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Genetic Diversity of Red Onion Using Microsatellite (SSRs) Markers

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Abstract **Original Research Article**

Onion is one of the most widely grown vegetable crops, but also one of the least genetically characterized. It is diploid (2n=16) and allogamous, which makes its selection program a little more demanding. Molecular markers make selection easier. The variability of individual phenotypes is a peculiarity of the living world. Some of these variations are expressed at the phenotypic level (morphology, physiology, behavior, etc.), while others are hidden, and their detection requires the use of adapted techniques (variability of proteins or DNA sequences). For this purpose, 23 onion varieties collected by INRAN (Institut National de la Recherche Agronomique) from Niger and the three onion powder mixes received from R&D Nestlé Abidjan. The genetic analysis was performed using molecular markers (SSRs). The results demonstrated a large inter variability in the onion varieties obtained from INRAN collection. An equivalent source of genetic diversity is also observed inside the main number of these varieties expertized.

Key words: genetic diversity, onion, polymorphism**,** molecular markers, Niger.

INTRODUCTION

In Niger, horticultural production, including vegetables, is one of the mainstays of the national economy, accounting for a significant share of irrigated agriculture and crops. The most profitable crops are tomatoes, cabbage, potatoes and onions. Onions are the country's second most important export crop, after cowpeas. Onions are grown all over the country, but the Tahoua region in the northeast alone accounts for over 70% of national production. The most widely grown variety is the violet de Galmi.

The strengths of onion production in Niger are the potential for irrigable land, much of which is suitable for onion cultivation, the politics of the authorities to support irrigated cultivation, and the professionalization of producers. Despite this, the onion production faces a number of constraints (edapho-climatic, socio-economic and biotic).

In addition to these constraints, work carried out by the Institut de Recherche en Afrique Tropicale (IRAT) and the Institut National de la Recherche Agronomique du Niger (INRAN) has highlighted the problem of varietal purity. This is a very important issue, as it affects production, marketing and, consequently, exports, which generate foreign currency for the country.

However, this variability is poorly documented, making it difficult to ensure proper maintenance. In addition, seed production is not carried out according to the rules, accelerating the genetic erosion of the different ecotypes. In the various production zones, farmers use seeds from different origins, thereby accelerating the genetic erosion of their ecotypes.

Attempts to purify and maintain the purity of certain local varieties were, however, carried out by INRAN as part of the Projet de Promotion et d'Exportation des Produits Agro-pastoraux (PPAEP) between 2002 and 2004, and as part of its collaboration with AVRDC. This work did not lead to any tangible results to save the onion variety in Niger.

The objective of this study was to assess the genetic diversity of 23 onion varieties using SSR markers, as well as their homogeneity.

MATERIAL AND METHODS

The two tables 1 & 2 below list, respectively, the 23 onion varieties collected by INRAN (Institut National de la Recherche Agronomique) from Niger and the three onion powder mixes received from R&D Nestlé Abidjan.

Table 1: List of onion varieties. For each variety two bulbs (x and y) have been fingerprinted in order to check the genetic homogeneity

Lab code	Name	INRAN code
1	Rose de Diffa	RsDa
2	Blanc de Soukoukoutan	BSn
3	Madarounfa Dan Radi	MfaDR
4	Violet de Galmi Ayerou	VGAy
5	Tawraka	Twa
6	Violet de Galmi Gaya	VGGa
7	Tassa Galmi	TG
8	Ayerou	Ay
9	Albassa Guidan Idder	AGI
10	Guidan Magagi	GM
11	Kankare	Ke
12	Violet de Galmi Diffa	VGDa
13	Youri	Yi
14	Yaourizé	YaI
15	Blanc de Soumarana	Bsa
16	Rouge de Gaidam	RgGa
17	Blanc de Galmi	BGi
18	Blanc de Diffa	BDa
19	Jan Iri	JaI
20	Violet de Galmi	VG
21	Ja Albassa	JaA
22	Blanc de Gotheye	BGo
23	Madarounfa	AMfa

Table 2: Dried onion powder listed according to the onion variety

Lab code	Name
P1	"Violet de Galmi
P2	Madarounfa Dan Radi
P3	Ayerou

DNA Extraction

The DNA extractions were performed using DNeasy 24 Plant Maxi Kit QIAGEN on 1g of ground onion bulb or 0.5g onion powder mix. This technology involves a lysis step followed by filtration and centrifugation steps that lead to the elimination of proteins, polysaccharides and cell debris. The DNA is then purified through a silica membrane after binding, washing and elution steps. The DNA was used as 20-fold dilutions for the analysis.

