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RESEARCH ARTICLE

RESISTANT COCOA GENOTYPES SELECTED AGAINST *PHYTOPHTHORAMEGAKARYA* IN THE RECIPROCAL RECURRENT SELECTION PROGRAM

Inago Caudou Trebissou[1], Gnion Mathias Tahi[1] and Klotioloma Coulibaly[2]

1. Cacao Genetic Improvement Laboratory, CNRA Research Station of Divo, Divo, Côte d'Ivoire.
2. Cacao Phytopathology Laboratory, CNRA Research Station of Divo, Divo, Côte d'Ivoire.

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Abstract

Black pod rot of cacao caused by stramenopiles of the genus Phytophthora can lead to up to 60% yield losses in endemic regions of Côte d'Ivoire. To reduce the field incidence of this disease, genetic control emerges as an alternative solution capable of providing resistant varieties to farmers.This study was conducted on 93 cocoa genotypes and three reference controls with Variables sensitivities. The inoculation technique used was that described by Nyassé, and the strain utilized was a highly aggressive isolate of P. megakarya. The Blaha scale was applied to assign infection scores to each leaf disk.The results of hierarchical cluster analysis on principal components (HCPC), followed by Correspondence Factor Analysis (CFA), revealed that the genotypes were divided into five homogeneous groups based on infection score frequencies. Twenty-eight genotypes in cluster 1 were classified as highly resistant to P. megakarya. Twenty other genotypes belonging to cluster 2 were classified as resistant to the pathogen. These genotypes will be used as progenitors in the genetic improvement program to create and disseminate resistant descendants against this phytopathogen.

Introduction:-

In cocoa (*Theobromacacao* L.), Black pod rot is caused by stramenopiles of the genus *Phytophthora*(Baldauf, 2008; Coulibaly et al., 2018) and represents the main source of economic losses globally(Nembot Fomba, 2021). In Côte d'Ivoire, two *Phytophthora* species are pathogenic to cocoa: *Phytophthorapalmivora*, a cosmopolitan species, and *Phytophthoramegakarya*, an endemic species in production areas of Central and West Africa (Coulibaly et al., 2022). *P. megakarya* is the most aggressive, causing between 60% and 100% yield losses in endemic areas. The spread of brown pod rot caused by P. megakarya to new production areas poses a growing threat to food security.

Controlling brown pod rot has always been a priority in cocoa genetic improvement programs in Côte d'Ivoire. Successive waves of hybrids distributed to producers aimed to not only increase productivity and bean size but also better address constraints of the time, such as mirid insects and brown pod rot caused by P. palmivora. However, with the emergence of *P. megakarya*, selecting resistant varieties to this pathogen is now essential to mitigate economic losses and ensure the sustainability of Ivorian orchards.

Corresponding Author:Inago Caudou Trebissou
Address:Cacao Genetic Improvement Laboratory, CNRA Research Station of Divo, Divo, Côte d'Ivoire.

The main cocoa breeding program in Côte d'Ivoire is the Reciprocal Recurrent Selection (RRS) programbased on two base populations with complementary traits, this program aims to create, after several cycles of crossing, a composite population integrating selected traits from the parents. The first cycle, initiated in 1990, was completed in 1999 (Lachenaud et al., 2001), and the second cycle began in 2000 with the establishment of intragroup recombination plots and an intergroup test. After more than ten years of data collection, the second cycle is nearing completion, and the progenitors for the third cycle have been selected based on a selection index incorporating multiple criteria(Tahi et al., 2017; Trebissou et al., 2021). However, resistance to *P. megakarya* was not included in these selection criteria.

This study therefore aims to identify, within this reciprocal recurrent selection program, sources of resistance to *P. megakarya*, in order to use them as parent plants and thereby enhance the resistance of hybrids to be disseminated in the future.

