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Species relationship in the *Pennisetum* gene pool: enzyme polymorphism

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Summary. Variation in leaf esterases (EST), 6-phosphogluconate dehydrogenase (PGD), shikimate dehydrogenase (SKDH), leucine aminopeptidase (AMP), phosphoglucomutase (PGM) and malate dehydrogenase (MDH) is reported in the *Pennisetum* gene pool. In the primary gene pool, polymorphism for EST, AMP, SKDH was very high, as compared to the near-monomorphic isozymes of PGD. Two loci controlling leaf esterases *Est-1* and *Est-2*, were identified in the primary gene pool. Differences in allelic frequency distribution of the polymorphic *Est-1* locus occur between the cultivated and wild pearl millet. The prevalent alleles of *Est-1* are absent in *P. purpureum* Schumach (secondary gene pool). A monomorphic band of the α-esterase-specific *Est-2* locus was identified in most of the secondary gene pool accessions, *P. squamulatum* Fresen and an accession of *P. pedicellatum*. SKDH and EST revealed differences between most of the tertiary gene pool species. By contrast, a PGD zymogram was prevalent in several species of different sectional taxa. Gene duplication for PGD isozymes occurs in the diploid species, *P. ramosum*, of the tertiary gene pool. Heterodimers of PGD and EST were observed in the hybrid between pearl millet and *P. squamulatum*, whereas a monomeric structure characterized SKDH and AMP.

Key words: *Pennisetum* – Gene pool – Isozyme variation – Esterases – Sectional taxa

Introduction

The hybridization of pearl millet [*Pennisetum glaucum* (L.) R. Br.] with a number of *Pennisetum* species reveals varying levels of species relationships and provides a basis for gene pool classification. A polymorphic group of wild annual diploid relatives of pearl millet previously recognized as distinct species (Clayton 1972) has been shown to posses similar karyotypes (Jauhar 1981) and hybridize readily with pearl millet to produce highly fertile hybrids, which together constitute the primary gene pool (Brunken 1977; Harlan 1975). In triploid hybrids between pearl millet (AA genome) and *P. purpureum* Schumach (A′A′BB genomes), the prevalence of seven bivalents (AA′) and seven univalents (B) has led to the suggestion of a high genomic affinity between the species, as well as establishing *P. purpureum* as the secondary gene pool of pearl millet (Harlan 1975, Jauhar 1981). Varying levels of partial homology between hybrids of pearl millet and other *Pennisetum* species such as *P. setaceum* (Forsk.) Chiov., *P. squamulatum* Fresen., *P. schweinfurthii* Pilger and *P. orientale* L. C. Rich. have been reported (Dujardin and Hanna 1984; Hanna 1979; Hanna and Dujardin 1986; Jauhar 1981; Patil and Singh 1964). The confounding effects of autosyndesis in some of these hybrids makes it difficult to establish precise homoeology within the tertiary gene pool species.

Phylogenetic studies using chloroplast DNA showed highly conserved sequences in the inverted repeat regions of the chloroplast genome in pearl millet and the tertiary gene pool species, but not in restriction sites of single-copy regions (Clegg et al. 1984). Studies of mitochondrial DNA revealed considerable differences between pearl millet and seven *Pennisetum* species in their restriction endonuclease fragments and hybridization patterns (Chowdhury and Smith 1988). Hybridization fragments (probed with six mitochondrial gene sequences) of *P. purpureum* (secondary gene pool) were similar to *P. squamulatum* (tertiary gene pool). Both species were more closely related to pearl millet than the other tertiary gene pool species.

The use of isozyme analysis for genetic and evolutionary studies of plants has been well documented (Crawford 1983). In the genus *Pennisetum*, a considerable number of isozyme studies have been reported in pearl millet, but comparatively few accessions of ssp. *monodii* (Maire) Brunken in the primary gene pool have been studied (Banuett-Bourrillon and Hauge 1979; Tostain 1985; Tostain et al. 1987). Studies on other *Pennisetum* species are also limited. In this paper, we report on the level of polymorphism associated with esterases (EST), 6-phosphogluconate dehydrogenase (PGD), shikimate dehydrogenase (SKDH), leucine aminopeptidase (AMP), phosphoglucomutase (PGM) and malate dehydrogenase (MDH) in the *Pennisetum* gene pools. On the basis of the variation patterns observed in the primary gene pool, comparisons of the degree of species relatedness were explored in the secondary and tertiary gene pools.

