Creation and study of the Pinot noir variety lineage

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Summary: The objective of the study presented here is to obtain pure line genotypes able to transmit to cross progeny the most useful characters to wine grapes, particularly the berry colour of Pinot noir. From the very heterogenous Pinot noir variety we chose a clone INRA-Colmar as the initial parent of a generation series. In the first 3 generations of self pollination we selected only red berries genotypes. After the 4th generation we made 'pedigree' selections with seedling separation, retaining not only the red berries population but also some white ones. This series of generations shows only a weak 'inbreeding' effect. Numerous populations with strong vigour and high fertility were observed. During these crosses, types of bunches and berries have appeared which were very different in shape from the original parent. Types range from the round berries of the Pinot noir to elliptical, ovoid, troncovoid, cylindrical or other kinds of berries. Berry size is also highly variable. We obtained bunches from small to very big with variable compactness. We obtained homogenous populations for such characters as berry colour, sexual type, vigour and fertility. These characters can be considered as homozygous after the 6th generation. This study shows the necessity of making numerous generations of self pollination to obtain homozygous forms of useful characters, which are generally polygenic. These observations must be confirmed by test crosses and in the meantime the self pollination program will be continued to enable us to fix the unstable characters.

Key words: Pinot noir, variety of vine, crossing, self pollination, seedling, selection, pure line, flower, berry, bunch, growth.

Introduction

The research for 'parents' able to transmit to progeny some characters which allow the improvement of red wine grape varieties has been often studied by different authors with the help of complex selection strategies.

At the Laboratoire de Viticulture de la Station de Recherches Vigne et Vin de l'Institut National de Recherches Agronomiques de Colmar, we have obtained numerous progenies by simple or multiple crosses.

In the Pinot noir improvement process, we have found that many of the seedlings from crosses involving Pinot noir as parent have white or little coloured berries and the black berries progeny yield a low quality wine.

These reasons induced us to study the possibilities of obtaining one or several homozygous genotypes for some characters such as sexual type, fertility and black berry colour with a high pigment content, after successive self pollinated generations having as an initial parent a Pinot noir variety clone. Up to now we obtained abundant new plant material whose main characteristics we are going to explain.

Materials and methods

As the initial parent we chose the Pinot noir clone selected by INRA Colmar. It is the most cultivated clone because of its quality.

We realized 6 generations in a greenhouse, one every 2 years, one for seed germination and floral initiation and another year for self pollination, fructification and grape vintage.

In the first 3 self pollinated generations, we did not make a pedigree selection, to avoid too many families. The selection during these 3 generations eliminated all individuals with weak vigour, weak fertility (or sterility), and red, little coloured or white grapes so that only the black wine grape populations of the Pinot noir type were retained. All the female seedlings and some with pollination problems were also eliminated.

After the 4th generation, we made a pedigree selection retaining not only the red berries populations but also some white genotypes with interesting characteristics. The retained genotypes should also generate enough seedlings to permit us to study disjunction.

Table 1 shows our selection diagram and the number of genotypes obtained and selected per generation. Table 2 documents different observations and notations recorded during different generations. In Tables 3, 5 and 6 the notes describing the flower, berry and bunch characteristics are defined.

