Authentication of swine derived products by means of SNP and STR markers

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INTRODUCTION

The current DNA analysis technologies are a robust, relatively cheap and sensitive tools for genetic identification and product authentication easily automatized. Up to date, *STRs* (*Short Tandem Repeats*) or microsatellites are the most utilized genetic markers because they are highly informative, evenly distributed in genome (Bishop *et al.*, 1993; Baron *et al.*, 2002). On the other hand, *SNPs (Single Nucleotide Polymorphisms*) represent an innovative class of markers because they are abundant, genetically more stable and their score is unambiguous (Syvänen, 2001; Vignal *et al.*, 2002).

AIM

To evaluate *STR* and *SNP* markers to authenticate an individual mono-breed product.

MATERIAL AND METHODS

❖ **Genomic DNA extraction from:**

- blood of 160 individuals [Duroc (N=16), Landrace (N=17), Large White (N=18), Pietrain (N=13), Apulo-Calabrese (Calabrese) (N=48) and Casertana (N=48)];
- individual mono-breed products (fresh or dry cured 'fiocco' and ham) of 25 autochthonous pigs (23 'Casertana' and 2 'Calabrese') and 4 commercial pig hybrids; the latter were voluntarily used as controls.

❖ **Markers employed:**

- 18 STRs selected according to ISAG /FAO (2004) guidelines;
- 47 SNPs: selected by *in silico* analysis (http://www.ncbi.nlm.nih.gov).

❖ **Genotyping:**

- STRs
  - 4 multiplex-PCR whose protocols are available to the Authors;
  - separation by ABI PRISM 310 Genetic Analyzer;
  - allele size (bp) calling by GenMapper 4.0 software;
- SNPs genotyped in outsourcing at KBioscience (UK) by KASPUR system (http://kbioscience.co.uk).

❖ **Data elaboration by software:**

- *GeneClass* ver. 2.0 (http://www.ensam.inra.fr/URLB/geneclass/geneclass.html)
- *Structure* (pritch.bsd.uchicago.edu/software).

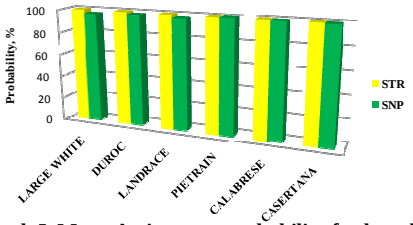
RESULTS

❖ **Dataset**

Results of assignment test (GeneClass ver.2.0) are showed in in the table I and graph I.

Table I. *STRs* and *STSs*. Number of typified subjects assigned and not assigned, distinctly for breed.

BREED	SUBJECTS, N						
	TYPIFIED	ASSIGNED				NOT ASSIGNED	
		P>99%		*P* <99%			
		STRs	SNPs	STRs	SNPs	STRs	SNPs
LARGE WHITE	18	18	12	0	3	0	3
DUROC	16	16	15	0	1	0	0
LANDRACE	17	17	15	0	2	0	0
PIETRAIN	13	11	9	1	0	1	4
CALABRESE	48	48	45	0	2	0	1
CASERTANA	48	47	45	0	2	1	1

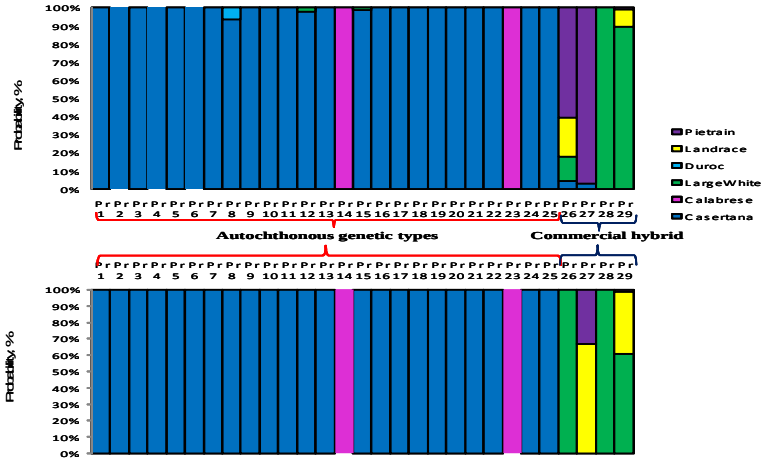


Graph I. Mean Assignment probability for breed by means of the two marker panel.

The two dataset were able to correctly assign to the breed of origin 98,7 % and 94,4% products using STRs and SNPs, respectively.

❖ **Authentication test: *'field experiment'* on 29 individual mono-breed products**

The results of assignment test carried out by GeneClass are showed in graph II *(a)* and *(b)* .



Graph II. *(a) STRs* and *(b) STSs*. Assignment probability (%) of each product to its breed of origin .

From graph II *(a)* and *(b)* it emerges:

- the 23 products declared as 'Casertana' were correctly assigned to their breed of origin with an average probability of 100% for *STRs* and 99.4% for *SNPs*;
- the two products declared as 'Calabrese' were correctly assigned to their breed of origin with an average probability of 100% for both *STRs* and *SNPs;*
- the assignment of products from commercial hybrids (pr. 26÷29) to one or more breeds was marker dependent.

These results were confirmed by *STRUCTURE* software, even if with a *mean assignment probability* equal to 90 % for *SNPs* and 83.5 for *STRs;* this difference was probably due to the different algorithm employed.

CONCLUSIONS

Within the limits of the observation field, our results show that the assignment of the products to the breed of origin seems to be promising and that both types of markers employed are potentially effective tools. Nevetheless, for their large scale application as diagnostic tool, it would be desirable to enlarge the size both of dataset and product sampling.

Aknowledgements: *dressed pork factory 'Di Maria' Cese Alte – Circello (BN);* **Fundings:** Ministry of Agricultural, Forestry and Food Policy (project acronym: SelMol); Campania Region

61th Annual Meeting of the European Association for Animal Production (EAAP), August 23rd- 27st 2010, Heraklion, CRETE ISLAND, GREECE