Material And Methods:-

Plant Material

1. Ninety-three genotypes from the RRS program (Tahi et al., 2017) were evaluated in this study.
2. Three reference controls: SCA6 (resistant), PA150 (moderately resistant), and NA32 (susceptible)

Fungal Material

The fungal material used to evaluate the resistance of RRS genotypes to Phytophthora spp. was the highly aggressive isolate 13P30-1 of *P. megakarya*(Ali et al., 2017)

Methods:

Inoculum Preparation

P. *megakarya* strains used in this study were isolated from immature pods naturally infected by the stramenopile in endemic areas. Necrotic parts of pods were cultured on 1.5% agar water medium. Incubation was done in the dark at 26°C for seven days, followed by purification and sub-culturing on "pea" medium (Coulibaly et al., 2013). To reactivate isolate aggressiveness, an explant from each culture was inoculated onto mature pods. An aggressiveness test was performed, and the most aggressive isolate was selected.

Cultures of the selected isolate were grown on solid "pea" medium in Roux bottles. Incubation was carried out at 26°C for six days under a 12-hour photoperiod. Zoospore release was induced by flooding the culture medium with 40 ml of sterile distilled water followed by thermal shock (4°C for 15 minutes, then under a 60-watt incandescent lamp for 45 minutes). The zoospore suspension was calibrated using a Malassezhemocytometer to a concentration of 3×10^5 zoospores per ml (Ali et al., 2017).

Leaf Sample Collection and Preparation

Two to three healthy leaves were collected from semi-hardened branches under partial shade at the Divo research station on the day before the evaluation, between 8 a.m. and 10 a.m., and transported to the laboratory on the same day. Once at the laboratory, sterile sponges soaked in distilled water were placed in lightproof trays. The marked leaf samples were placed with their underside on the sponges, and the setup was hermetically sealed with a black tarp and kept overnight in a 25°C chamber. The leaves were conditioned in this way to make them more receptive to inoculation. The following day, 40 leaf discs with a diameter of 15 mm were cut from the leaves of each genotype using a punch.

Experimental Design

The inoculation of the 93 genotypes and the three controls was carried out in two batches. The first batch consisted of 49 genotypes and the three controls, while the second batch included 44 other genotypes and the three controls. During the evaluation of each batch, the experimental design was a completely randomized block with four sub-blocks represented by trays. Each tray contained 50 rows arranged in two rows of 25 lines. The rows were randomly randomized from one tray to another. Each row consisted of ten leaf discs from the same genotype. The three controls were represented in each tray by ten leaf discs each. A total of forty leaf discs per evaluated genotype and per control were used for each batch. Two repetitions were performed for each batch (Efombagn et al., 2011), resulting in 80 leaf discs for each genotype and 160 leaf discs for the controls.

Inoculation of Leaf Discs

The inoculation technique followed the method described by Nyassé (Nyassé et al., 1995). After inoculation, the trays were hermetically sealed with a black plastic tarp. Observations were made seven days after inoculation, focusing on symptom identification and scoring based on Blaha's scale(Bowers &Tondje, 2006)(Figure 1):

- **No symptom development** = 0 (highly resistant)
- **Penetration points** = 1 (resistant)
- **Connected points** = 2 (moderately resistant)
- **Reticulated necrotic aspect** = 3 (moderately susceptible)
- **Marbled necrosis** = 4 (susceptible)
- **True necrosis**= 5 (highly susceptible)

Statistical Data Analysis

The chi-square test was used to evaluate the significance of differences among genotypes based on the infection scores obtained.

A hierarchical ascending classification on principal components was performed using the "FactoMineR" package in R (Lê et al., 2008). This method combined correspondence analysis (CA) to reduce the data into principal variables and hierarchical ascending classification (HAC) to group observations into homogeneous clusters based on their similarity, measured by the chi-square distance.

Results And Discussion:

Results:-

Adjustment for Manipulation Effects

The 93 genotypes and the three reference controls were evaluated in two repetitions. For each control, the infection scores obtained during the four manipulations were not significantly different. Therefore, adjustments for "manipulation effects" were not required to compare the evaluated genotypes with the reference controls.

Formation of Groups Based on Significant Infection Score Frequencies

The Pearson chi-square test of independence revealed significant differences among the genotypes in their infection scores. The evaluated genotypes were divided into five groups with the following characteristics **(Table 1)** :

- **Group 1**: Comprised of genotypes with a significantly high frequency of infection score 1 across their leaf discs.
- **Group 2**: Comprised of genotypes with a significantly high frequency of infection scores 1 and 2 across their leaf discs.
- **Group 3**: Comprised of genotypes with a significantly high frequency of infection scores 2 and 3 across their leaf discs.
- **Group 4**: Comprised of genotypes with a significantly high frequency of infection score 3 and a few scores of 4 across their leaf discs.
- **Group 5**: Comprised of genotypes with a significantly high frequency of infection score 3 and a higher frequency of score 4 compared to Group

Distribution of Genotypes Across Groups

The genotypes were classified into five homogeneous groups **(Figure 2)** based on their infection score frequencies using hierarchical clustering on principal components (HCPC).

