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Isozyme polymorphism and organization of the agamic complex of the *Maximae* (*Panicum maximum* Jacq., *P. infestum* Anders, and *P. trichocladum* K. Schum.) in Tanzania

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Abstract The tribe *Maximae* (*Panicum maximum* Jacq., *P. infestum* Anders., *P. trichocladum* K. Schum.) includes two sympatric pools with different modes of reproduction and ploidy levels: an apomictic and tetraploid pool on the one hand, and a smaller, sexual and diploid pool on the other. From an analysis of isozyme polymor-

Introduction

The evolutionary potentialities of agamic plants have long been considered to be low owing to the absence of sexuality (Darlington 1939; Stebbins 1941; see Marshall

to the groups observed by Pernès (1975) from morphological traits (Assienan et al. 1993).

In the present paper, we compare, qualitatively and quantitatively, the isozyme polymorphism of Tanzanian sexuals and apomicts. We also study the typology and the geographical distribution of this polymorphism, as well as the relationship of *P. maximum* with *P. infestum*. The discussion addresses: (1) the origin of C types in relation to the known existence of gene flow; (2) the similarity and differences of polymorphism in the two pools; (3) the polymorphism and the frequency of rare alleles, and lastly (4) the heterozygosity of individuals in relation to their ploidy level.

Materials and methods

Plant material

Twenty diploid sexuals of *P. maximum* and 313 tetraploid apomicts were collected in Tanzania and in Kenya during two field trips in 1967 and 1969 (Combes 1975; Pernès 1975). These perennial clones were maintained as a living collection at the research station of Adiopodoumé (Côte-d'Ivoire).

Our sample includes all 94 clones from Tanzania. It covers the two geographical zones where the diploids were found and thus represents the heart of the centre of diversity. It comprises 20 sexual clones, 63

brown or red, corresponding to the decomposition of α-naphthyl acetate (αEST) or β-naphtyl acetate (βEST), respectively. In the diploid plants, the βEST bands are doublets. Nevertheless, some previous studies on apomicts showed the existence of single bands of βEST, particularly in *P. infestum*, as well as in the C types.

Several loci of the enzyme systems studied here were encountered in diploids. These are *Aat-1* (two alleles A1 and A2), *Mdh1* (two alleles A0 and A1) and *Mdh-2* (one allele B1) – these loci give a non-thermosensitive enzyme with heterodimers, *Mdh-3* (three alleles C1, C2 and C3), *Pgd-1* (one allele A1), *Pgd-2* (two alleles B0 and B1), *Pgd-3* (one allele C1), *Pgd-4* (one allele D1), *Gpi-1* (four alleles A1 to A4), *Pgm-1* (two alleles A1 and A2), *Est-1* (two alleles A0 and A1), *Est-2* (two alleles A0 and A1) and *Est-3* (two alleles B0 and B1). The last two esterase loci coded for the β-naphthyl esterases.

Notations

For the monmeric enzymes, all bands were noted. For the dimeric enzymes, only the bands corresponding to a homodimer were taken into account.

Each band is identified by a name (for example, AAT-A2), where the first part indicates the enzyme system (AAT). When inheritance was already known, the second part of the name specifies the locus (A) and the allele (2). These known loci are represented by the letters A, B, C and D. The letter X indicates an unknown inheritance (for the bands specific to the apomicts).

The bands were sorted into six classes, according to their frequency: always present bands ($f_j = 100\%$); very frequent bands ($100\% > f_j > 90\%$); frequent bands ($90\% > f_j > 60\%$); fairly frequent bands ($60\% > f_j > 40\%$); infrequent bands ($40\% > f_j > 10\%$); and lastly, absent or rare bands ($f_j < 10\%$).