DNA Amplification

The amplification is performed for each SSR marker with two specific primers and one of them is labelled with a fluochrome allowing the detection of the PCR fragment. The primers are used as 0,5pM (final concentration). The amplifications are performed into the thermo cycler PCR Applied Biosystems 9700 as follows: 10min at 94°C, 35 cycles with 30 sec at 94°C – 30sec at 50°C – 1 min at 72°C and a final extension at 72°C for 7 min.

Signal Detection

The PCR products are then added to a mix of formamide and the GeneScan 500 LIZ Size Standard. As the primers forward are labelled with FAM or VIC fluorochroms, two amplified DNA PCR SSRs could be mixed together. After a denaturation step, the plate is placed in the genetic Analyser 3500xL and the capillary electrophoresis is managed following the supplier instructions. The experimental data were analysed by GENEMAPPER (Applied Biosystems software).

Data Scoring for Onion Bulbs

We assumed that our studied cultivated onion varieties were diploid with two alleles for a given locus. For each genotype and each SSR marker, data were scored in an excel file as follows: “2” when the plant is homozygous for a given allele or “1” for each allele when the plant is heterozygous.

Statistical analysis is performed using NCSS2007 software (Hintze, J. 2001) from the excel data file. A dendrogram is drawn with Ward’s minimum variance as clustering method and Euclidian distance from raw data obtained from the SSR set used.

Data Scoring for Genetic Diversity Study on Dehydrated Onion Mix

In this case, more alleles can be amplified because these samples could contain different onion genotypes. Therefore, each signal (allele) must be

selected. During the GENEMAPPER analysis each allele must be recorded with its size (bp) and the area of the detected signal associated to a given allele. The final result are given by the percentages of each allele. Only the allele which are representing more than 5% of the total are kept.

SSR Marker Selection

52 SSR markers were tested for the selection and amplified on 12 DNA onion bulbs. They were then selected according to their polymorphism and their quality. A total of 13 of them were kept for the final analysis. They are listed in the table 3 below.

Table 3: SSR markers selected for the diversity study. The microsatellites highlighted in yellow are also used for DNA amplification on onion powders

Name	Forward primer	Reverse primer	Labelling
ACM004	TCGTTCTTTAGAACACGTTAGG	GTCGGCGGATATAGTGACA	FAM
ACM006	GCAGTTCTCCCTTTGTAAAATCA	GTGATGGATGAGTGGATGGA	FAM
ACM024	CCCCATTTTCTTCATTTTCTCA	TGCTGTTGCTGTTGTTGTTG	FAM
ACM045	AAAACGAAGCAACAAACAAAA	CGACGAAGGTCATAAGTAGGC	FAM
ACM068	GAAGGTGAAGGTGTACGGT	CAAATGGCTGCAATAAGCAA	VIC
ACM082	CACCGTTCCTCAGCTCACTT	AGAGGGACGAAATGAAAGCA	FAM
ACM091	TCTCCTCCTCTAACCAGCCA	GGTGCTCCAGTTGAGCTTTC	FAM
ACM097	TTCTCAGCTGCAATGGTGAC	ACGTGCTTGGGCATTATTTC	FAM
ACM099	CATTTCTCCCTTCTGCTTTGTT	TTTGGATTGTGAGTTTGGCA	FAM
ACM102	TGGATTTGTGAACAACCGAA	GATGCAGGCAGTGTTTTGAA	VIC
ACM119	TTTCAGCAACATAGTATTGCGTC	TCTTCGGGATTGGTATGGAG	VIC
ACM138	ACGGTTTGATGCACAAGATG	CCAACCAACAGTTGATACTGC	VIC
ACM152	TCCCAAGAGTCCAAGAATGG	TGTTCTCCCTTAAACAGTGCAA	VIC

RESULTS AND DISCUSSION

1. Genetic diversity on onion collection

Using a collection of 23 varieties with two bulbs per genotype, a total of 46 alleles were detected using the 13 SSR markers, the average is 3.5 alleles per locus. The heterozygosity rate was estimated for each genotype and varies from 8 to 77% which reveals a large range of variability for this trait.

The PIC (Polymorphism Index Content) value was evaluated for each SSR and varies from 0.34 to 0.69. The average PIC value is above 0.5, it indicates a good genetic information level on this onion sampling for this SSR set.

