

ETUDE DE LA VIABILITÉ DES GRAINS DE POLLEN DE ONZE VARIETES DE TOMATES (*Solanum lycopersicum* L.) CULTIVEES A KISANGANI (RD Congo)

[STUDY OF THE VIABILITY OF POLLEN GRAINS OF ELEVEN VARIETIES OF TOMATOES (*Solanum lycopersicum* L.) CULTIVATED IN KISANGANI (DR Congo)]

O. Lokonga¹, D. Dhed'a¹, L. Okungo², and W. Oleko¹

¹Departement of Biotechnologies Sciences, Faculty of Sciences, B.P. 2012, University of Kisangani, RD Congo

²Departement of Phytotechnic, Faculty of Agronomic Science, B.P. 1232, Faculty Institute of Agronomic Sciences of Yangambi, Kisangani, RD Congo

Copyright © 2018 ISSR Journals. This is an open access article distributed under the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT: As regards the study of *in vitro* pollinic fertility, the best conditions for germination and elongation of the pollinic tube are obtained at 20 g/l sucrose, 0.62 g/l of boracic. On the whole, foreign varieties (Carotina, Marmande, Makis, Opal, Roma) have a weak indicator of viability as compared to local varieties. Fertility of different flowers analyzed according to their position on the plant, is generally decreasing from bottom to top. These results could explain some cases of failure of fertilization when crossing-over different varieties of tomatoes. The results also show the importance of choice of the flower used when sampling pollen grains.

KEYWORDS: viability, pollen grains, varieties, tomatoes (*Solanum lycopersicum* L.).

RESUME: En ce qui concerne l'étude de la fertilité pollinique *in vitro*, les meilleures conditions pour la germination et l'élongation du tube pollinique sont réalisées à 20gr/l de saccharose, 0,62 gr/l d'acide borique. Dans l'ensemble, les variétés étrangères (Carotina, Marmande, Makis, Opal, Roma) ont un indicateur de viabilité faible par rapport aux variétés locales. Quant à la fertilité de différentes fleurs analysées suivant leur position sur la plante, elle est généralement décroissante de bas vers le haut. Ces résultats expliqueraient certains échecs de fertilisation lors de croisement entre différentes variétés de tomates et montrent l'importance de choix de la fleur servant pour le prélèvement des grains de pollen.

MOTS-CLEFS: viabilité, grains de pollen, variétés, tomates (*Solanum lycopersicum* L.).

1 INTRODUCTION

So that the reproduction of tomato proceeds correctly, it is necessary that pollen has a reproductive good quality. This quality recovers two aspects, the physiological quality of pollen as gametophyte male and a its capacity to achieve the totality of the program of reproduction male [1,2]. Thus, so that the male cycle of reproduction is achieved suitably, pollen must initially be viable when it is released from the anther and must have a good longevity, i.e. to remain viable sufficiently a long time until reaching a specific mark [3]. Then, pollen must be able to germinate on the mark and to produce a pollen tube which reaches an ovule [4]. Lastly, it must release from the gametes able to fertilize the polar oosphere and cores, which has its aptitude to fertilize.

The whole of these characteristics is gathered under the term of reproductive quality that certain authors include under the term of viability of pollen [3]. The reproductive quality of pollen will be made up of its viability, its capacity to be germinated and its aptitude to be fertilized. It is possible to evaluate viability by testing the capacity of germination of pollen, its metabolic activity (enzymatic) or the presence of the cytoplasm [5]. According to Dafni and Firmage [3], the reproductive quality of pollen depends on many factors which can be intrinsic, morphological or environmental, like the microsporogenesis, the metabolism of pollen, the morphology of the flowers and the anthers, the period of antheses, the pollinating vector, secretions of insects, the relative humidity and the temperature.

The viability of pollen conditions the success of fecundation at tomato and it varies much after its emission of the anther. The viability of pollen can be affected by the temperature and moisture [5]. The action of a temperature excessively high on the tomato flower affects the male bodies and appears according to several aspects. The malformation of the anthers preventing the release of pollen with maturity and an insufficiency of the germination of pollen and growth of the pollen tubes.

According to the component to be tested, the reproductive quality of pollen can be evaluated by various methods. Those make it possible to evaluate the effect of a given factor or to make sure of the quality of pollen before the practice of pollinations manual [6, 7, 8]. In our case, we want to reassure ourselves if all the local and foreign varieties have the same pollinic fertility and if this fertility varies according to the position of the flower on the plant.

The specific objective of this Study is to evaluate the pollinic fertility of the local and foreign varieties of tomato studied. This is important before planning hybridizations to be carried out and the choices of the flowers with this intention.

2 VEGETABLE EQUIPMENT

Vegetable equipment consists of local varieties (Red round, Purple round, Red flattened, Purple flattened, Red lengthened and Purple lengthened) and foreign (Carotina, Makis, Marmande, Opal, Roma).

3 METHODS

The capacity to be germinated of pollen can be evaluated by tests of *in vitro* germination. The capacity to be germinated of pollen is measured by calculating the percentage of pollen grains germinated in a sample [9]. For that, the pollen grains are put to germinate *in vitro* on an adapted medium of germination. *In vitro* germination is a technique which consists in depositing pollen grains on a solid medium and to observe them under microscope to visualize the formed pollen tubes [10]. This method however requires an important work of adaptation of the germinative medium to the species considered, more particularly with regard to the source of carbon.

Indeed, the saccharose concentration necessary to *in vitro* germination of pollen varies between 2% and 40% according to the species. The saccharose optimum must be given for each species using tests of germination to various concentrations. A too weak saccharose concentration involves a low level of germination and little developed pollen tubes whereas a too high concentration causes the bursting of the pollen tubes [7].