The allelic structure of most polyploid clones (simplex, duplex

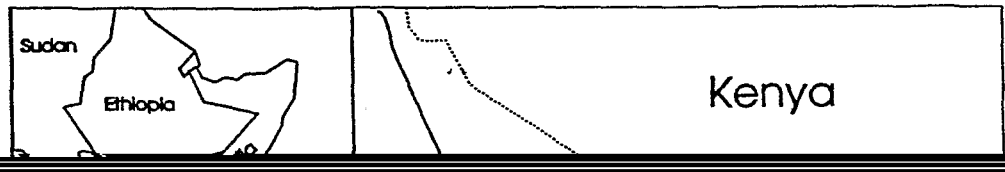

Fig. 1 Geographical distribution of clones. Clones of groups C, S and A are represented in *italic*, *bold* and *normal*, respectively. The three geographical zones (Korogwe-Vugiri, Kilosa-

Table 1 Band frequencies (1) in all studied geotypes, (2) in the three groups A, S and C, and (3) in the three regions Bagamoyo, Korogwe et Kilosa. For each band, Fisher's test combined with Ryan's correction allowed multiple comparisons of proportions. Indices (a, b, c for the types of plants, and m, n, o for the regions) indicate the result of comparisons, e.g. for MDH-C2, S is not different from A, while C is different from S and A

Band		Types of plants				Geographical distribution		
		All clones	S	C	A	Bagamoyo	Korogwe	Kilosa
AAT	A1	0.95	0.85^{a}	0.82^{a}	1.00^{a}	0.85^{m}	1.00^{m}	1.00^{m}
	A2	0.59	0.20^{a}	1.00^{c}	0.64^{b}	0.91^{m}	0.33^{n}	0.48^{n}
	X1	0.05	0.00^{a}	0.00^{a}	0.08^{a}	0.03^{m}	0.00^{m}	0.15^{m}
MDH	C1	1.00	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{m}	1.00^{m}	1.00^{m}
	C2	0.21	0.05^{a}	0.91^{b}	0.14^{a}	0.47^{m}	0.06^{n}	0.07^{n}
	C3	0.10	0.20^{b}	0.09^{a}	0.06^{a}	0.03^{m}	0.24^{n}	0.00^{m}
	B1	1.00	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{m}	1.00^{m}	1.00^{m}
	A1	0.66	0.25^{a}	1.00^{c}	0.70^{b}	0.88^{m}	0.58^{n}	0.40^{n}

that, in diploids, the three added loci were homozygous with null alleles. All intermediate assumptions may exist, but only some crosses at the tetraploid level using sexual colchiploidised plants as female (obligate apomixis) will be able to provide a definite answer.

Quantitative comparison of enzymatic diversity between sexuals and apomicts

The percentage of polymorphic loci in apomicts (groups

Genetic organisation in groups A, S and C

Comparison of groups for the heterozygosity of individuals

The band richness of individuals (NBI) reflects the level of heterozygosity in diploids and in tetraploids. For all individuals, NBI varied from 8 to 16. Nevertheless, some very highly significant differences existed between the group ($F_{2;90} = 11.00$). The C group, with 13.3 bands per individual (expected mean), contrasted significantly

having numerous isozymes introgressed from *P. infestum*. Sub-groups A2 and A3 differed from the the latter by the absence of the band MDH-C2, and the presence

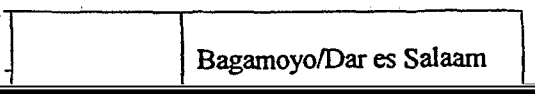

Table 2 Monomer frequencies in the three geographical zones. For each monomer, a χ^2 test was used to verify the frequency equality of the monomer in the three groups. When the test lead to rejection of the null hypothesis, a 2-by-2 comparison of groups was applied