Table 4: The allele number and the PIC value for each SSR locus studied

SSR	Nb allèles	PIC
ACM006	4	**0.69**
ACM024	6	**0.52**
ACM045	4	**0.61**
ACM102	4	**0.52**
ACM119	3	**0.54**
ACM082	3	**0.34**
ACM091	4	**0.59**
ACM138	5	**0.57**
ACM068	2	**0.33**
ACM097	2	**0.49**
ACM099	3	**0.56**
ACM152	3	**0.50**
ACM004	3	**0.40**

A hierarchical ascendant classification was performed with the molecular data obtained from the 23 varieties (two bulbs for each). The dendrogram issued from this statistical analysis clearly shows that only five varieties out of the 23 tested has a similar DNA profile between the two bulbs studied. These five onion varieties are Violet de Galmi Ayerou (VGAy), Kankare (Ke), Youri (Yi), Rouge de Guidam (RgGa) and Blanc de Diffa (BDa). We could expect a better genetic homogeneity for these five varieties but it must be confirmed by the analysis of a larger number of bulbs.

Among the other 18 onion varieties analyzed the genetic distances are similar as well as between the two onion bulbs in a given variety (intra variability) than between bulbs from different onion varieties. (inter variability). This data strongly suggests that the onion varieties are not vegetatively propagated but are rather

multiplied by seeds. This trait is supported by the allogamous status of the onion species.