Materials and methods

The plant material consisted of 4 and 17 pearl millet inbreds and landraces, respectively. Other species studied in the *Pennisetum* gene pool are as follows: (i) primary gene pool – 44 accessions of *P. glaucum* spp. *monodii* and *stenostachyum* collected from the Sahel region of Africa; (ii) secondary gene pool – 15 accessions of *P. purpureum*; (iii) tertiary gene pool – *P. ramson* (Hochst.) Schweinf., *P. massaicum* Stapf, *P. villosum* R. Brown ex Fresen., *P. setaceum*, *P. squamulatum*, *P. schweinfurthii*, *P. polystachion* (L.) Schult, *P. pedicellatum* Trin, *P. orientale*, *P. nervosum* (Hers) Trin., *P. flaccidum* Griseb, *P. alopecuroides*, *P. hohenackeri* Hochst. ex Steud. and *P. subangustum* (Schumach.) (Hanna 1987). With the exception of the last three species, at least two accessions were analyzed from each tertiary gene pool species. Intergenomic effects of enzyme expression were studied using interspecific hybrids of pearl millet × *P. squamulatum* and pearl millet × *P. purpureum*.

Leaf tissue for enzyme analysis was taken from seedlings or the furled leaf of axillary shoots from adult plants grown under greenhouse and field conditions. Leaf tissue was homogenized in a buffer (200 mg/600 µl) consisting of 0.2 *M* TRIS-HCL, 20% sucrose and 2% 2-mercaptoethanol, pH 6.8, in an iced mortar.

Homogenates were centrifuged at 16,000 × g for 15 min at 4 °C. The supernatant was then frozen until it was ready to be analyzed.

Enzymes were separated on a vertical polyacrylamide gel slab (175 × 162 × 0.75 mm) with an upper and lower tank buffer of 0.25 *M* TRIS plus 0.19 *M* glycine and 0.25 *M* TRIS, pH 8.5, respectively. The resolving gel was 7.5% acrylamide, 0.2% methylene (bis) acrylamide and 0.375 *M* TRIS, pH 8.8; the stacking gel was 5.3% acrylamide, 0.14% bis and 0.138 *M* TRIS, pH 6.8. Electrophoresis was conducted at 15 mA/gel at 2°–4 °C until the bromophenol blue tracking dye reached 0.5 cm from the gel bottom. Gels were stained for AMP, SKDH, PGD, PGM and MDH, according to the method described by Vallejos (1983). Esterase activity was detected using either 2 ml of 1% α-naphthyl acetate (in 60% acetone) or a mixture of 1% α- and β-naphthyl acetate in 100 ml of 70 m*M* phosphate buffer, pH 7.0, containing 40 mg fast blue RR salt. The relative mobility (RF) of the enzyme bands was calculated with respect to the migrating front of the bromophenol blue tracking dye.

Results and discussion

Esterases

Primary gene pool. Esterases (EST) were extensively studied because of the very good resolution obtained and because of the interspecific differences (Fig. 1 A) revealed in the entire *Pennisetum* gene pool when compared with the other enzymes. Esterase bands with specificity for the α-naphthyl acetate substrate were predominantly in the cathodal migrating zone. Most of the β-esterases were in the anodal migrating zone where polymorphism within species, especially the primary gene pool, was observed (Fig. 1 A and B). An α-esterase band, Rf 0.62 (Fig. 1), was identified in the entire primary gene pool in addition to two highly frequent α-esterase bands (Rfs 0.66 and 0.71). In comparing the leaf tissues of seedlings and axilary shoots, no differences were observed in the β-esterases and the monomorphic α-esterase band (Rf 0.62).