- **Cluster 1**: Contains 28 genotypes, including the resistant controls SCA6 (C77) and PA150 (C76). These genotypes are classified as highly resistant to Phytophthora megakarya**(Figure 3).**
- **Cluster 2**: Includes 20 genotypes also classified as resistant to Phytophthora megakarya**(Figure 4).**
- **Clusters 3, 4, and 5**: Contain the remaining genotypes **(Figure 5 & 6)**. The susceptible control NA32 (C75) was classified in Cluster 5.

Discussion:

The results obtained demonstrate the existence of significant genetic diversity among the 93 cacao genotypes evaluated for their resistance to *P. megakarya*. Hierarchical clustering based on principal components (HCPC) identified five homogeneous groups, each characterized by specific frequencies of infection scores.

Group 1, consisting of 28 genotypes, including the resistant controls SCA6 (C77) and PA150 (C76), is distinguished by a significantly high frequency of score 1, characteristic of genotypes highly resistant to *P. megakarya*. These genotypes could serve as a solid foundation for breeding programs as parental lines to improve the resistance of offspring in the next cycle.

Group 2, comprising 20 genotypes with a significant proportion of scores 1 and 2, reflects a moderate tolerance to the disease. While these genotypes are less resistant than those in Group 1, they show promising potential in terms of production and resilience to the disease in systems where pathogen pressure remains moderate.

Groups 3, 4, and 5, containing increasingly sensitive genotypes, highlight the vulnerability of part of the cacao germplasm to this pathogen. These genotypes could either be excluded from areas where the disease is prevalent or used in crossing programs with more resistant genotypes to improve the resistance of their offspring.

The variability observed in infection scores among leaf discs within the same genotype suggests horizontal and polygenic resistance, which aligns with the work of Rêgo (Rego et al., 2022; Rego et al., 2023). This diversity, present within the breeding program, contributes to the resilience of cacao trees to various biotic and abiotic constraints. It enables genotypes to adapt to changing environments and the rapid evolution of pathogens, ensuring greater sustainability of crops. The use of resistant genotypes or their descendants in the field holds promising prospects for reducing harvest losses caused by P. megakarya, the most formidable species under natural infection conditions. Furthermore, although genotypes sensitive to Phytophthora megakarya are not suitable for combating this pathogen, they may serve as valuable material in managing other constraints, such as drought tolerance or swollen shoot disease.

Conclusion And Perspectives:

The results of this study identified significant genetic diversity among the cacao genotypes evaluated for their resistance to P. megakarya. Twenty-six genotypes were classified as highly resistant, while twenty others showed lesser resistance. These resistant or tolerant genotypes can be integrated into the cacao breeding program to slow the spread of this pathogen in orchards. The horizontal and polygenic resistance observed in these genotypes provides cacao trees with resilience to various biotic and abiotic constraints, ensuring better crop sustainability in ever-changing environments.

However, further studies should assess the correlation between laboratory resistance tests, such as leaf disc inoculation, and the rate of pod rot observed in the field to better understand the relationship between the two tests. Moreover, integrating resistant genotypes into multilocal trials would help confirm their performance across different environments and identify potential genotype-environment interactions. The use of molecular markers to characterize the genetic bases of resistance would facilitate marker-assisted selection and the development of more robust cacao varieties adapted to diverse conditions.

Figure 1:-Different sensitivity scores of genotypes after artificial inoculation of leaf discs according to the Blaha rating scale.

Table 1:- Formation of Clusterbased on the significance of infection score frequencies.