For much of species, boric acid and calcium are necessary to the germination of pollen. The boric acid normally brought by the style and the mark at these species facilitates the consumption of sugars and plays a part in the production of pectin in the pollen tube [7]. Calcium present at the surface of certain pollen grains is implied in the pectin synthesis and the control of the osmotic conditions [7]. *In vitro* germination provides a controlled experimental system, allows an important repeatability and is fast to set up.

However, it does not reproduce the growth completely *in vivo* [11]. Indeed, with a medium of germination highly optimized, the formed pollen tubes reach only 30 to 40% length of the tubes developed *in vivo* and of the structural anomalies are frequently observed. Moreover, the existing dynamic interaction between pollen and the pistil remain impossible to reproduce *in vitro* [11]. This study of the reproductive quality of the pollen of *solanum lycopersicum* was carried out by using pollen coming directly from the anthers. The anthers were excised.

The anthers are broken in order to release pollen in the culture medium. The unit is maintained 4:00 at the temperature of laboratory to induce germination. After incubation, 2 drops of acetic carmine 5% were added. After 10 minutes of colouring, the pollen grains are then observed under the inverted microscope (Brand Motoc AE31) in order to determine the percentage of germination on the various varieties. A pollen grain was considered germinated when the length of the pollen tube was greater than the diameter of the grain. The test of germination was applied to five flowers laid out with different levels, for the local and foreign varieties. The flowers are dissected to take the anthers and the anthers are broken in order to release the pollen which one sowed on the various culture media below.

3.1 RESEARCH OF THE OPTIMUM CONDITIONS FOR IN VITRO GERMINATION OF THE POLLEN GRAINS

To search the conditions supporting the germination of in vitro pollen grains, 4 series of culture media of Murashige and Skoog ms [12] were prepared:

Agar medium containing macroelements ms saccharose enriched into 2% Saccharose.

Medium agar saccharose enriched in micronutrients MS.

Midium agar saccharose enriched in vitamins MS.

Medium agar saccharose enriched out of growth regulators (AIA: Acid acetic 3-indol and BAP: 6-Benzyl aminopurine).

The detailed composition of medium MS is given to table 1.

Table 1: Composition of medium of Murashige and Skoog[12]

		Concentration in	
		mg/l	µM
Macroelements	NH ₄ NO ₃	1.650	20.650
	KNO ₃	1.900	18.810
	CaCl ₂ .2H ₂ O	440	2.990
	MgSO ₄ .7H ₂ O	370	1.500
	KH ₂ PO ₄	400	2.970
Microéléments	H ₃ BO ₃	6,18	100
	MnSO ₄ .H ₂ O	16,9	100
	ZnSO ₄ .7H ₂ O	8,6	30
	KI	0,83	5
	Na ₂ MoO ₄ .2H ₂ O	0,24	1
	CoCl ₂ .5H ₂ O	0,024	0,1
	CuSO ₄ .5H ₂ O	0,025	0,1
Iron	FeSO ₄ .7H ₂ O	27,8	100
	Na ₂ . EDTA.2H ₂ O	37,22	100
Antioxydant	ac. Ascorbic	10	56,78
Vitamins	Wisteria	2	-
	Thiamin	0,5	-
	Ac. Nicotinique	0,5	-
	Pyridoxin	0,4	-
Growth regulators	AIA	0,17	1
	BAP		
	- Proliferation	2,25	10
	-Regeneration	0,23	1
Source of carbon	Saccharose	30.000	87.642
Gelling	Agar	5.000	-
	or gelerite	2.000	-

3.2 SOWING OF POLLEN GRAINS THE POLLEN

Grains were collected flowers between 7 a.m. and 8 hours 30 minutes, then sown on the various culture media so - high. The observation under the photonic microscope was carried out in order to detect the germination which results in the pollen emission of tube.

3.3 ENUMERATION AND MEASUREMENT POLLEN LENGTHS OF TUBES

The enumeration of the pollen grains consisted in counting all the pollen grains germinated and not germinated present in the microscopic field in order to determine the rate of germination. The measure of length of the pollen tubes of the

germinated pollen grains was made using the ocular micrometer. The micrometric factor was given by using the ocular micrometer by reference to the objective micrometer.

The culture of pollen grains and germination *in vitro* are illustrated by figure 1.