Table 1: Selection diagram

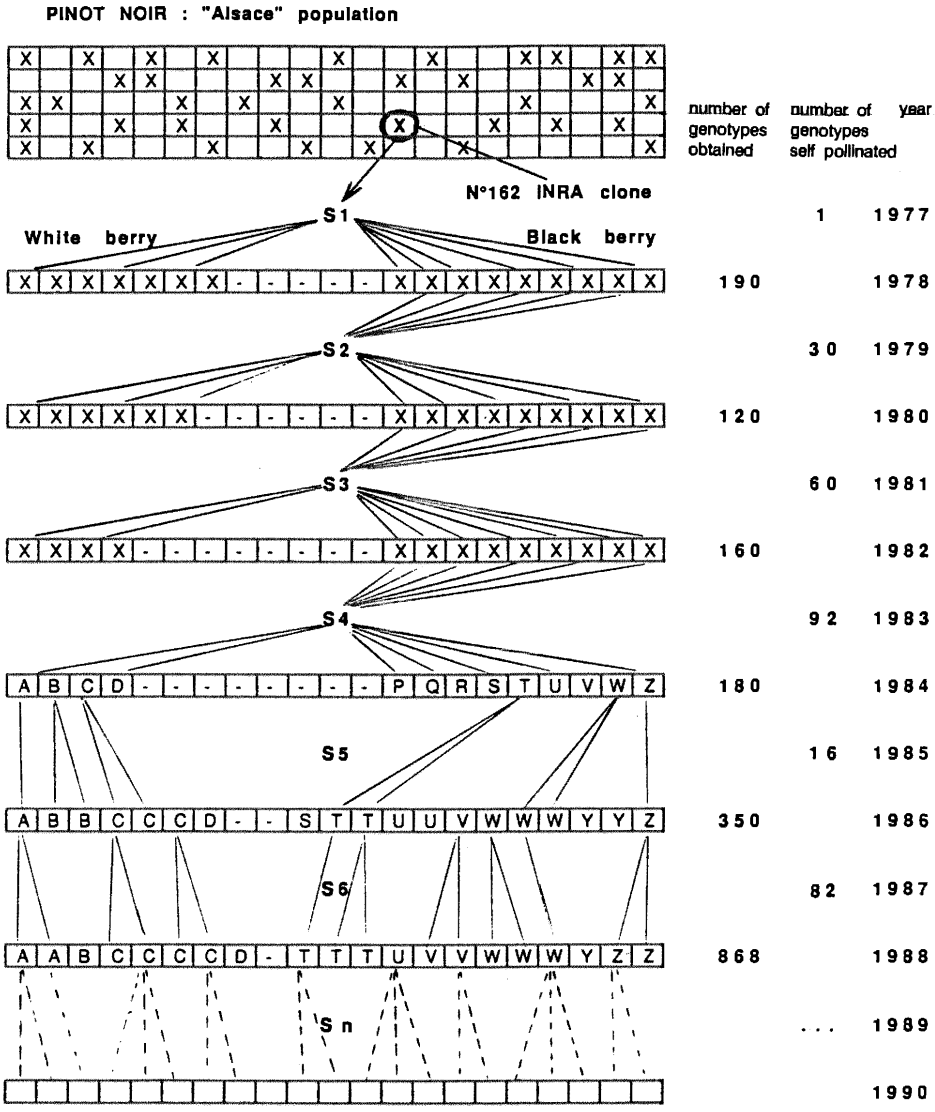

Table 2: Notes taken during the 4th-5th and 6th self pollinated generations

	1st year in greenhouse culture		
	Seedling vigor at the end of the 1st year		
	Base diameter		
	20° internode diameter after the first tendril		
	Length of 20 internodes		
Leaves colouring at the end of vegetation cycle : red colouring or not			
	2nd year in greenhouse culture		
Before	**flowering**		**Code O.I.V.**
	5 buds fertility, number of inflorescences per shoot		-
	Class of inflorescences importance		-
During	**flowering**		
	Early coulure		
	Sexual type		-
	Coulure		-
During	**veraison**		
BUNCH	Size		-
	Length		-
	Density		-
BERRY	Color	+ additional colors	-
	Size		-
	Shape	+ additional shapes	-
	Flavor		-
	Coulure and millerandage		
Ampelographic	**notes**		
	Leaf shapes		-
	Lobing degrees		
	Seedlings vigor by shoots development measures		-

Results

From 1st to 3rd self pollinated generation

During the first 3 self pollinated generations, we only retained the hermaphroditic progenies with black berries of the Pinot noir type.

4th generation (S4)

The 4th generation is composed of 92 genotypes.

Here we observed high vigour with low variation, expressed by length of shoots and by the diameter of the principal axis (Table 4). In the greenhouse, this vigour was the same as that we obtained from a simple cross with *Vitis vinifera* varieties.

Average fertility is 0.7 but with 9 sterile genotypes and 10 weakly fertile: 21 having a fertility level higher than 1.0, i. e. more than one inflorescence per shoot.