	Cluster 1			
	Intern %	Global %	P.value	V.test
Frequency 1	**56,31**	29,29	**7,38 e -249**	**33,69**
Frequency 2	-	-	-	-
Frequency 3	11,91	35,69	2,31 e -204	-30,5
Frequency 4	0,34	3,96	2,91 e -37	-12,75
	Cluster2			

	Intern %	Global %	P.value	V.test
Frequency 1	**33,37**	**29,29**	**7,57 e -5**	**3,96**
Frequency 2	**37,75**	**31,05**	**1,83 e -10**	**6,37**
Frequency 3	28,37	35,69	4,85 e-12	-6,91
Frequency 4	0,5	3,95	4,12 e-21	-9,43
	Cluster3			
	Intern %	Global %	P.value	V.test
Frequency 1	14,69	29,29	1,24 e -93	-20,53
Frequency 2	**34,14**	**31,05**	**4,78 e-5**	**4,07**
Frequency 3	**48,05**	**35,69**	**1,18 e -55**	**15,71**
Frequency 4	3,12	3,95	8,88 e -03	-2,61
	Cluster4			
	Intern %	Global %	P.value	V.test
Frequency 1	4,89	29,29	1,13 e-82	-19,26
Frequency 2	18,52	31,05	9,22 e -19	-8,84
Frequency 3	**67,04**	35,69	**1,35 e-89**	**20,07**
Frequency 4	**9,54**	3,95	**5,13 e-15**	**7,82**
	Cluster5			
	Intern %	Global %	P.value	V.test
Frequency 1	5,42	29,29	1,36 e -41	-13,51
Frequency 2	13,33	31,05	2,48 e-20	-9,24
Frequency 3	**53,75**	35,69	**1,23 e-16**	**8,28**
Frequency 4	**27,5**	3,95	**1,18 e-81**	**19,14**

Significant values in bold

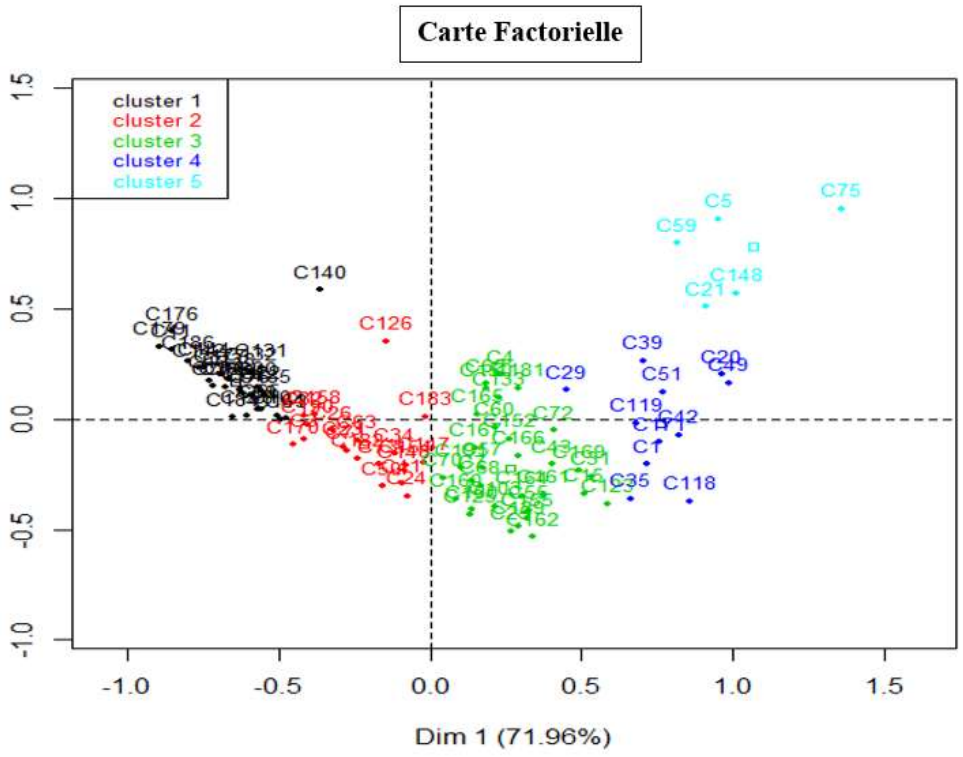

Figure 2:-Distribution of individuals across clusters based on hierarchical clustering on principal components.