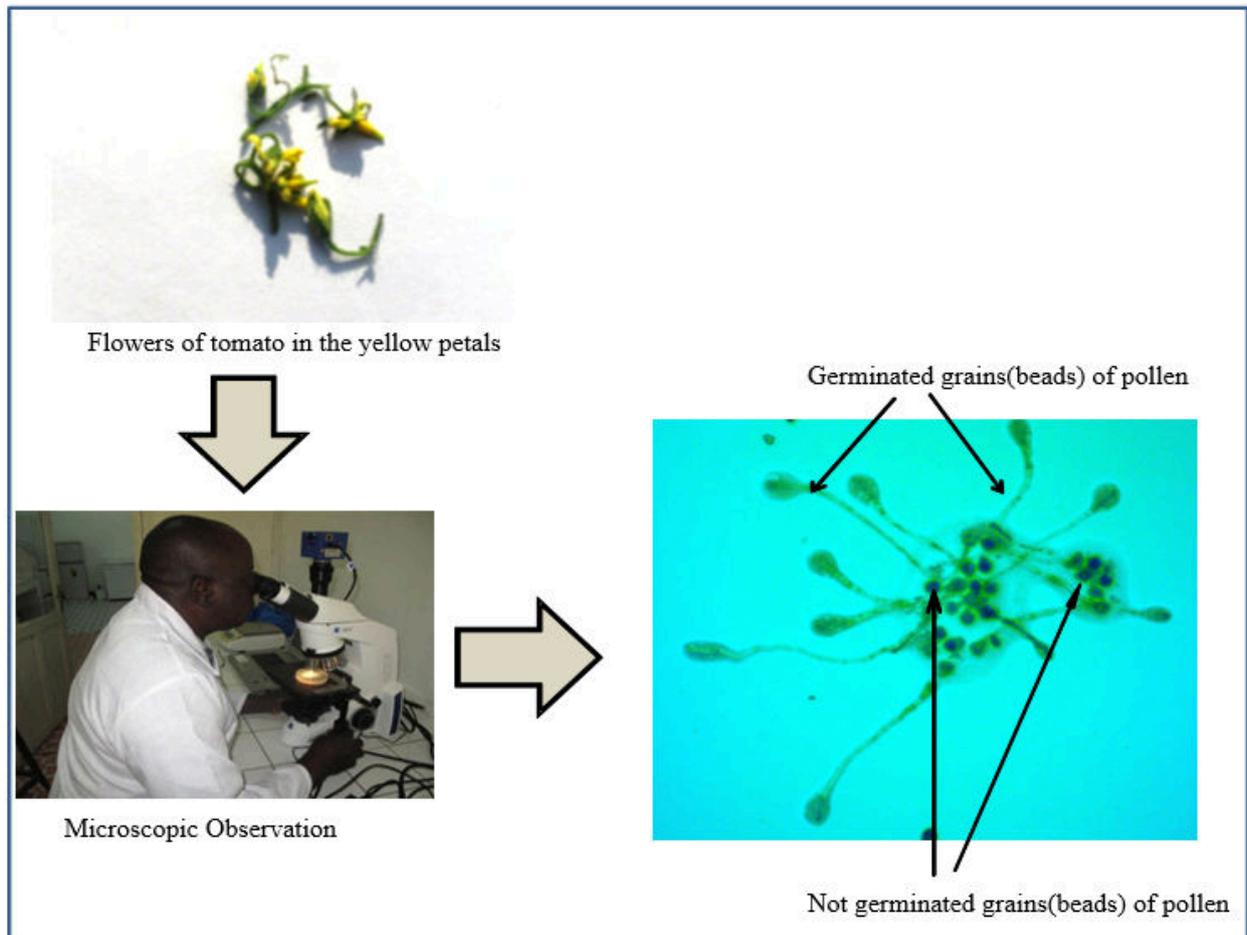


Fig. 1. Culture of pollen grains and its germination *in vitro*. Contrary to the not germinated pollen grains the pollen grains considered as germinated present a pollen tube of which the length is higher than the diameter of the grain.

4 DATA ANALYSIS FOR THE POLLINIC FERTILITY

To determine the indicator of viability in order to compare the various varieties of studied tomatoes we resorted to the formula of Dhed'a and Mackiewicz [13]:

$$IV = \frac{A \times B}{1000}$$

Where indicating IV= of viability

A= averages of the sprouted grains

B= averages pollen lengths of tubes in μm .

5 RESULTS AND DISCUSSIONS

Table 2 gives the results of *in vitro* germination of pollen obtained for various mediums of orientation tests tested on the local form Purple-round.

Table 2: Germination of pollen on the difference studied mediums

Mediums	Germination of pollen
M1	-
M2	+
M3	-
M4	-

Legend:

- : no germination

+: germination

M1 = Medium agar saccharose (2%) enriched in Macroelements;

M2 = Medium agar saccharose (2%) enriched in Microéléments (boric Acid 0,062%);

M3 = Medium agar saccharose (2%) enriched in Vitamin;

M4 = Medium agar saccharose (2%) enriched out of Growth regulators (1µM AIA + 1µM BAP).

It arises from the observation of this table 45 that the medium containing the microéléments of Murashige and Skoog [12] allow the germination of the pollen grains.

The medium M2 shows that, only boric acid with 0,062%, and the saccharose with 2% allowed *in vitro* germination of the pollen grains resulting in the emission of the pollen tube.

Indeed, Boron in the form of the boric "Acid" compound is undoubtedly the factor chimiotropic responsible for the emission of the pollen tube at *Solanum lycopersicum* . From this point of view, our results corroborate those of the other authors who announce that calcium and Boron would play a beneficial part for the germination of pollen [14, 15].

5.1 RESEARCH OF THE OPTIMAL SACCHAROSE CONCENTRATION FOR THE GERMINATION OF POLLEN

The percentages of germination, length of the pollen tubes and the indicators of viability to the various saccharose concentrations are consigned in figures 2, 3 and 4 at the local form purple round. The median values of the indicators of viability of the foreign and local varieties of tomato are presented in table 3.

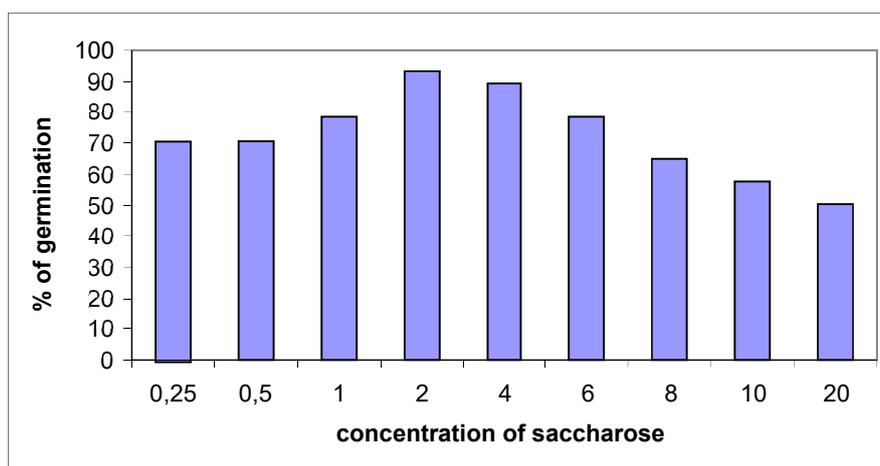


Fig. 2. Variation of the percentage of germination of pollen to the various Saccharose concentrations (%).