The sexual type, which is a very important criterion between generations, could be clearly determined for 49 genotypes: 11 are female and 38 hermaphroditic. The female types were eliminated.

Table 3: Notes code

FLOWER SEX physiological		BERRY COLOUR		BERRY SHAPE	
CODE OIV: 151		225		223	
1	male	1v	white-green	1	flat
3	hermaphrodite	+ 1j	white-yellow	2	slightly flat
5	female	2	rose	3	roundish
		3	red	4	short elliptic
		4	red-grey	5	ovate
		5	dark-red-violet	6	obtuse-ovate
		6	black	7	obovate acuminada
		+ 6a	blue-black	8a	cylindric
		+ 6b	dull-black	+ 8b	wide cylindric
		7	red-black	9	long elliptic
				10	arched
				+codes 11 to 31 after GALET P.	

BERRY LENGHT		BUNCH SIZE		BUNCH LENGTH	
221		202		203	
1	very short	1	very small	1	<= 10 cm
3	short	3	small	3	15 cm
5	medium	5	medium	5	20 cm
7	long	7	large	7	25 cm
9	very long	9	very large	9	30 cm

(+ = additional classes)

Table 4: Disjunctions in S4

Observations:	number of genotypes	average	minim.	maxim.	coeff. variation
Base diameter	92	6.9	4.8	9	17,80%
Diameter 20°eye	92	6.2	4.5	8.1	12,80%
Length 20 internodes	92	173.7	110.0	245.0	15,70%
Fertility	85	0.7	0	1.7	-

Notes by class		(class=effectif)						Pinot Noir = class:
Sex	49	1=38	3=11	5=0				3 heterozygous
Bunch size	50	1=18	3=26	5=6				1
Length	50	1=23	3=24	5=3				1
Density	40	3=11	5=12	7=17				3
Colour	49	1=9	3=10	6=30				6
Form	50	3=30	4=15	5=2	6=1	7=1	8b=1	3
Size	42	3=7	5=28	7=7				3
Coulure	49	0=44	5=5					0
	number of plants observed							