Band		Frequencies			Test results			
		Bagamoyo	Korogwe	Kilosa	Global	S vs C	S vs A	C vs A
AAT	A1	0.85	1.00	1.00	**	N.S.	*	*
	A2	0.91	0.33	0.48	***	***	***	*
	X1	0.03	0.00	0.15	N.S.	–	–	–
MDH	C1	1.00	1.00	1.00	N.S.	–	–	–
	C2	0.47	0.06	0.07	***	***	N.S.	***
	C3	0.03	0.24	0.00	*	N.S.	N.S.	N.S.
	B1	1.00	1.00	1.00	N.S.	–	–	–
	A1	0.88	0.58	0.48	***	***	**	*
	X1	0.09	0.03	0.41	**	N.S.	**	N.S.
PGD	A1	1.00	1.00	1.00	N.S.	–	–	–
	B1	0.00	0.09	0.00	N.S.	–	–	–
	C1	1.00	1.00	1.00	N.S.	–	–	–
	D1	1.00	1.00	1.00	N.S.	–	–	–
GPI	A1	0.00	0.21	0.00	***	*	***	N.S.
	A2	0.47	0.33	0.59	***	N.S.	•	N.S.
	A3	1.00	0.94	1.00	*	N.S.	*	N.S.
	A4	0.00	0.03	0.00	N.S.	–	–	–
PGM	A1	0.12	0.33	0.04	***	N.S.	***	**
	A2	1.00	0.97	0.96	N.S.	–	–	–
	X1	0.09	0.00	0.00	**	•	N.S.	**
	X2	0.09	0.03	0.00	N.S.	–	–	–
β-EST	A1	0.77	0.79	0.56	•	N.S.	N.S.	N.S.
	B1	0.15	0.06	0.11	N.S.	–	–	–
	X1	0.03	0.03	0.00	N.S.	–	–	–
	X2	0.03	0.00	0.00	N.S.			
	X3	0.09	0.00	0.04	*	*	N.S.	*
	X4	0.21	0.00	0.00	***	**	N.S.	***
	X5	0.18	0.00	0.00	***	**	N.S.	**
α-EST	A1	0.27	0.33	0.26	N.S.	–	–	–

pattern can be considered as representative of the mean enzymatic pattern of all clones.

The enzymatic diversity of the Korogwe-Vugiri region was less marked, with 2.15 alleles per locus, Nevertheless, this estimator was still strongly influenced by rate alleles ($A' = 1.62$). The enzymatic polymorphism of sexuals was higher here than that of the sympatric apomicts, and was characterised by a higher frequency of bands MDH-C3, GPI-A1 and PGM-A1. The diversity of apomicts and that of sexuals overlapped only partially.

The third region, Kilosa-Morogoro, appeared as the lowest polymorphic zone. Except for one C type, this region included only apomictic genotypes, *P. maximum* s.s. The number of alleles per locus was lower ($A = 1.85$), but was less influenced by rare alleles ($A' = 1.62$). Only one band (MDH-X1) was more frequent.

The decreasing regional sorting, represented by Bagamoyo, Korogwe, and Kilosa, corresponded to "sexuality with C types", "sexuality without C types", and "non-sexuality with very rate C types". Note that without the rare alleles, the diversity in Korogwe and Kilosa became similar.

A highly significant regional effect, which was independent of the frequencies of types of plants (A, S or C), existed for NBI ($F_{2;90} = 5.99$). Once again, the Bagamoyo region (NBI = 12.36) contrasted with that of Kilosa (NBI = 11.15). This was due essentially to the existence in the first region of clones of *P. maximum* introgressed by *P. infestum* (sub-group A1). Note also the intermediate location of Korogwe (NBI = 11.90).

Only one trait was practically invariant between regions. This was the percentage of polymorphic loci, for which the slight superiority of the Korogwe region (%P = 0.692) over the regions of Bagamoyo and Kilosa (%P = 0.62) is, in fact, due only to the presence of a rare allele, B1, at the *Pgd-2* locus.

Discussion

Mutual gene flow between the two pools of the agamic complex of the *Maximae* has been previously emphasized (Savidan and Pernès 1982). The emergence of tetraploid sexuals in between-diploid crosses (about 0.5% of progenies), or after pollination of a diploid

sexual plant by an apomict, allows gene flow from the sexual pool to the apomictic pool (Savidan and Pernès 1982). Theoretically, these tetraploid sexuals disappear rapidly (Pernès 1975; Williams 1975) explaining their absence among collected accessions (Pernès 1975). Their

Inheritance studies of enzymatic traits in diploid sexuals (Assienan et al. 1993) showed the existence of segregation distortion for alleles C1, C2 and C3 of the *Mdh-3* locus (corresponding to the bands MDH-C1, MDH-C2, MDH-C3, respectively). In particular,

1987), as well as in other allogamous and anemophilous plants (Hamrick and Godt 1989).

The presence of gene flow explains the similarities between sexual and apomictic pools (see discussion

References

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