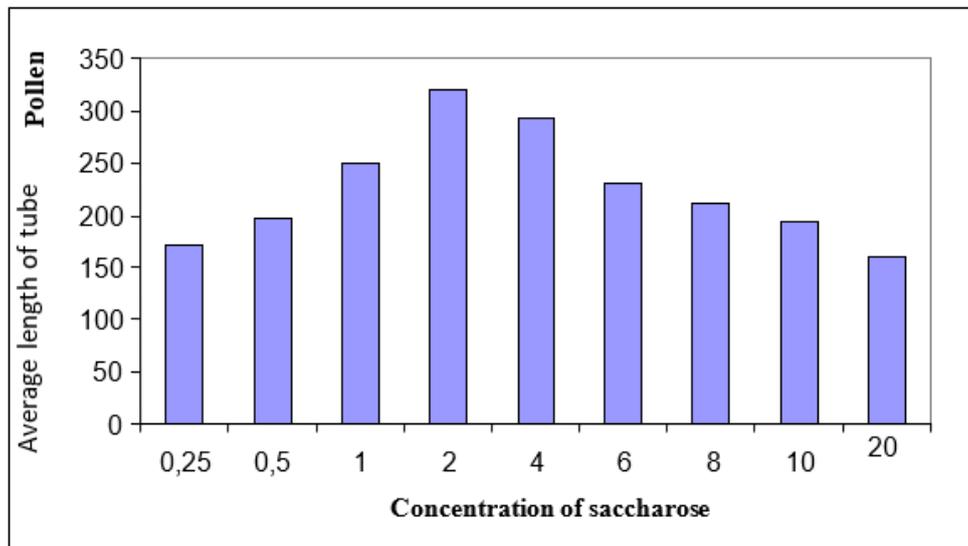


Fig. 3. Variation in the length of pollen tubes (in μm) to the various saccharose concentrations

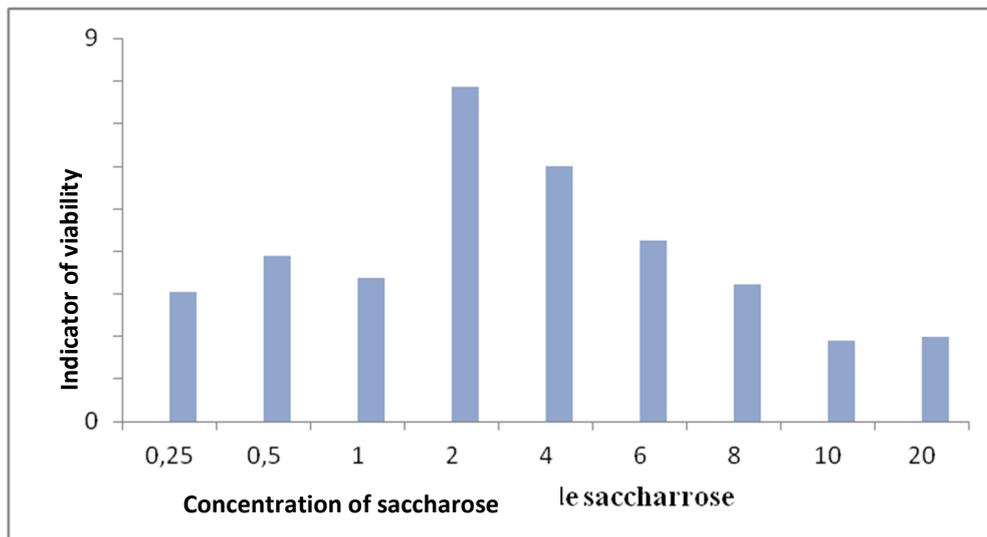


Fig. 4. Variation of indicator of viability to the various saccharose concentrations

By looking at the data of figures 2, 3 and 4 we note that % of germination, the length of pollen tubes and the indicator of viability are higher with the saccharose 2% concentration. With this concentration, this average remains always high as well for the number of the pollen grains germinated as for the length of the pollen tubes. These results approach those obtained by other researchers for various crop plants. Scarlat [16] obtained a better germination of the pollen grains of *Solanum lycopersicum*, *Soybean hispida* and *Allium cepa* respectively to 0, 5%, 2% and 10% of glucose. Maisonneuve and Nijs [17] studied the germination of pollen grains of some tomato lines according to the growth of the plant at various temperatures and they obtained a good growth of pollen grains after six hours of incubation to 22°C. Dhed'a and Mackiewicz [13] showed that the optimal conditions for *in vitro* germination of the pollen grains are carried out to saccharose 6% for *Psophocarpus tetragonalobus* (L) cd. and at 20% for *Psophocarpus scandens* (Endl) Verde.

Prat [18] also observed the germination of pollen grains of lily (*Lilium longiflorum*) after 1 to 4 hours of incubation, in a medium made up of 10mg of boric acid, 30 mg calcium nitrate, saccharose 10g and 0,5% of agar. V iron [19] for its part obtained the germination of tomato pollen grains after 4 hours of incubation, at the temperature of the greenhouse to the darkness, by using a plug of germination containing 3.5 ppm from boric acid and saccharose 5%. It obtained 10% of germination at the mutants KB and WT of the variety Micro-Tom (*Solanum lycopersicum esculentum*) and more than de 42% of germination at the wild plants of the same variety. Compared to these results, all our varieties showed rate of higher germination.

5.2 VIABILITY OF POLLEN OF VARIOUS VARIETIES

Figures 5 and 6 show the indicators of viabilities of the various varieties studied during the first and of the second culture.