Table 5: O.I.V code no. 223

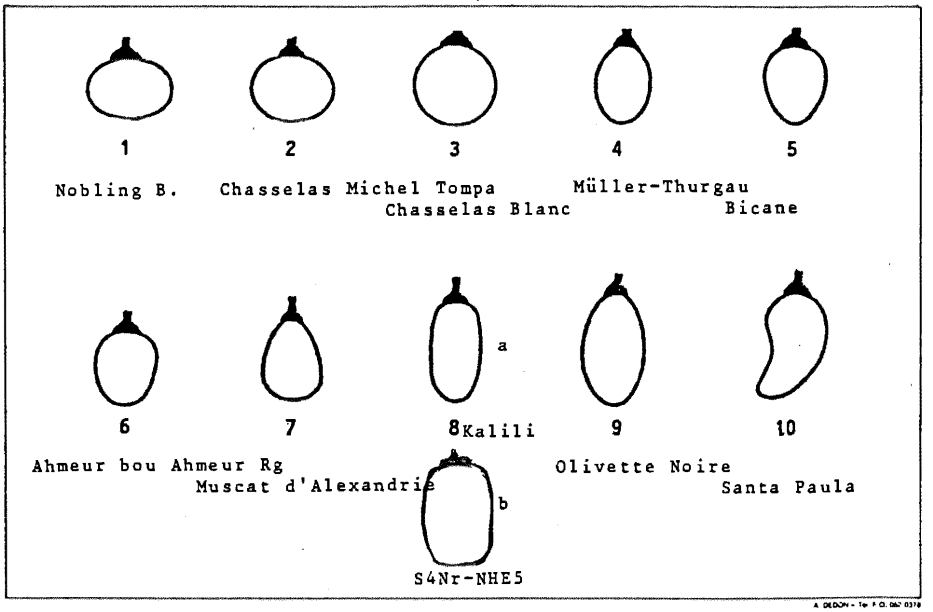

Table 6: Berry shapes according to GALET

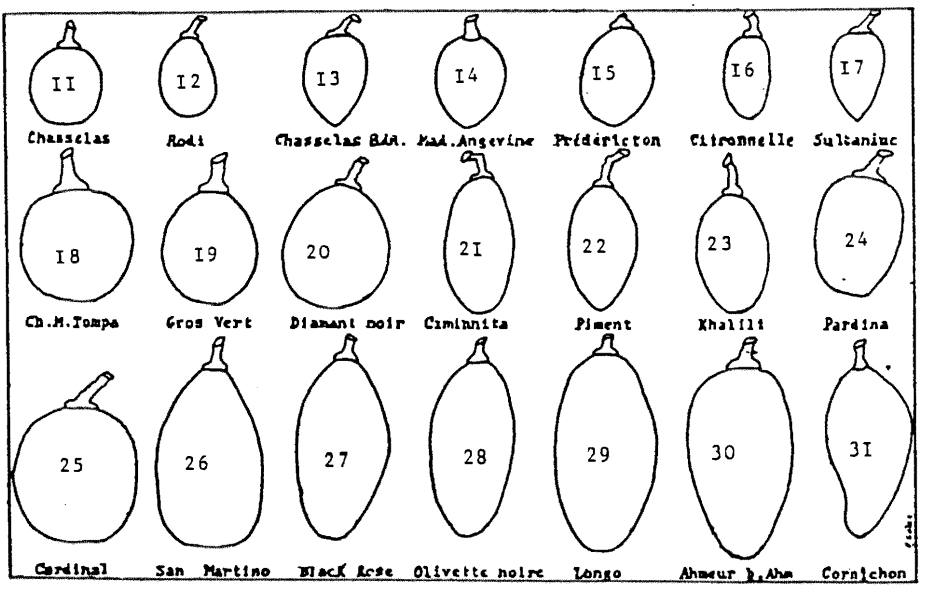

Table 7: Disjunction in S5 of the main S4 genotypes having S6 progenies

S4								S5																								Nb. of S5	
								effect.	inflor. nb				sex		berry colour				berry shape						size bunch				length bunch					giving	effectif
	NI	SX	CB	FB	GO	DM	LG			NI				SX			CB					FB						DM				LG				1 progeny	in
									0	1	2	3	3	5	1	3	5	6	3	4	5	6	7	9	1	3	5	7	1	3	5	7	9	in S6	S6
NHF0	4	3	1	3	5	5	3	70	9	28	19	6	41		39				30			2	5	3	13	16	11		12	17	11	1		19	243
NHN0	1	3	1	4	7	5	3	69	13	31	20	1	44	4	40				14	3	16	2			11	13	11	1	24	12	1			14	150
NHE0	2	3	3	4	5	1	1	5		1			1		1																			1	10
NHE9	1	3	3	3				11		6			5	1				3				2												1	1
NHF5	2	3	3	6	5	3	3	35	4	6	21	2	7		1		8	8							10	1	3		15					2	4
NHE1	2	3	6	3	5	1	1	10		6	3		9					8	5			3			2	6	1		2	6	0	1		3	28
NHF4	2	3	6	3	5	5	3	17	2	9	6		11			3		8	11						5	6			5	6				4	60
NHF6	1	3	6	4	5	1	1	14	3	6	3		5	1				8	1		5	2			3	5			8					6	21
NHI6	2	3	6	3	3	3	3	29	2	11	15		17			1		10	4			5			10	7			10	7				12	84
NHL6	2	3	6	3	5	5	5	16	3	2	10		5	1				6	3			3			1	3	2		2	1	3			3	34
NHN4	1	3	6	4	5	3	3	11	3	3	3		6		2			4		1	4			1	1	2	3		6					4	4
NHN6	1	3	6	3	7	3	3	13	2	6	3		6					7	5			3			2	3	3		2	1	4	1		3	4
NHN7	1	3	6	4	5	1	1	37	13	16	5		13	4				14	4	2	3	5			7	5			14					8	10
								total																										total	total
								337																										80	653

At this stage (S4), the bunch characters are similar to those of Pinot noir but some seedlings differ substantially and we can see in Table 4 how the length and compactness of the bunches increase.