Although the Rf 0.71 band overlapped with the polymorphic β-esterases, two migrating zones could be established: the anodal β-esterases and cathodal α-esterases, designated as *Est-1* and *Est-2* (Fig. 1 B), respectively, in the primary gene pool. A similar zonation has been reported in grain esterases of pearl millet cultivars from West Africa and are known to be controlled by two loci, *Est-A* and *B* (Tostain and Riandey 1984), which correspond to the leaf *Est-1* and *Est-2* patterns observed in this study. In the leaf *Est-1*, seven single-banded variants – *Est-1a*, *-1b*, *-1c*, *-1d*, *-1e*, *-1f* and *-1g* – considered to be alleles of this locus, were identified (Fig. 1 B). In addition, various combinations of these alleles with three-banded *Est-1* phenotypes (Fig. 1 B) expected of dimeric enzymes occurred in heterozygous plants. The heterogeneity of each accession in the pearl millet landraces and the primary gene pool was evident from the non-occurrence of completely homozygous alleles in randomly analyzed plants within an accession. The proportion of homozygous to heterozygous *Est-1* phenotypes was much higher in the wild and weedy relatives than in the pearl millet landraces.

Analysis of the *Est-1* locus from field-grown plants taken from representative accessions of the primary gene pool from Senegal, Mali and Niger showed an allelic frequency distribution that differed from those of the pearl millet landraces (Table 1).

Table 1. Frequency (%) of *Est-1* homozygous phenotypes in the primary gene pool from Senegal, Mali and Niger

	Est-1a	*-1b*	*-1c*	*-1d*	*-1e*	*-1f*	*-1g*
Wild pearl millet	0.0	10.9	7.8	50.0	3.1	18.8	9.4
Landrace	0.0	53.3	13.3	10.0	0.0	16.7	6.7

