PRESANTATION OF A FAST TECHNICS FOR THE DESCRIPTION OF THE CHROMOSOMIC CHART OF A VEGETABLE SPECIES

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Manuscript received: February 21, 2004; Reviewed: April 13, 2004; Accepted for publication: April 16, 2004

ABSTRACT

The caryologic technics aim to determine the level of polyploidy of a population or a species. The information brought for the knowledge of the chromosomal number of a species, is of various nature. Thus for several years the caryology has made it possible to better understand the taxonomy of a species, its tendencies evolutionary, its geographical distribution and the phenomena of its speciation [16]. Any time the chromosomal number, although being able to characterize a plant or a group of plants is not a character constant.It can vary inside a group, tribe, family or kind, and within same species[12,15,16]. The technics of identification of the most known chromosomes are: traditional technics based on the histological cut, the Technics of Feulgen which has like action to colour in purple red the DNA chromosomal [6,7,8] and technics of bands or mechanisms that it brings into play are related to the physicochemical properties of the chromatin and the condensed form of the chromosomes. To highlight the chromosomes of a species we observed somatic mitosis carried out bolsters of young roots. The principal stages of the technics has to follow are: the pretreatment, fixing, storage, the hydrolysis, colouring, the assembly and observation. All this work was completed on young roots of *Solanum sodomeum* L. or the number of the chromosome is still questioned.

KEYWORDS: caryology, species, chromosomes, chromatin, mitosis, somatic cell

DETAILED ABSTRACT

The caryologic technics aim to determine the level of polyploidy of a population or of a species. To highlight the chromosomes of a species, we observed somatic mitosis of the meristeme bolsters of young roots. The experiment at summer realized on young roots of *Solanum sodomeum* L, or numbers it chromosomes is still questioned. *Solanum sodomeum* L, fact part of the family of solanaceous, which is presented in form a very thorny shrub, her sheets and its stems are provided by spines which are right, long-lived and can measure up to 1 cm length [2,5,9]. Its fruits are used in traditional medicine as infusion for the sterility of the women and like abortive [1,4,5].

The plant contains alkaloids steroïdic and saponides [4,10,13]. Among alkaloids we found alkaloids steroïdic like the solanine.

Our study consists in following the process of mitosis which proceeds in the cells of the root's meristeme of *Solanum sodomeum* L, the objective is to specify the optimal conditions allowing the appearance of a better metaphase because this stage facilitates the chromosomal enumeration. The methods used for our work are: the setting in germination of seeds; cuts built on roots out of transverse section; fixing is carried out after the pre treatment with of Carnoy I and Carnoy II during 12h with 63h. Pretreatments to colchicine were carried out with various proportionings for obtention of a metaphasic plate. With end to obtain a good colouring of the cores we heated the root cuts posed between blade and plate until the evaporation of acetic Carmine.

The microscopic examination (G X 40) of the root points after crushing between blade and plate of *Solanum sodomeum* L highlighted morphological only one standard of cells which are small, of oval form has cores well colour and more or less bulky. The great majority present a core in Interphase or Prophase, some cells only in Telophase or Anaphase, of this fact it is necessary to use the colchicine has various concentrations and lasts various time of pretreatments

Analysis of our results shows that the weak concentrations of the colchicine (0,25% and 0,5%) do not allow to examine the chromosomes but to concentration of (0,75%) with a pretreatment from 4 to 5 hours give better results but with a concentration of (1,25%) with a pretreatment from 4 to 5 hours the chromosomes are well individualized with the enlargement (G X 100).

The hydrolisis with HCI made it possible to soften the fabrics which become easier spread out and support a good colouring with ascetic Carmine and hot A allowed a good colouring and a good enumeration of the chromosomes.

INTRODUCTION

The solanaceous form ones of families of most important at Angiospermes. They contain approximately 90 kinds; including 3000 species of which approximately thousand belong to the kind Solanum [7,8,10]. This one contains some wild species, such as *Solanum sodomeum* L commonly known under the name of " morelle of Sodome ". In the world it is also registered under the name of *Solanum linnaenum* Hepper and Gager . In spite of its spontaneousness this plant is of important economic interest, seen its medicinal values and its resistance to the diseases with which the other solanaceous ones are sensitive such as the verticillose [15,16].