With regard to berries, the original form is class 3, but we see many genotypes of classes 4, 5, 6, 7 and even 8. From size observations it appeared that 83 % of the berries are thicker than those of the initial parent and 25 % of them are very thick.

5th generation (S5)

During the 5th generation obtained by self pollination from the 16 genotypes identified in the 4th generation, 80 genotypes were selected and self pollinated.

When we study the sexual type of the progenies of the 4th generation of self pollination genotypes (Table 7) we notice that 7 genotypes produced only seedlings with flowers of class 3 (hermaphroditic); 6 other genotypes produced only black berries and 4 produced berries of different classes.

Table 8: S5 general characters by S4 family

family identification	vigor	height June-87	buds coulour	leaves size	lobing degrees	lobes number
NHE1	strong	2	red-green	very large	deeply lobed	5
NHE9	medium	1-1.5	red	medium	deeply lobed	5
NHF4	strong	2	red-green	very large	lobed	3 - 5
NHF5	strong	2	red	medium	deeply lobed	5
NHF6	strong	2	very dark-red	small to medium	unlobed	3
NHI6	strong	2	red-green	large	lobed	3 - 5
NHK7	weak to med.	1	red-green	medium	lobed	3 - 5
NHL5	strong	2	red-green	medium to large	deeply lobed	5
NHL6	strong	2	red	large	deeply lobed	5
NHM3	very weak	< 1	red-green	small	lobed	3 - 5
NHM6	very strong	> 2	red	very large	deeply lobed	5
NHN4	strong	2	red	medium	deeply lobed	5
NHN6	medium	1-1.5	red-green	medium	lobed	3 - 5
NHN7	medium	1-1.5	red	medium	unlobed	1 - 2
NHN0	strong	2	red	medium	deeply lobed	3 - 5

Table 9: Germination percentage by S4 family

S4 familly	Total number of seeds	Percentage of germination
NHN6	227	6.6
NHE1	649	7.4
NHE9	57	8.8
NHL6	560	15.3
NHF5	101	21.8
NHE0	268	23.5
NHN7	185	41.1
NHN4	194	43.3
NHI6	486	43.4
NHF4	445	47.8
NHF0	2367	54.1
NHF8	74	59.5
NHN0	1183	61.1

Only 1 genotype of the 4th generation is stable for this character: NHF4. The remaining S4 progenies have berries identical to the original Pinot noir but also progenies with extremely different berry shape such as NHF0: 8a.

The size of the bunches varies greatly but concerning the length notation of the bunches, 4 genotypes yield a stable progeny for this character. Table 8 shows the general characters of the different families. We can see that only 1 family has a weak vigour, 9 show strong vigour and the other 5 are medium. In S4, the coefficient of correlation between vigour and fertility is 0.49: the passage to each following generation induces an automatic selection for vigour. The size of the leaves is not to be considered as an important character because we know that its expression in a greenhouse has no correlation with that in the vineyard.

6th generation (S6)

The 6th generation includes 82 progenies from 16 genotypes of S5 coming from 13 genotypes of S4.

Table 10: Disjunction of the sexual type in S4-S5-S6

S4			S5		S6	
	H	F	H	F	H	F
NHE0	1		1	0	4	0
NHE1	1		9	0	18	0
NHE9	1		5	1	4	0
NHF0	1		41	0	129	0
NHF4	1		11	0	14	0
NHF5	1		7	0	1	1
NHF6	1		5	1	10	1
NHI6	1		17	0	30	4
NHL6	1		5	1	9	1
NHN0	1		44	4	50	5
NHN4	1		6	0	11	0
NHN6	1		6	0	0	0
NHN7	1		13	4	24	2
	H	F	H	F	H	F
Effect.	13	0	170	11	304	14

Table 11: Disjunction of the berry colour in S4-S5-S6

S4Nr	colour	S5			code colour							S6			code colour						
		1v	1j	2	3	4	5	6	6+	6t	7	1v	1j	2	3	4	5	6	6+	6t	7
NHE0	3 red	1										4									
NHE1	6 black							8				2						12			
NHE9	6 black							3										1			
NHF0	1 white	39										102									
NHF4	6 black				3			8				3						46			
NHF5	3 red	1					8	8												•	
NHF6	6 black							8				3						6			
NHI6	6 black				1			10										30			
NHL6	6 black							6										9			
NHN0	1 white	40										46									
NHN4	6 black	2						4				6			1			3			
NHN6	6 black							7													
NHN7	6 black							14						3				19			
Effect.	13	83			4			76				166		3	1			126			