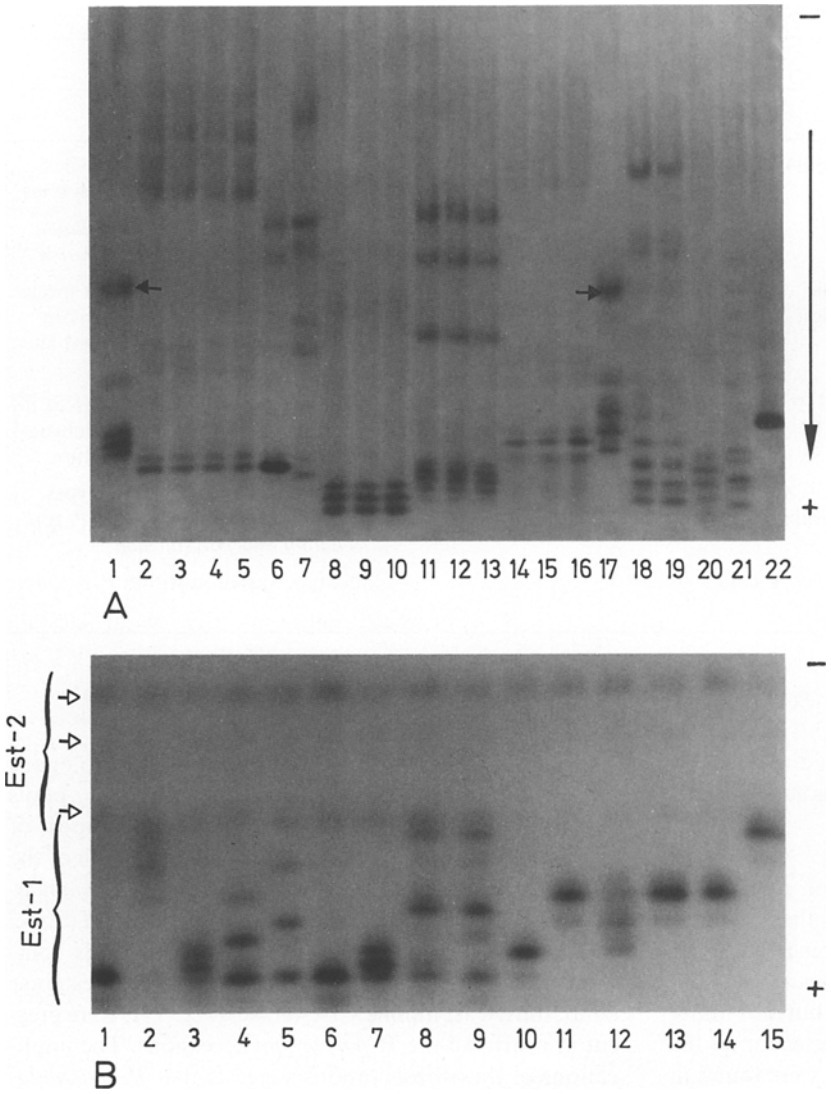

Fig. 1. A Leaf esterase zymograms of *Pennisetum* species. The *vertical arrow* indicates the direction of migration. *Lanes 1* and *17*, *P. glaucum*; *2–5*, *P. massaicum*; *6*, *P. ramosum*; *7*, *P. alopecuroides*; *8–10*, *P. divisum*; *11–13*, *P. villosum*; *14–16*, *P. nervosum*; *18–19*, *P. squamulatum*; *20–21*, *P. polystachion*; *22*, *P. hohenackeri*. The *horizontal arrow* (*lanes 1* and *17*) point to an α-esterase band (Rf 0.62) common to the primary gene pool. **B** Polymorphic β-esterases of *Est-1* and the near-monomorphic α-esterase of *Est-2* of the primary gene pool. The *arrow* in 0.66 and 0.71 in order of increaseing anodal migration. Homozygous alleles at *Est-1* are shown in *lanes 1* and *6-1g*; *lane 10-1f*; *lanes 11, 13, 14-1d*; *lane 15-1b*. The other lanes depict various heterozygous combinations of *Est-1*

The *Est-1 d* allele, which accounted for half of the total homozygous allelic variation, accounted for only 10% of the variation in the landraces. The opposite trend in variation was observed with the *Est-1 b* allele (Table 1). This change in gene frequency between the wild and pearl millet landraces may implicate selection for the *Est-1 b* phenotype as a trait associated with domestication. However, it appears unlikely since linkage studies, principal component and discriminant analysis (Tostain 1985; Tostain et al. 1987) of several enzymes in the primary gene pool have been reported not to show any associations of plant characters such as height and maturity with esterases. The *Est-1 a* phenotype, which was absent from accessions in Table 1, occurred in both pearl millet landraces and ssp. *monodii* accessions from Mauritania and Chad.

Secondary gene pool. Of the 15 accessions analyzed in the secondary gene pool, 13 possessed a single-banded anodal β-esterase, Rf 0.88, while the other two were characterized by different three-banded phenotypes. All three variant forms of *P. purpureum* occur outside the mobility range of the homozygous *Est-1* alleles of the primary gene pool. However, a rare variant heterozygous with *Est-1 f* was identified in one plant from an accession of the ssp. *monodii* (from Mali), which possessed equivalent mobility to the Rf 0.88 band of *P. purpureum*. Given the tetraploid level of *P. purpureum*, it appears the added B genome has had very little effect in increasing the number of anodal β-esterases. It may be inferred from these observations that either a rare *Est-1* phenotype from the primary gene pool (A genome) was involved in the natural synthesis of the allotetraploid, *P. purpureum*

Table 2. Summary of some of the major features of isozyme variation in the *Pennisetum* gene pool

Enzyme	Gene pool		
	Primary	Secondary	Tertiary
Est-1	7 alleles (Rf range, 0.70–0.84)	3 variants; frequent phenotype, Rf=0.88	Distinct interspecific phenotypes; polymorphism within some species
Est-2 Rf=0.62 Rf=0.66	Monomorphic very frequent	Monomorphic very frequent	Monomorphic in *P. squamulatum* monomorphic in *P. squamulatum*
PGD	PGDb and c-monomorphic PGDa – unstable expression	Monomorphic for PGDa b, a/b[a], b/c[a]	PGDb prevalent in nearly all species. PGDa, b, a/b[a], monomorphic in *P. ramosum* (gene duplication) and several polyploid species
SKDG	4 alleles; SKDHa (Rf 0.43, 0.45) b (0.45, 0.48), c (0.48, 0.52) d (0.45, 0.52)	Monomorphic for SKDHa	One monomorphic band present in species of sections Heterostachya, Brevivalvula and Eupennisetum
AMP	8 bands (Rf 0.52, 0.54, 0.56, 0.57, 0.59, 0.61, 0.63, 0.65). Variability with accessions	Monomorphic bands (Rf 0.54, 0.56, 0.61)	Distinct interspecific phenotypes. Highly polymorphic within *P. pedicellatum* and *polystachion*
PGM	PGMc and d frequent alleles in land races		Ecotype variation within *P. orientale*

[a] Bands of intermediate mobility

(A'A'BB) or, subsequent to amphidiploidization, the *Est-1* locus derived from the A genome has substantially been modified.

