Introduction

Chemical components of seeds have an important impact on tree adaptation and future distribution of species in the perspectives of climatic changes. In this study, we try to address the historical, environmental, climatic and genetic causes of the variation of seed’s composition in Vitellaria paradoxa, a major species in African agroforestry systems. We present here results concerning the great variability observed in chemical trait variation, a phylogeny reconstruction phylogeography of the species and preliminary results concerning the genetic diversity patterns of SAD gene (a key gene responsible to stearic/oleic acid ratio) and VTE2-2 (strongly involved in tocopherol biosynthesis pathway).

Material and Methods

- **Material**: DNA was extracted from 110 trees belonging to 44 populations distributed on 12 countries. These 110 genotypes, 24 individuals have been used for gene sequencing (Figure 1).

- **Phenotype determination**: After a petrol-ether extraction of seed’s fat, Fatty Acid (FA) composition of seeds was determined by CPG analysis, and tocopherol content by HPLC.

- **Neutral diversity analysis and Biogeography phylogeography assessment**: 8 nuclear SSR markers were used to assess neutral diversity on the natural area. Several markers including 5 cpSSR and 2 chloroplasts sequences were used in order to propose a phylogenetic history address phylogeography of Vitellaria paradoxa.

- **Candidate genes diversity**: SAD gene diversity was assessed by cloning and sequencing Introns 2 of the gene, as Exons 7 to 9 of VTE2-2 (Homogenisate phytol transferase) was directly sequenced from PCR products.

Results & Discussion

- **Chemical Traits Variations**: A huge variation in seeds content was has been observed through the natural area (Figure 2). We expected a gradient variation for Oleic/Stearic acids and tocopherol content towards North to South axis in each country sampled (Senegal, Mali, Burkina, Ghana and Uganda).

- **Neutral diversity and Biogeography of Vitellaria paradoxa**: The study of 8 nuclear SSR markers revealed a very low FST (0.08) across the natural area, suggesting a huge gene flow and a weak genetic drift. Nucleotide polymorphism of TrnK and TrnQ from chloroplast genome revealed only 3 SNPs distributed on 2kb and did not allowed us to build a clear phylogeography of the species. The 5 cpSSR allowed us to test evolution history of the species as suggested by the chlorotype distribution (Figure 3 and 4). This result suggested at the Western part of the area, a large seed flow, and at the Eastern part, a strongly structured genetic diversity. This could result from a combined theory of glacial refuges, human practices in the western part, and geographical barriers at the Eastern part of the area.

- **Description of the nucleotide variability in the SAD genes**: We found 2 expressed SAD genes in Vitellaria paradoxa. This duplication has never been observed yet in plants. This discovery has been then a huge problem for genotyping, as the two copies are very closely related to each other. Only 8 SNPs and 2 Indels have been revealed between the 2 copies on 2kb comparison (Figure 5). One of the copies (SAD1) seems non-functional, as in silico translation lead to a truncated protein, due to a stop codon at the end of the first exon. Based on the polymorphism observed in Intron 2, SAD1 seems also to accumulate twice more mutations than SAD2. VTE2-2 (Figure 6) shows a weak nucleotide diversity, with only 5 SNPs detected on 52 haplotypes. A summary of nucleotide variation is shown Table 1.

- **Natural Selection Assessment**: We calculated Tajima’s D (D) for Intron 2 of the both SAD copies. Although these are non-coding sequences, we can notice that SAD1 exhibit non significant negative Tajima’S D, whereas SAD2 shows a largely significant D=-2.5142 (p<0.001) (Table 1) suggesting this sequence is under a purifying selection. However, these results must be considered with caution because negative Tajima’S D could also result from a demographic process, as population expansion. These results need to be completed by an analysis of diversity at a smaller scale level (a country), in order to limit demographic effects on the polymorphism observed.

Perspectives

- SNPs typing of the remaining Vitellaria paradoxa genotypes and smaller scale gene typing.
- Association studies: Chemical traits/Genotypes for North-South gradients.
- Neutral marker genotyping of new populations from Nigeria and Soudan to better assess phylogeography patterns.