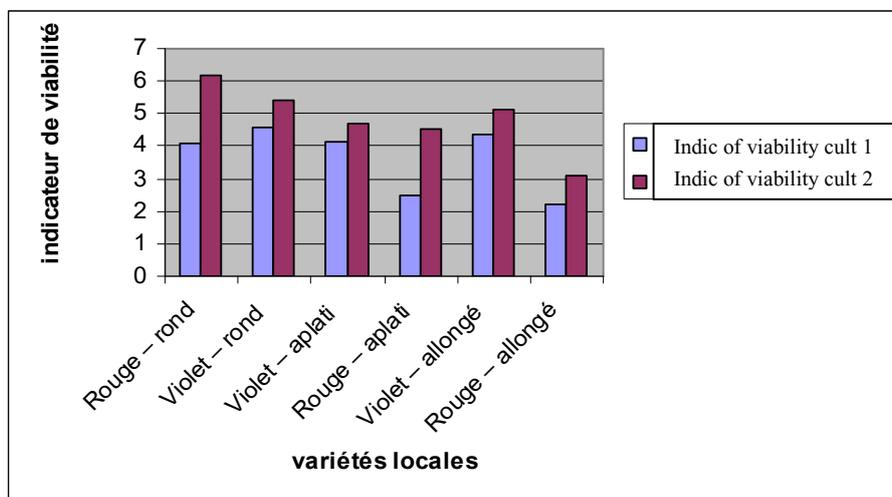


Fig. 5. Indicator of viability of local varieties

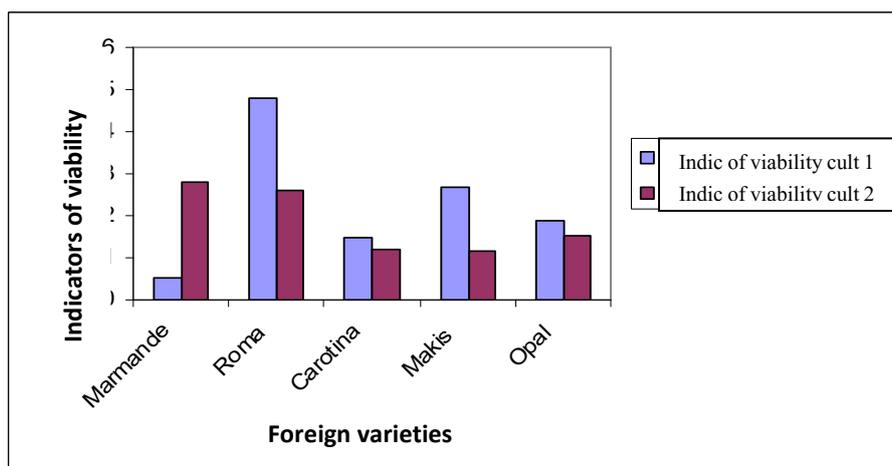


Fig. 6. Indicator of viability of the foreign varieties

Figures 5 and 6 show that the local varieties (Red round, Purple round, Purple lengthened) and the foreign variety Roma have an indicator of viability being around 4 higher than the other forms at the first generation. The phenotypes (flattened Red and lengthened Red) of the variety local as well as the foreign varieties (Makis and Opal) have an indicator of viability around 2. Generally the behaviors of the varieties remained the same ones during two cultures. Indeed, the local varieties have compared an indicator of viability higher to the foreign varieties.

The analysis of the second culture shows that the value of indicator of viability for all the local forms (3.1 to 6.2) and for all the foreign varieties remained the same one. This light increase in the value of indicator of viability for the local phenotypes would be due to a better choice of the lines to the first culture and the climatic variations. Whereas the variation at the foreign varieties could be caused by a reduction in the genetic potentialities and in the conditions of the medium [20]. The improvement of this value of indicator of viability at Marmande can be explained by the climatic variations. These results also state that the local forms (Red round, Purple round, Purple flattened, Red flattened, Purple lengthened and Red lengthened) have an indicator of viability higher than the foreign varieties (Marmande, Roma, Carotina, Makis and Opal) with the second

culture. However, the trend remained the same one for each variety during 2 cultures. The average indicators of viability of the local and foreign varieties during two cultures are included in table 3.

Table 3: Average indicator of viability of the local varieties and foreign

Varietes	IV
Local	
Round red	3,028
purple Round	2,787
Purple flattened	2,274
flattened Red	2,044
Purple lengthened	3,354
Red lengthened	2,675
Etrangères	
Marmande	1,763
Roma	1,65
Carotina	1,768
Makis	1,216
Opal	1,176

The reading of table 46 makes it possible to note that the average indicator of viability differs in general from one variety to another as well for the local varieties as foreign. For the local varieties of tomatoes, it varies from 2.044 to 3.354. On the other hand for the foreign varieties the indicator of viability is registered in the fork going from 1.176 to 1.763. For the local varieties of tomatoes, the Purple one lengthened and Red round are characterized by the highest indicators. In addition for the foreign forms, Marmande, Makis and Roma have the highest indicators of viabilities. These differences are ascribable to the genetic characteristics of the varieties.

Table 4 summarizes the median values of the indicators of viability of the local and foreign varieties.

Table 4: Median values of the indicators of viability of the local forms and foreign

Varieties	Average of IV
Local	2,69
Foreign	1,41

It rises of this table 4 that the local varieties presented the highest indicator of viability compared to the foreign cultivars. These different performances are due to the fact that the local forms are in their ecological medium.

5.3 FERTILITY OF THE FLOWERS ACCORDING TO THEIR POSITION ON THE PLANT

The average results of the indicators of viability of the various flowers produced according to their position on the plants of the various varieties during two cultures are illustrated on figures 7 and 8.