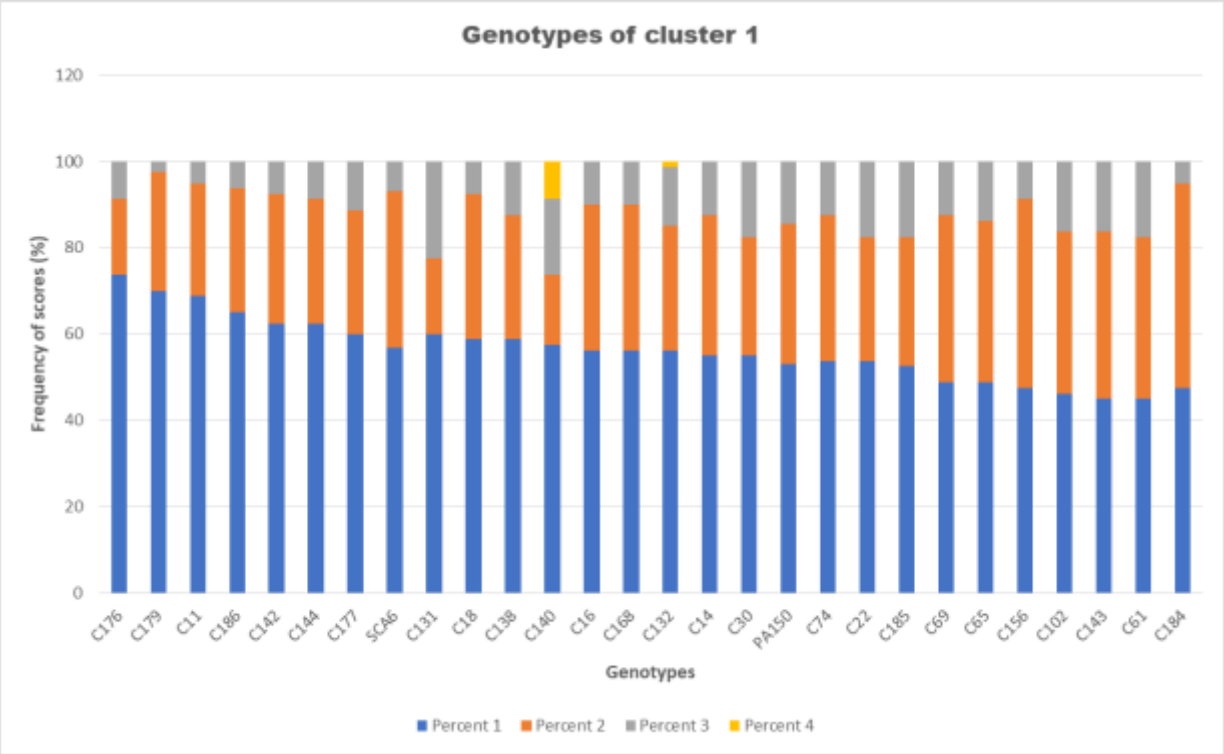

Figure 3:-Genotypes of cluster 1.