In Table 9 we can see the seed germination power leading to the seedlings of the 6th generation of self pollination. This power of germination, as per cent, is extremely variable between the S4 families. The vigour in S6 remains strong. Expressed by the measure of the growth of the 1st year primary axis, the vigour differs from 1 to 5.75 m at the end of the development with an average of 2.80 m for 655 plants. The average of shoot growth per family differs in the 2nd year of greenhouse culture from 1.90 to 2.90 m. The fertility level varies from 1 to 3 and was noted for 455 of the 655 plants.

270 plants, i. e. 59.3 %, are fertile. 30.3 % could not be determined because there were only 1 or 2 buds or they had not burst.

In S6 progenies the percentage of fertile plants is very variable but no progeny is stable. As to the sexual type (Table 10) checked in S5, there were 8 progenies out of 13 with no plants with female flowers. However in S6 the plants of these same progenies presented a few female plants. Nevertheless, we obtained some other progenies of sexual type hermaphroditic homozygous. After flowering, we made a notation about coulure and millerandage. We observed large variability on coulure notations of progenies with an average of 3.0 in most of the families and general average of 3.2, which is a high value. 6 progenies had a total coulure on the 82 progenies.

Millerandage notes are as variable as coulure notes, of the same size and with a general average of 3.1.

Table 11 shows the disjunction of berry colour regrouped with regard to S4 families. NHE0, NHF0 and NHN0 families are homogeneous: white berries. NHE9, NHI6 and NHL6 are also homogeneous but with black berries. The other families have heterogeneous progenies. The disjunction of the berry shape is shown in Table 12 where we presented the main shapes. In this table we notice the dispersion of the berry shape within some families and the distance compared to the original shape of the Pinot noir.

6th generation: the progenies

After studying all 82 S6 progenies in relation to generations S5 and S4, we wanted to know if there existed progenies with several homogeneous characters. In many cases we found a homogeneity concerning the colour and the shape of the berry, the vigour of the plant or the shape of the leave. But of the 82 progenies, 1 only was homogeneous for all observed traits. S4.NHN7-S5.REZ2-S6.THP2 to THR1 shown in Table 13.

All the notations on bunches were stable, the values of growth were extremely similar, only fertility was variable. However, the relatively low number of plants does not allow us to conclude with certainty that it is a lineage. More observations will be necessary as well as culture in the vineyard.

Discussion

The aim of the successive self pollinated generations is to obtain pure lines, i. e. individuals that are genetically identical and completely homozygous. However, self pollination induces the well-known inbreeding phenomenons (Levadoux 1950; and other authors): weak percentage of germination, high seedling mortality, low vigour of the survivors and low fertility or even sterility.

In the 6 successive self pollinated generations of the Pinot noir it is obvious that an automatic selection was made with the elimination of seedlings which are weak (appearance of a high percentage of lethal or sublethal genes), sterile, sensitive to coulure and millerandage and/or have female flowers.

Every time it was possible, we applied some technics, which permitted us to preserve a maximum of seedlings in each generation by the amelioration of the percentage of seed germination (Balthazard 1979) and by the use of new breeding techniques.

Table 12: Disjunction of the berry shape in S4-S5-S6

Code	OIV	and	Galet													ovate														
								flat		roundish		obtuse-ovate and obovate acuminada												cylindric						
S4		S5						S6	2	3	20	5	6	7	12	17	22	24	26	4	8	9	10	15	16	19	21	25	27	29
		3	4	5	6	7	9	1	18	11		13	30								23	28	31							
												14																		
NHE0	4		1							4																				
NHE1	3	5			3				4	11										1										
NHE9	3				2															1										
NHF0	3	30			2	5	3	1	3	14		13	11			1	10	3		19	7	5	1		5		1			
NHF4	3	11						1	6	7																				
NHF5	6																													
NHF6	4	1		5	2							8								3										
NHI6	3	4			5				1	6		13								8										
NHL6	3	3			3					4										4		1								
NHN0	4	14	3	16	2				3	14		17	1							6		1								
NHN4	4		1	4			1			1		6					1					2					1			
NHN7	4	4	2	3	5					3		16	1							2										
NHN6	3	5			3																									
		77	7	28	27	5	4	2	17	64	0	73	13	0	0	1	11	3	0	44	7	9	1	0	5	0	2	0	0	0