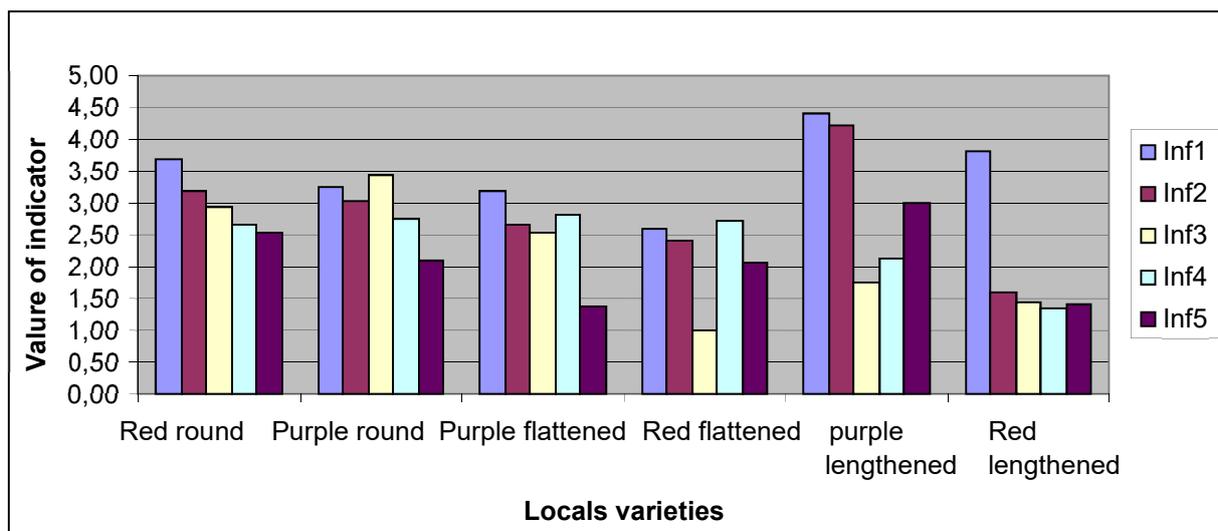


Fig. 7. Average of the indicators of viability of the various flowers produced according to their position on the plant for the locals varieties during two cultures

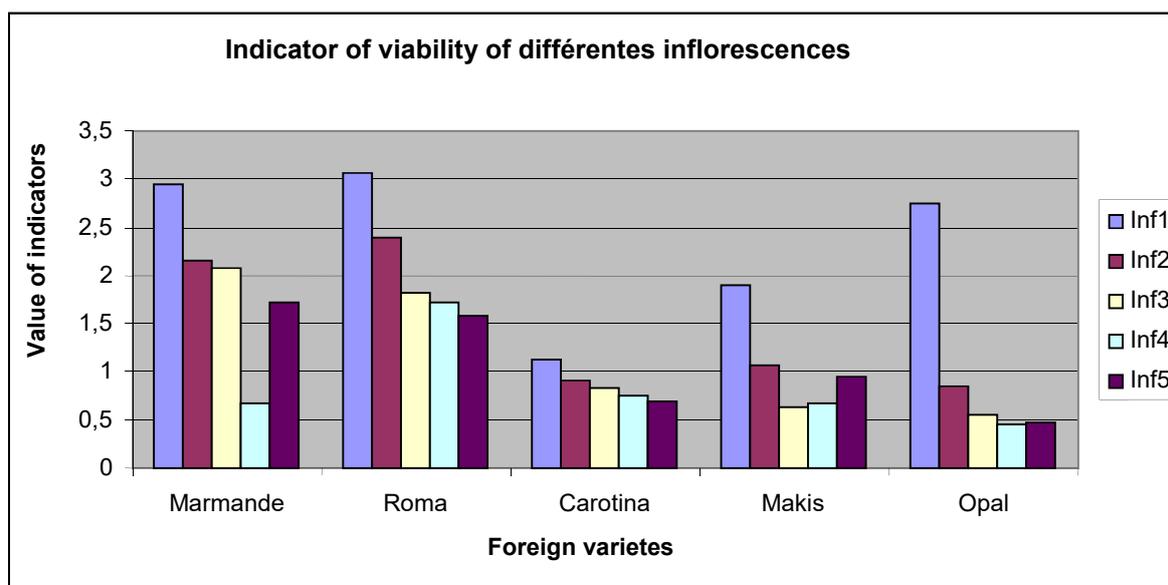


Fig. 8. Average of the indicators of viability of the various flowers produced according to their position on the plant for the foreign varieties during two cultures

It arises from the observation of figures 7 and 8 that the fertility upwards decrease of bottom for the local forms round Red and lengthened Red and at almost all the foreign varieties. The local varieties Purple round, purple flattened, red flattened and Purple lengthened do not present any trend. These results show that it would be more interesting to carry out the crossing with the first formed flowers.

This observation would explain certain failures of crossing observed by using the plants of different ages in particular between an early variety (local varieties) and a late variety (foreign varieties). The differences in results noted by the study of fertility of the flowers at the same plant confirms on the whole the assumption of Rabiet [21] which stresses that the production of pollen by flower at the same plant is variable and that several factors intervene to mark this difference (age of the plant, strength, illumination).

The comparison of the local and foreign varieties on the basis of indicator of viability during two cultures gave the results of the analysis of the variance consigned in tables 5 and 6.

Table 5: Summary of the ANOVA for the first culture

Source of Variation	SCE	Ddl	CM	FC	Ft (5%)	Decision
Total	55,14	54	-	-	-	-
Repetition	12,5	4	3,12	12,89	2,61	S
Treatment (varietes)	32,94	10	3,29	13,59	2,08	S
Résidual	9,69	40	0,24			

From the analysis of table 5 relating to the analysis of the variance, it arises that the difference is statistically significant between the repetitions and the varieties of tomato with regard to their indicator of viability. The comparison of the averages on the basis of test of the smallest significant difference with data results condensed in table 6.

