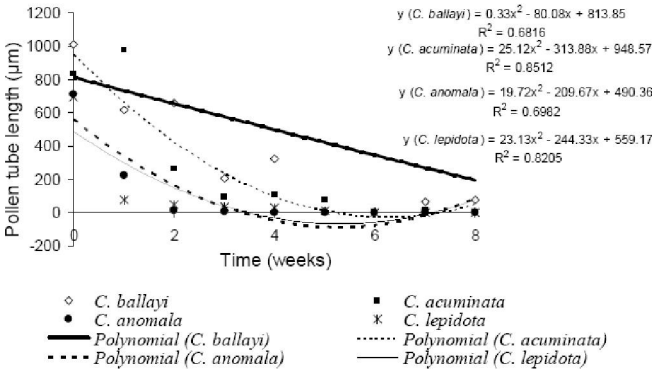

Figure 2 : Pollen tube elongation of non dehydrated pollen grains of four *Cola* species (*C. ballayi, C. acuminate, C. anomala and C. lepidota*) stored in deep freezer (-20°C).

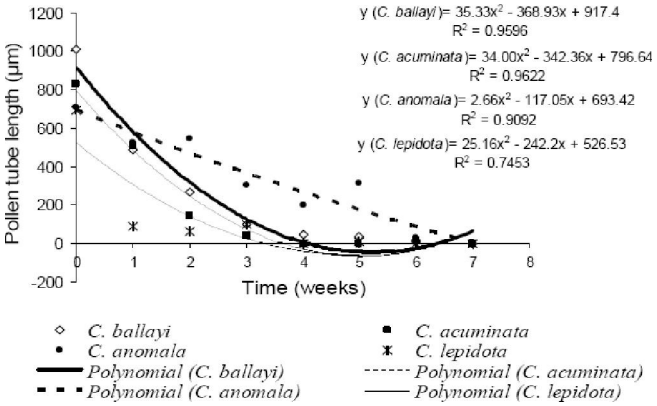

Figure 3 : Variation of pollen tube lengths of non-dehydrated pollen grains of four *Cola* species (*C. ballayi, C. acuminata, C. anomala and C. lepidota*) stored in refrigerator (10°C).

strongly reduced after one week of storage in deep freezer. The regression equation of *C. ballayi* with the smaller amplitude (0.33) showed the slight reduction of the pollen tube lengths with time. The pollen tube lengths at 6 weeks after storage in deep freezer were around zero for all the four *Cola* species. The pollen of *C. anomala* didn't grow after 2 weeks of storage in deep freezer.

The regression equation of the pollen tube lengths of *C. ballayi, C. acuminata and C. lepidota* stored in the refrigerator gave the very high amplitudes of 35.33, 34.00 and 25.16 respectively; showing the strong reductions of the pollen tube lengths with duration of storage. The regression equation of the pollen tube length of *C. anomala* stored in the refrigerator gave the smallest amplitude (2.66) indicating the slight reduction of the pollen tube length with time. The pollen grains of *C. anomala* still grow up to 316.78 µm after 5 weeks of storage in the refrigerator.

DISCUSSION

Pollen and seeds are plant organs that are generally much more tolerant to dehydration[10]. But the low germination rates of dehydrated pollen of *Cola* indicate that the changes occurred after dehydration were not reversible[19] and these pollens are less resistant to dehydration. The 2 weeks dehydration may have irreversibly affected the water and carbohydrates contents of pollen[9]. Also, the lost of pollen structural integrity is one of the principal causes of the pollen viability decreasing[4].

Our results showed that the non dehydrated pollen of *Cola* presents the good ability of germination. This indicates that the water content of fresh *Cola* spp. pollen was good for germination. As many plants of *Malvaceae* family, mature pollen of *Cola* are classified amongst the partially dehydrated pollen[7, 17].

C. anomala pollen showed a good longevity when stored in the refrigerator at temperature 10°C. Similar results demonstrating that pollen viability was highly dependent on relative humidity of the atmosphere have been published earlier[18]. However, the germination rates were higher in *C. ballayi* and *C. acuminata* pollen stored at –20°C. These findings corroborated the results obtained by many authors[6, 8, 20, 21] demonstrating that pollen viability was higher at low temperature. The reduction of germination capacity of non-dehydrated pollen grains stored at 10°C and at -20°C may be due to an inactivation of essential germination enzymes and substrates. Profound qualitative and quantitative changes in carbohydrates content occur during pollen storage[9] and the accumulation of secondary metabolites as organic acids during storage could subsequently inhibit pollen tube growth.

Entomophilous species are generally considered to have longer viability because the pollen grains must wait for pollinators[16]. Although Cola plants are entomophilous species, results obtained in this study showed that *C. lepidota* seems to have a short period of viability while pollen viability of *C. ballayi, C. acuminata* and *C. anomala* seem to be longer.

The pollen tube lengths of *Cola* spp. decreased with time when stored in deep freezer (-20°C) and in the refrigerator (10°C). These results suggest that the

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storage could have reduced the quantity of nutritive compounds of the pollen. Loguercio[11] stated that the variation in pollen tube elongation might reflect a characteristic genotypic constitution of gametophytic populations. The present results show that the elongation is more dependent on the age of pollen than on the pollen specie.

CONCLUSION

This study revealed that germination capacity of *C. anomala* pollen was better preserved than the other *Cola* pollen stored at -20°C. During storage, the pollen tube length decreases whether the pollen grains were preserved at 10°C or at -20°C. These results could be used in *Cola* breeding programs.

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