The plant is presented in the form shrub which according to the bibliography [4,13,16], in Algeria is in the areas of Tell (Algiers, Bone, the calle, Constantine) where it pushes on the edges of the ways, the falls, the sand beaches and the fallow [3,4,13]. The species *Solanum sodomeum* L is originating in south of America and of tropical and one finds it sub spontaneous in Morocco [1,5,6,8] and we see disappearances in Algeria where the grounds on which it pushed are recovered little by little for constructions. The plant is presented in form of a very thorny shrub, its sheets and its stems are provided with thorns which are right long-lived and can measure until a1 cm length [2,5,9].

Very little studied, the plant presents a very major root system, its length can easily exceed 2m, the flowers either solitary, or are grouped by two according to the bibliography [3,13,16], grouped in cyme composed of 12 flowers, with one to two flower hermaphrodites. The flower is a pedoncule with a corolla purplished out of star of 2,5 with 3cm of diameter, hermaphrodite and of pentamerous type (5P + 5S +5E + 2C). The flowers persist on the plant during all the year, but more abundant in winter and in spring in the Mediterranean zone. The fruit is a bay of round form of marbled green color of young white with plight and yellow color bursting like a lemon with maturity. It has a sweet savour at the beginning which becomes thereafter acid and bitter [1,4,5,9,10]. Its fruits are used in traditional medicine as infusion for the sterility of the women in Zaire and like abortive [1,4,5]. According of the other people it is toxic and contains gluco-alkaloids and saponides [4,10,13]. Among alkaloids one found alkaloids steroïdic as the solanine which is abundant in all fabrics of various species of *Solanum* [3, 5, 6].

It was announced that work on this plant is very rare and incomplete. With end to supplement our knowledge on this shrub we tried to undertake a caryologic study to allow us to understand the phytogenetic relations and the process of speciation. Our experiment consists in following the process of mitosis which proceeds in the cells of the root meristem after germination of seeds. The objectif is to specify the optimal conditions allowing the emergence of a better metaphasic phase. This stage facilitates the enumeration and consequently the determination of the chromosomic chart to us.

MATERIAL AND METHODS

The products used are:

• The fixers used are those proposed by Carnoy (1986) called Carnoy I (30ml ethanol + 10ml acetic acid) and Carnoy II (60ml ethanol + chloroform 30ml + 10ml acetic acid). Account is held owing to the fact that the fixers block any evolution of cellular division making it possible to preserve structural integrity of the chromosomes [14,15].

• The dye used is acetic carmine (1mg of acetic carmine + 100ml distilled water). Colouring recommended is that with Feulgen reagent described for the first time in 1926 but other dyes is also employed to give a red colouring to the chromosomes, ex: acetic carmine, acetic orceine, the nigrazine etc.[8,14].

• The hydrolize is made by hydrochloric acid 1N and 5N. It is recommended to obtain a good spreading out of the cells and chromosomes between blade and plate following a good hydrochloric softening of fabrics in the presence of the acid followed whith explanation of the cytoplasm.

• The pretreatment is made by soak fabrics in division in an antimitotic agent which causes to block mitotic divisions at the metaphase stage and to contract the chromosomes. The agents used are the powder colchicine of several concentrations:

0,25 Mg in 100ml distilled water = 0,25%

0,50 Mg in 100ml distilled water = 0,50%

0,75 Mg in 100ml distilled water = 0,75%

1 Mg in 100ml distilled water = 1%

1,25 Mg in 100ml distilled water = 1,25%

• For the vegetable material we use: seeds of *Solanum sodomeum* L, coming from bays collected in the month of august on a shrub which pushed on the beach of Zemouri El Bahri (County of Boumerdes) of the Mediterranean.

The methods used for our work are:

The setting in germination of seeds was carried out in boxes of Petri (15 grain/boxe) papered filter paper and cotton soaked in water with tap. They are then covered and placed at an ambient temperature (25°C approximately).

The cuts are built on long roots of 0,5 with 1cm, one practises transverse sections with a very thin blade, then they are rinsed with water tap.

Pretreatments are practised on the cuts. We introduce the cuts into the colchicine with various concentrations: 0,25%, 0,5%, 0,75%, 1% and 1,25% during one hour

• Fixing are carried out after the pretreatments, when the cuts are firstly rinsed in water then placed in the fixers Carnoy I and Carnoy II then placed at the refrigerator. The duration of fixing is different: 12h, 18h, 24h, 63h.