Table 6: Results of the test of ppds for the first culture

	V-AL 3,1	R-R 3,0	V-R 2,91	V-AP 2,51	R-AP 2,16	RO 2,11	MM 1,91	R-AL 1,72	MA 1,04	OPA 1,01	CARO 0,86
V-AL 3,1	-	0,1	0,19	0,59*	0,94**	0,99**	1,19***	1,38***	2,06***	2,09***	2,24***
R-R 3,0		-	0,09	0,49	0,84**	0,89**	1,09***	1,28***	1,96***	1,99***	2,14***
V-R 2,91			-	0,4	0,75*	0,80**	1,00**	1,19***	1,87***	1,90***	2,05***
V-AP 2,51				-	0,35	0,4	0,60*	0,79***	1,47***	1,50***	1,65***
R-AP 2,16					-	0,05	0,25	0,32	1,12***	1,15***	1,30***
RO 2,11						-	0,2	0,39	1,07***	1,10***	1,25***
MM 1,91							-	0,19	0,87**	0,91**	1,05***
R-AL 1,71								-	0,68*	0,71*	0,86**
MA 1,04									-	0,03	0,18
OPA 1,01										-	0,15
CARO 0,86											-

The results of this table 6 show that the Purple indicator of viability of the variety lengthened is similar to the indicators of Red round round and Purple viability of the varieties but differs significantly from the variety Purple flattened, highly of flattened the Red varieties and Roma and very highly of Marmande, lengthened Red, Makis, Opal and Carotina.

Same manner, the indicator of viability of the Red variety round is similar to those of varieties Purple flattened round and Purple, but differs highly from flattened Red and Roma, very highly of Marmande, lengthened Red, Lemurs, Opal and Carotina. The Purple indicator of viability of the variety round is similar to that of Purple flattened, but differs significantly from the Red variety flattened, highly from Roma and Marmande, and very highly of lengthened Red, of Makis, Opal and Carotina.

However the indicator of viability of Purple flattened is similar to those of flattened Red and Roma but differs significantly from Marmande, highly from Red lengthened and very highly of Makis, Opal and Carotina. Ultimately, the remains of the indicators follow the diagram hereafter: the indicator of viability of flattened Red is similar to those of Roma, Marmande and lengthened Rouge but differs very highly from those of Makis, Opal and Carotina. Now, the indicator of viability of the Roma variety resembles those of Marmande and flattened Rouge but differs very highly from those of Makis, Opal and Carotina. On this optics it takes shape at the horizon which the indicator of Marmande covers a character of similarity to that of lengthened Red but differs highly from Makis and Opal and very highly from Carotina. Lastly, the indicator of viability of the Red variety lengthened differs significantly from Makis and Opal and highly from the Carotina variety.

Table 7: Summary of the ANOVA for the second culture

Source of Variation	SCE	ddl	CM	FC	Ft (5%)	Décision
Total	78,174	54	-	-	-	-
Repetition	23,567	4	5,891	13,242	2,61	S
Treatment (varietes)	36,81	10	3,681	8,273	2,08	S
Residual	17,797	40	0,444			

It arises from table 7 relating to the analysis of the variance which the difference is statistically significant between the repetitions and the varieties of tomato with regard to their indicator of viability. The comparison of the averages on the basis of test of the smallest significant difference to give the results summarized in table 8.

Table 8: Results of the test of ppds for the second culture

	V-AL 3,60	R-AL 3,32	R-R 3,05	V-R 2,66	V-AP 2,03	R-AP 1,92	CARO 1,67	MM 1,6	MA 1,38	OPA 1,33	RO 1,18
V-AL 3,60	-	0,28	0,55	0,94*	1,57***	1,68***	1,93***	2***	2,22***	2,27***	2,47***
R-AL 3,32		-	0,27	0,66	1,29**	1,64***	1,65***	1,72***	1,94***	1,99***	2,14***
R-R 3,05			-	0,39	1,02*	1,13**	1,38**	1,45***	1,67***	1,72***	1,87***
V-R 2,66				-	0,63	0,74*	0,99*	1,06**	1,28**	1,33**	1,48***
V-AP 2,03					-	0,11	0,36	0,45	0,65	0,7	0,85*
R-AP 1,92						-	0,25	0,32	0,54	0,59	0,74*
CARO 1,67							-	0,07	0,29	0,34	0,49
MM 1,60								-	0,22	0,27	0,42
MA 1,38									-	0,05	0,2
OPA 1,33										-	0,15
RO 1,18											-

The analysis of table 8 gives off with sufficiency a series of related indicators of which here content: the first articulation is focused on the Purple indicator of viability of the variety lengthened which approaches appreciably those of the varieties Red lengthened and Red round, but differs significantly from Purple round and very highly from Purple flattened, Red flattened, Carotina, Marmande, Makis, Opal and of Roma.

In this order of idea the indicator of viability of lengthened Red is added which is strongly similar to those of round and Purple Red round, but differs highly from that of Purple flattened and very highly of flattened Red, Carotina, Marmande, Makis, Opal and in this context, it arises that the indicator of viability of round Red is identical to that of Purple round, but differs significantly from that of Purple-flattened, highly of those of flattened Red and Carotina, and very highly of Marmande, Makis, Opal, and Roma.

It is followed moreover that the indicator of viability of Purple round is similar to that of Purple flattened, but differs from there significantly from those of flattened Red, Carotina, and highly of Marmande, Makis, Opal and very highly of Roma. On this basic feature, the indicator of viability of Purple flattened is similar to those of flattened Red, Carotina, Marmande, Makis, Opal, but differs significantly from that of Roma.

In addition, the indicator of viability of flattened Red resembles those of Carotina, Marmande, Makis, and Opal but differs significantly from Roma. The indicator of viability of the Carotina variety is similar to those of Marmande, Makis, Opal, and Roma.