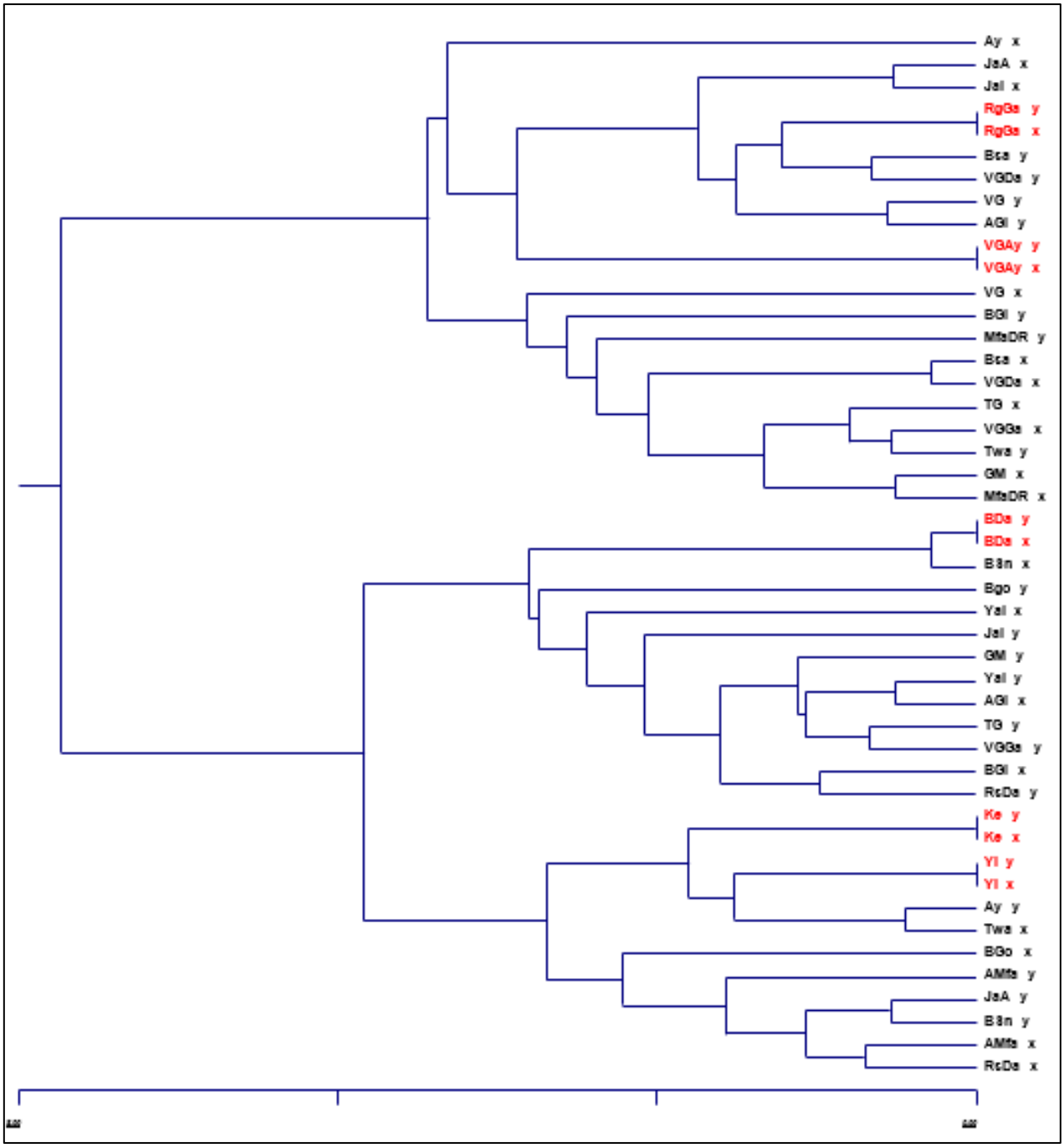

Figure 1: Dendrogram realized with 46 bulb samples using the set of 13 SSRs. The onion varieties indicated in red have the same genetic background on the two bulbs (x and Y) tested. The code of the onion varieties is given in Table 1

2. DNA fingerprint on onion mix

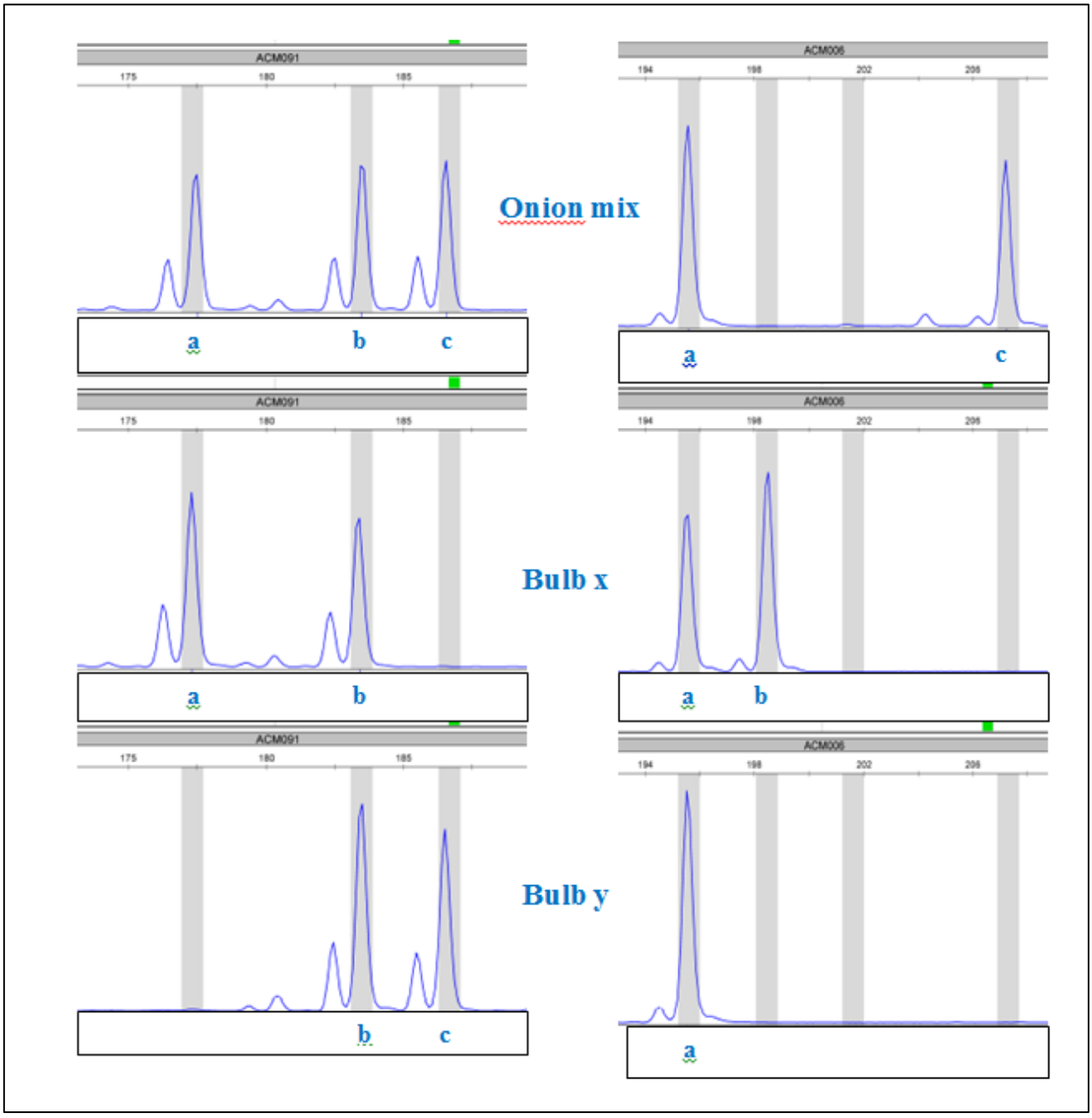

Figure 2: DNA fingerprint showing SSR genetic profiles on onion mixes with two bulbs used as controls. On the left part is showing the DNA study of "Madarounfa dan Radi" with the SSR ACM091 while the right part is illustrated using the variety "Violet de galmi Gaya" with the microsatellite ACM006