Most of the time, we obtained from plants of the 4th, 5th and 6th generation families normal vigour that allowed the plant to initiate flowers.

From a genetical point of view, we chose as an initial parent a clone selected from a variety population including numerous types.

The starting variability was quite narrow. According to GALAIS (1981), the inbreeding effect can increase variability and therefore the progress by generation but also the duration of the

Table 13: NHN7-REZ2 progeny

S4	S5	S6	growth 1st year			Fertility		Colour	Shape	Sex	Growth
			a	b	b-a	oeil 1	oeil 2	shoot	leave		2nd year
NHN7	REZ2	THP0	281	380	99	2	2	1	2	H	120
"	"	THP2	101	189	88	0	0	1	2		98
"	"	THP3	216	345	129	0	0	1	2		131
"	"	THP4	235	342	107	0	0	1	2		158
"	"	THP5	246	367	121	2	1	1	2	H	128
"	"	THP6	265	384	119	1	0	1	2	H	137
"	"	THP7	264	385	121	0	0	1	2		140
"	"	THP8	243	347	104	1	0	1	2	H	152
"	"	THP9	273	374	101	0	1	1	2	H	122
"	"	THR1	247	355	108	1	0	1	2	H	130
Average			237.1	346.8	109.7						131.6
Coeff.of variation %			21.7	16.7	11.4						12.9

		S6	Coulure	Mille-randage	Shape berry	Colour berry	Size bunch	Length bunch	Compact. bunch		
		THP0	3	4	ovate	black	small	very small	compact		
		THP2									
		THP3									
		THP4									
		THP5	4	4	ovate	black	small	very short	compact		
		THP6			ovate	black	small	very short	medium		
		THP7									
		THP8	3	4	ovate	black	small	very short	medium		
		THP9	4	4	ovate	black	small	very short	medium		
		THR1	3	4	ovate	black	small	very short	medium		

Table 14: Summary: sex, colour and shape

	Sex		Colour	berry	Shape	berry
S4	S5	S6	S5	S6	S5	S6
NHE0	*	*	*	*		(*)
NHE1	*	*				
NHE9		*	(*)	(*)		
NHF0	*	*	*	*		
NHF4		*				
NHF5						
NHF6						
NHI6				*		
NHL6			*	*		
NHN0			*	*		
NHN4	*	*				
NHN6	*		*			
NHN7						

*= Character considered as been homozygous

selection cycle. Concerning the sexual type of the flowers, the progress to homozygoty is very slow and corroborates, if necessary, that sexual type is dependent on a complex multiallelic system with effect of relations of epistasie and of occasional dosage (CARBONNEAU 1983). The berry colour, the dimension and length of the bunches become quickly stable.

Concerning the shape of the berries we observed a very large dispersion tat also became stable during the following generations (Table 14). After 6 generations of self pollination and among 82 progenies, only 1 is homogeneous and can be considered as a pure line. The progress towards homozygoty as in the case of the sexual type quoted before is extremely slow: the noticed characters are under the dependence of sexual genes.

Conclusion

Summarizing the reported results leads us to the conclusion that in the case of the clone of Pinot noir chosen as the original parent it is necessary to carry out at least 6 generations of self pollination to obtain 1 homogeneous progeny or lineage among 82. This homogeneity concerns all the phenological characters expressed by the plant issued in the 2nd year of cultivation after sowing in a greenhouse. Many progenies are homogeneous only for one or several characters. This work will be continued with several more self pollinated generations and test crossing of the selections. The advantage of chosing one or the other obtained lineage will then be considered.

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