• For the hydrolisis two methods were used:

Hydrolize with Hydrochloric acide 1N during 10 minutes at a temperature of 60°C and hydrolize to HCl (5N) during 10 minutes then transfered in HCl (1N) during 5 minutes to ambient temperature

• So as to obtain a good colouring of the cores we heated the root cuts posed between blade and plate. We carried out a microscopic assembly thus where each root cut taken is fixed and crushed between blade and plate involved in acetic drop of Carmine. Microscopic examination (G X 10) enables us to detect the cells in division quickly. The observation of the chromosomes is carried out with the enlargement (G X 40).

RESULTS AND DISCUSSION

Microscopic examination (G X 40) of the root points after crushing between blade and plate of *Solanum sodomeum* L highlighted morphological only one standard of cells which are small, of oval form at well coloured and more or less bulky cores. The great majority of the cells present a core in Interphase or in Prophase, some cells are only in Telophase or Anaphase. The results obtained according to the various pretreatments carried out are gathered in Table 1 The results obtained in Table 1 indicate to us that for the pretreatments carried out with the colchicine with 0,25%, obtention of metaphasic plate is very rare and this only after the pretreatment lasted 5 hours. It results from it that the colchicine with a concentration from only 0,25% does not present any effect at a weak duration of pretreatment. It takes a duration of minimum 5 hours to be able to detect the metaphasic plates.

For the pretreatments with the colchicine 0,5% the mitosis are done rare for the treatments of short duration (1 hour and 2 hours) while those of long duration (4 hours and 5 hours) are most effective since the number of metaphasic plates observed is larger. Thus using of the colchicine with 0,5% and after 4 hours duration of pretreatment gives very good results.

For the pretreatments with the colchicine of 0,75%, the metaphasic plates are very rare and are not visible that afterwards 5 hours of pretreatment. Durations lower than 5 hours seem to be insufficient for apparition of these plates. For the pretreatments with the colchicine with 1% the metaphasic plates are not visible afterwards 3 to 4 hours of treatment. Thus it's deduced that the colchicine with 1% doesn't have satisfactory effect way afterwards 4 hours of pretreatment.

The pretreatments with the colchicine with 1,25%, the metaphasic plates after 1 and 2 hours of pretreatment are rare but they appear clearer and more numerous after pretreatments of long duration (3h, 4h, 5h). What leads us to say that the colchicine to 1,25% watch its best effectiveness after a pretreatment exceeding 3 hours.

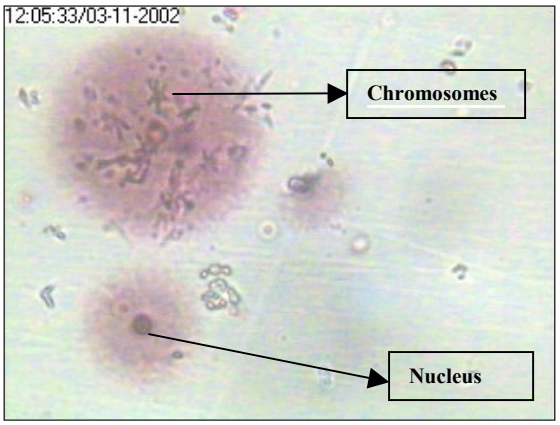

Figure 1: Chromosomes obtained at Colchicine 0,5% with pretreatment during 36 hours

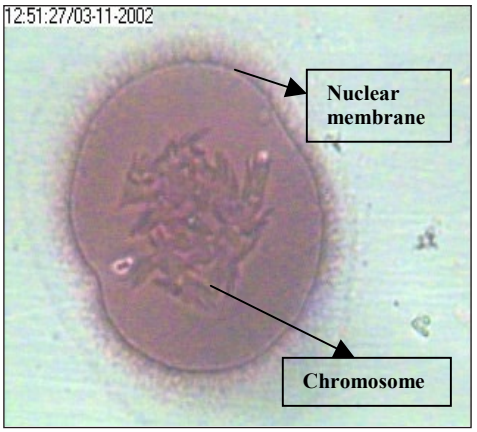

Figure 2: Chromosomes obtained at Colchicine 1% with pretreatment during 36 hours

CONCLUSION

The Analysis of our results shows that the weak concentrations of the colchicine used (0,25% and 0,5%) do not make it possible to examine the chromosomes and the metaphasic plates well. Indeed according to the bibliographical search [11,14,15], when the mitoclassic amounts of the agents are weak, the disjunction of the chromosomes is antiquated and two unequal cores are

Table 1: Pretreatment and obtaining the metaphasic plates at *Solanum sodomeum* L.