DNA in onion mixes obtained through the DNA extraction technique described above and gives good SSR fingerprints quality and allows the genetic analysis in onion powders.

We observed two situations. In the onion mix from Madarounfa Dan Radi variety we detected three alleles a b and c, also amplified in the two bulbs of this onion variety. In the second example, two alleles a and c are detected in the mix. The allele a is present in the two bulbs while allele c is not detected. As we noticed before these two varieties are not genetically homogeneous and the low number of bulbs tested are not be representative of the variability of each onion variety. In this regard, a good genetic evaluation of the diversity on the varieties with key agronomic or organoleptic traits of interest could be performed on the onion mixes in order to assess the level of genetic diversity among these onion batches.

Another important point to be considered is the reliability of the genetic origin for each onion variety according to the raw material chain procurement among

the different farms and period of the year. The onion varieties have to be considered as variety populations and should be characterized according to their allele's frequencies defining a genetic background more or less specific among the varieties tested.

DISCUSSIONS

A recent study on the application of microsatellites in plants showed that microsatellites are often used in the analysis of genetic variability in onion (Rajwan *et al.*, 2010). They have become the markers of choice because they are locus-specific, widespread in the genome, co-dominant and reproducible (Anil *et al.*, 2010). In this study, a significant difference in the polymorphism of markers used across onion ecotypes was noted. The majority have a PIC greater than 0.5 (Table 1), and are therefore more informative, as pointed out by Anil, (2010) markers with a PIC greater than 0.5 give much more information, and the latter are more widely used to distinguish the rate of polymorphisms according to De Woody cited by Anil *et al.*, 2010). The number of alleles is 46 with an average of 3.5 alleles/locus, close to the results found by Anil *et al.*, which are 54 alleles for an average of 2.84, when assessing the genetic diversity of onion in India using 19 microsatellite markers, 2010), when assessing the genetic diversity of onion in India using 19 microsatellite markers.

The results showed that all the groups contained ecotypes with different bulb colors and a wide range of similarity indices. The variability can be explained by the fact that they belong to different regions spread across the four agro-climatic zones (Boukary *et al.*, 2012) and are grown on different soil types, whereas grouping was not done on the basis of color, growing season or geographical provenance. There is also a lot of introduction through commercial exchanges (distribution houses, markets, etc.) and non-compliance with seed production standards, one of the foundations of intra-population diversity. Research results from the years (Nabos 1976), led to the selection of three varieties: Galmi violet for its keeping qualities, yield and taste, and two white varieties for their dry matter content and taste. Violet de Galmi, an export variety, is the most popular in West Africa (Rouamba *et al.*, 1993).

This observed diversity can be phenotypic, which is the interaction of the genotype with the environment. In fact, all the short-day onion ecotypes grown in Niger are highly cross-pollinated and can be found in all the country's agro-ecological zones. This is in line with the results obtained by Currah (2002), who pointed out that short-day and open-pollinated onions can show great genetic diversity compared with long-day or intermediate onions, which are more localized.

Sampling for the homogeneity test does not allow us to conclude whether these five ecotypes are homogeneous or not. However, it is important to stress that all these ecotypes were collected in remote locations or areas where onion production is not overly developed.

CONCLUSION

The genetic analysis performed using molecular markers (SSRs) demonstrates a large inter variability in the onion varieties obtained from INRAN collection. An equivalent source of genetic diversity is also observed inside the main number of these varieties expertized.

The DNA genetic study realized on the onion batches allows to trace the raw material all along the supply chain from farms to the gates of the factory. This DNA traceability tool could be an advantage for Nestlé to trace any specific onion variety with a specific genetic background in relationship with a key technologic or organoleptic trait of interest.

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(FAO). Contributions in English, French and Spanish are considered, and if accepted, will be published in the original language, with a summary in the other two. Further notes on contributions are given on, 15.

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