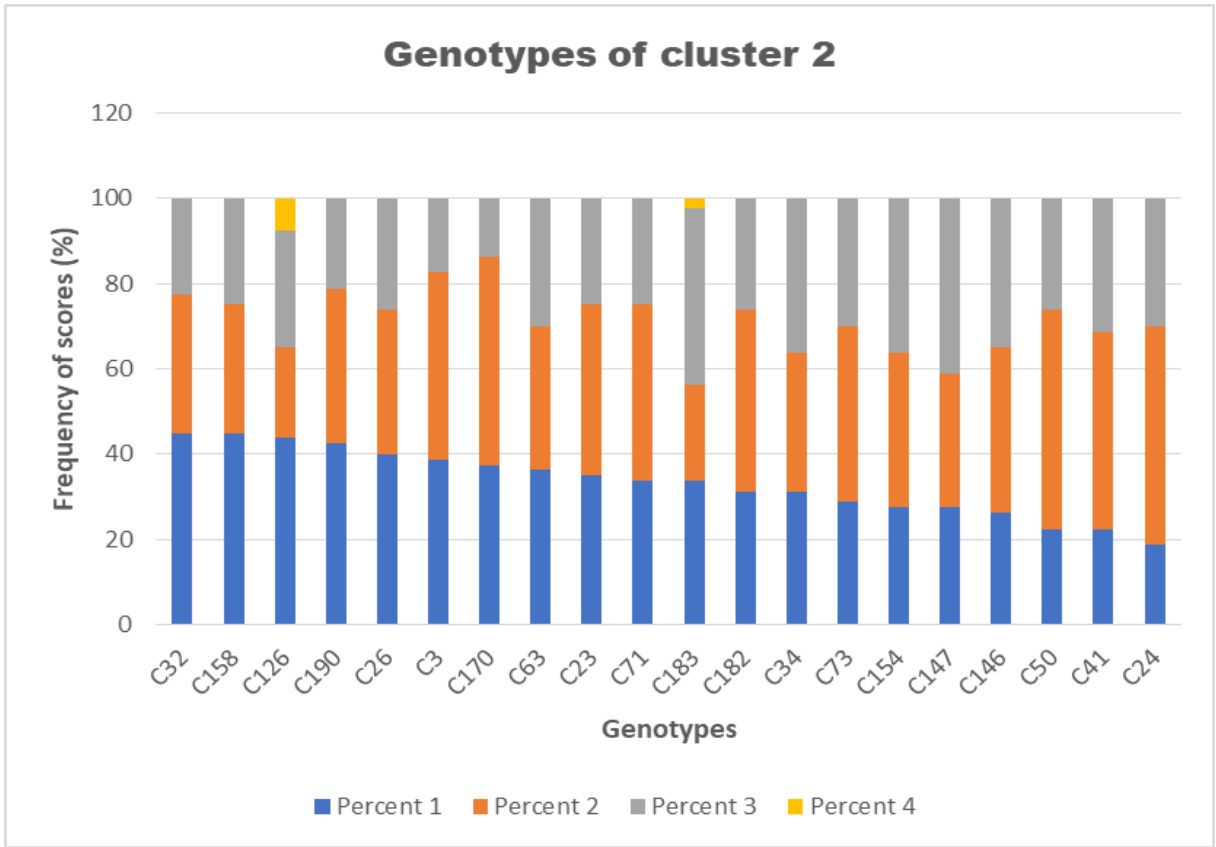

Figure 4:-Genotypes of cluster 2.