Therefore, the indicator of viability of the Opal variety is similar to that of Roma. These differences would be ascribable with the genetic constitution suitable for each variety. All the local varieties have an almost similar indicator of viability, excluded the forms flattened Red and lengthened Red which have indicators of viability lower than the others. The results got on the viability of the male gametes combined with those obtained on the agronomic characterization of the varieties of tomato during two cultures made it possible to select the foreign varieties Roma and Makis on the one hand and the six local varieties on the other hand in order to carry out hybridizations. In each variety, the plants heads of the lines were retained to carry out hybridization.

6 CONCLUSION

The study of the pollinic fertility in vitro of some varieties of tomatos indicated that the best conditions for the germination of pollen grains are carried out with 20 grams per liter of saccharose, 0.62 gr/l from boric acid and 10 gr/l of agar. All the studied varieties have an indicator of viability interesting except for the foreign varieties (Carotina, Marmande, Opal) and the forms flattened Red and Red lengthened for the local varieties.

The results in keeping with the fertility of various flowers analyzed according to their position on the plant, reveals that it is generally decreasing of bottom upwards. The first produced flowers show a percentage of fertility larger than those which follow the first flowerings.

These results would explain certain failures of fertilization at the time of crossing between the varieties of tomatoes and watch the importance of choice of the flower being useful for the taking away of the pollen grains. It can make it possible to draw up diagrams of the crossings with a greater chance of success, for a selection of the varieties of tomatoes adapted to the ecological conditions of Kisangani and its surroundings.

REFERENCES

- [1] J. Jahier, A.M. Chevre, F.Eber, R. Delourme, A.M.Tanguy. Techniques of vegetable cytogenetics INRA, Paris. pp 66-72 1992.
- [2] N. Tarchoun, H. Chalbi, , H.Harzallah. Study of the viability of the gametes and selection of lines for the nouaison at high temperature at tomato of season (*Lycopersicon esculentum*) en tunisie Ed. AUPELF-UREF. John Libbey Eurotext, paris, pp 271- 281. , 1993.
- [3] A.Dafni and D.Firmage. Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Systematics and Evolution, 222 (1-4), pp 113-132. , 2000.
- [4] E.Mayer and G. Gottsberger. Pollen viability in the genus *Silene* (Caryophyllaceae) and its evaluation by means of different test procedures. Flora, 195(4), pp 349-353. , 2000.
- [5] J.Lejoly. Valorization and conservation of the vegetable biodiversity. Notes with the use of the students of the diploma of thorough studies (D.E.A). Biology-agronomy.UNIKIS. , 2007.
- [6] J. Heslop-Harrison, Y. Heslop-Harrison, K.R. Shivanna. The evaluation of pollen quality, and a further appraisal of fluorochromatic (FCR) test procedure. Theoretical and Applied Genetics 67, pp 367-375., 1984.
- [7] C.A. Kearns and D.W. Inouye. Techniques for pollination biologists University of Colorado, Niwot, pp 13. , 1993.
- [8] O. Lokonga. Characterization of genetic diversity and pollinic fertility in vitro of the tomatoes (*Lycopersicon esculentum* Millet) of the area of Kisangani (DR CONGO), DEA, Ined. Unikis 63 P., 2008
- [9] O. Lokonga. Test of hybridization between the local forms and varieties introduced for obtaining new tomato genotypes (*solanum lycopersicum* L.) adapt to the ecological conditions of the area of Kisangani (DR CONGO), Thesis, Ined. Unikis 343P. , 2015
- [10] M. Nepi and G.G. Franchi. Cytochemistry of mature angiosperm pollen. Plant Physiology And Plant Molecular Biology 48, pp 461-491. , 2000
- [11] JL. Brewbaker and B.H Kwack. The essential role of calcium ion in pollen Cell Physiol 41, pp 1272-1278. , 1963.
- [12] L.P. Taylor and P.K. Hepler. Pollen germination and tube growth. Annual Review Of thaliana male-sterile mutants. Sexual Plant Reproduction 11(6), pp 297-322. , 1997.
- [13] T. Murashige and Skoog. A revised medium for rapid growth and bio-assays with tobacco callus tissue cultures, physiol. Plant 15, pp 473-497. , 1962.
- [14] D. Dhed'a and H. Mackiewicz. In vitro germination of the pollen grains of *Psophocarpus tatragnolobus* (L) DC *Psophocarpus scandens* (Endl) Verde, Ann. Fac. Sc. UNIKIS, N°3, pp 9-14. , 1985.
- [15] J. Louveaux and P. Pesson Pollination and vegetable Productivity, INRA, Paris, France. pp 440-480. , 1984.
- [16] C. Dibos. Interactions plants – pollinating characterization of the quality of the pollen of two cucurbitaceous during its ontogenesis, its presentation and its transport on the body of the domestic bee,thesis of doctorate.University Of Avignon and the countries of Vaucluse, 191P. , 2010.
- [17] A. Scarlat Temperature depend pollen and polen tube elongation in some culture plants. Ann. Univ. Bucaresti. S. Biologie, Annul. XXVII, pp 83-88. , 1978.
- [18] Maisonneuve et Den Nijs *In vitro* pollen germination and tube growth of tomato (*Lycopersicon esculentum* Mill) and its relation with plant growth.Biomedical and life sciences. Euphytica, springer Netherlands, Vol33, pp 833-840. , 1984.
- [19] R. Prat. Experimentation in vegetable biology and physiology, ed. Quae, 296 p., 2007.
- [20] N. Viron. Identification and validation of new genes candidates implied in the regulation of the development of the tomato fruit, new thesis, university of Bordeaux 1, 124 p., 2010.
- [21] F. Lints .Genetics, ed. Technique and documentations, Paris, 580p. , 1987.
- [22] E. Rabiet .Bee and pollination. Association of the autoedities authors, France, 81 p., 1986.