Pretreatment with colchicine (%)	Duration of Pretreatment (hours)	Duration of fixation (hours)	Hydrolize	Colouring	Observations
0,25	5	36	HCl (5N)+ HCl (1N) at Ambient. temp	Acetic Carmin	Good results after 5 hours
0,50	5 4	36 36	HCl(5)+ HCl(1N) at Ambient. temp	Acetic Carmin	The observations are clear
0,75	5 2	36 36	HCl(5N)+ HCl(1N) at Ambient. temp	Acetic Carmin	Better results after 36 hours
1	5 1 3	36 12 18	HCl(5N) + HCl(1N) at Ambient. temp	Acetic Carmin	Good observations -Chromosomes are opaques -Chromosomes are clear
1,25	5 1 2	36 12 36	HCl(5N) + HCl(1N) at Ambient. temp	Acetic Carmin	-Chromosomes visible but at 12 hours they are not clear

obtained. Sometimes under the influence of very weak concentrations of the colchicine, only the Prophase I is inhibited and one then obtains a stereoscopic cell and it's for that, that we find difficulties to count the chromosomes. With a concentration of 0,75% and some 4 hours and 5 hours pretreatments the best results give to determine the phases of the cellular divisions which lead to the enumeration of the chromosomes. The mitoclassic substances are of double interest, they make it possible to separate in experiments the various stages from the mitosis and analyse more precisely their unfolding and the enumeration of the chromosomes of each species. During our test the best results are obtained with the pretreatments with 1% and 1,25% but with the pretreatments of 1,25% and in long duration (4h, 5h) the chromosomes are well individualized and quite visible with the enlargement (G X 100). We notice also that a short duration of fixing (12h and 18h) seems to be insufficient. The best observations are obtained with pretreatments of long duration (24h and 36h).

REFERENCES

[1] BELLAKHDAR J.,1997 : La pharmacopée marocaine traditionnelle. Ed. Ibis, pres, 746P.

[2] BERKALOFF A., BOURGUET J., FAVARD P. et LACROIX J.C., 1987 : Biologie et physiologie cellulaire :chromosome, nucléole, enveloppe nucléaire. Ed. Hermonn,186P.

[3] BINET P. et BRUEL J.P.,1968 :Physiologie végétale – Tome III. Ed. Dain,1156P.

[4] BRUNETON J., 1997 : Pharmacognosie-phytochimie. Plantes médicinales. Ed. Lavoisier.

[5] BRUNETON J., 2001 : Plantes toxiques végétaux dangereuses pour l'homme et les animaux. 2ème Ed. médicales internationales. 564P.

[6]CRETEP.,1965 :Précisdebotanique :Systématique des angiospermes. Tome II. Ed. Masson,429P.

[7] DEYSSON G., 1975 : La cellule végétale. Structure et fonctionnement. Tome I 3ème Ed. Paris 298P.

[8] DURAND H et FAVARD L., 1974 : La cellule structure et anatomie moléculaire. Ed. Hermann. Paris, 383P.

[9] EMBERGER L., 1960 : Traité de botanique : Les végétaux vasculaires. Tome II. Fascicule II. Ed. Masson et Cie éditeurs 1539P.

[10] ESSAD, 1957 : La polyploïdie et ses aspects évolutifs en relation avec l'amélioration des plantes. Ann. Anuel. Plantes (2) PP 199-222.

[11] GORENFLOT R. et RAICU P.,1980 : Cytogénétique et évolution. Ed. Masson,181P.

[12] GRIFFITHS J.F., MILLER H., SUZUIT T.,

LEWONTIN C., et GELBART M.,1997 : Introduction à l'analyse génétique. Traduction française. Paris, 901P.

[13] INGRID J. et SCHOFELDER P., 1986 : Guide de la flore méditerranéenne. Ed. Hitier. 350P.

[14] JAHIER J. et CHEUREA. M., ERER F., DELOURMER., TANGUYA M., 1992 : Techniques de cytogénétique végétale. Ed. INRA. 184P.

[15] NOUGAREDE A.,1969 : Biologie végétale-Cytologie. Ed. Masson, 597P.

[16] PANFIL C., 1978 : Genetica, Editura Didacticâ si Pedagogicâ, Bucuresti, 365P.

[17] STANFIELD W.D.,1987 : Génétique, Courts et Problèmes. Ed. ISBN, 28P.