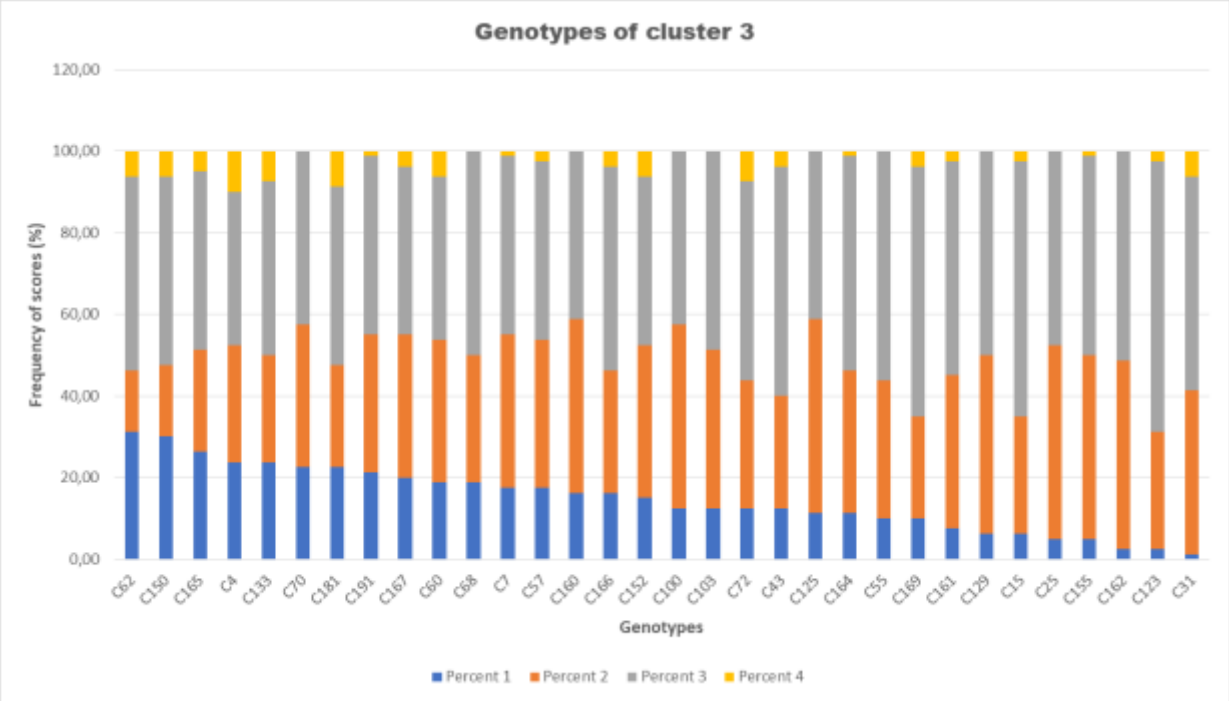

Figure 5:-Genotypes of cluster 3.

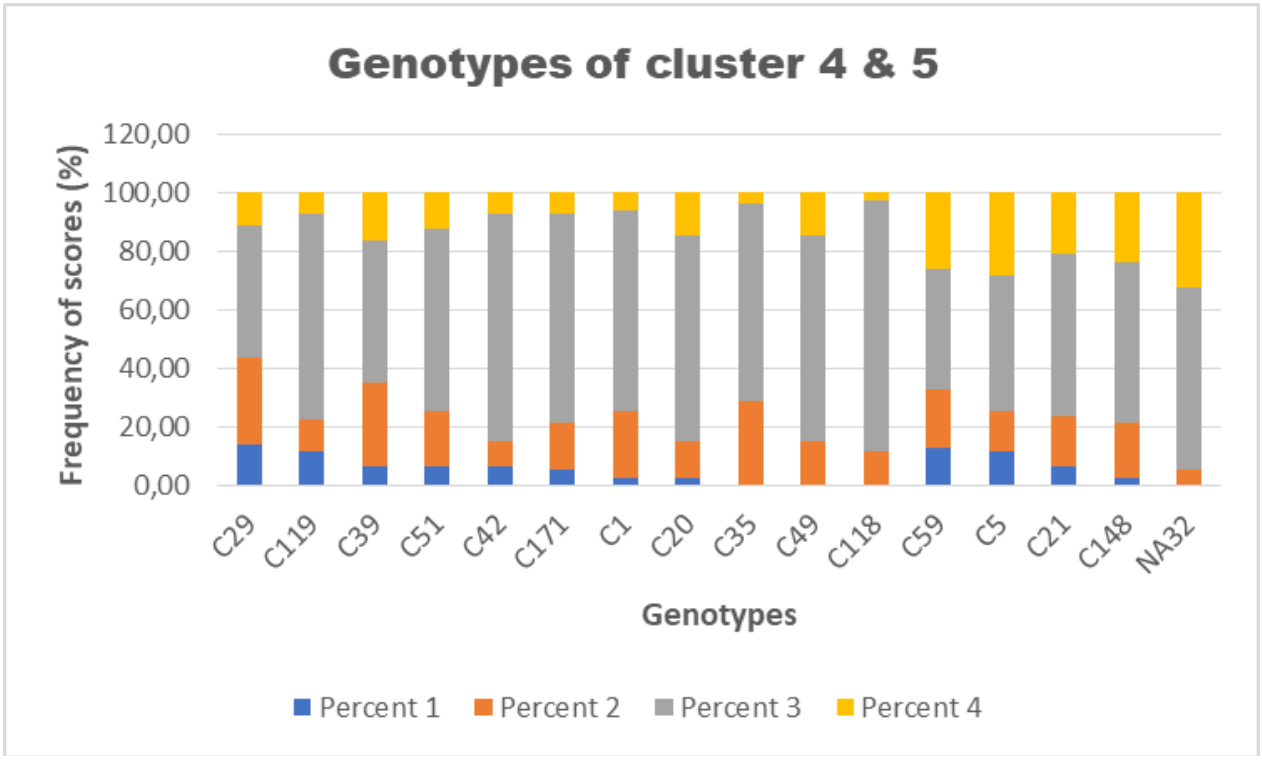

Figure 6:-Genotypes of cluster 4 & 5.

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