Tertiary gene pool. In the tertiary gene pool, most of the species possessed more anodal β-esterases than those of the *Est-1* mobility zone of the primary gene pool. The exceptions were *P. nervosum, P. hohenackeri* and *P. alopecuroides* accessions from China, but not those from South Africa. Differences within species for all the esterase isozymes of the tertiary gene pool were found in *P. setaceum, P. pedicellatum, P. polystachion* (Fig. 1) and *P. alopecuroides*. A varying degree of species relationships was observed in the sectional taxa. *P. ramosum* and *P. massaicum* of the section Eupennisetum (Stapf and Hubbard 1934) possessed anodal β-esterase isozymes of equivalent mobility but of different intensity, and were further differentiated by their cathodic esterases (Fig. 1).

Two of the four most anodal β-esterase bands of *P. squamulatum* (Fig. 1 A) which corresponded to the single-banded isozyme of Rf 0.88 and 0.91 associated with *P. schweinfurthii* and *P. orientale,* respectively, were observed in all accessions of each species. These three species belong to the section Heterostachya. Despite the differences for the β-esterase within *P. polystachion, P. pedicellatum* and *P. subangustum* (all of the Brevivalvula section), they were characterized by the presence of two α-esterase bands with high activity in seedlings.

The *Est-2* locus of α-esterase which includes the monomorphic band, Rf 0.62 (Fig. 1), and the prevalent band, Rf 0.66, was used to examine possible homologous regions in the secondary and tertiary gene pools (Table 2). One accession of *P. pedicellatum* and most of the secondary gene pool accessions possessed both of the α-esterases of *Est-2*. All five accessions of *P. squamulatum* analyzed had the monomorphic band of *Est-2* (Table 2). In addition, two other cathodal bands common to *P. squamulatum* (the least mobile – β-esterase band; third least mobile – α-esterase) (Fig. 1 A) were present in nearly all the *P. purpureum* accessions. The implications of these observations suggests that *P. squamulatum* shares genomic regions partially homologous to both *P. purpureum* and the primary gene pool. Further support for this observation has been derived from the cytogenetic study of single and double cross hybrids involving all three species (Dujardin and Hann 1984) and mitochondrial DNA hybridization patterns. The leaf esterase patterns of interspecific hybrids, pearl millet × *P. squamulatum* and pearl millet × *P. purpureum* revealed a heterodimeric band between the prevalent β-esterase, Rf 0.88, of *P. purpureum* and the *Est-1* locus in the latter hybrid; it was apparent that only one of the four most anodal β-esterase of *P. squamulatum* (Rf 0.85) formed a heterodimeric band with the *Est-1* locus of the primary gene pool.

6-Phosphogluconate dehydrogenase (PGD)

The isozymes of PGD were well resolved in most of the *Pennisetum* species. Leaf PGD from seedlings of all primary gene pool accessions from the Sahel, pearl millet

inbreds and landraces were monomorphic for two bands designated as PGDb and c (Fig. 2A). Variability caused by the presence or absence of a more cathodal band PGDa (Fig. 2A), was observed in the wild and cultivated pearl millets when the leaf tissue was taken from axillary shoots. A rare variant found in a single plant from an accession of ssp. *monodii* (from Senegal) possessed two additional anodal bands (Fig. 2A). Further genetic analysis of large populations (particularly from Senegal) is required to verify whether PGDa, b and c, are products of different loci or alleles. Tostain et al. (1987) have reported the presence of three loci controlling PGD in wild and cultivated pearl millet. Gene duplication in two of these loci and the presence of interlocus heterodimers were also observed.

In the secondary gene pool, at least five bands were common to all the accessions which included PGDa and b, intermediate bands between PGDb and c, and PGDa and b, plus a more anodal band than PGDa (Fig. 2B). Almost all tertiary gene pool species possessed the PGDb band. A three-banded PGD phenotype characterized by PGDa and PGDb and a band of intermediate mobility between them occurred in accessions of *P. polystachion* (Fig. 2B), *P. subangustum, P. pedicellatum, P. ramosum, P. hohenackeri* and *P. villosum*. In accessions where the enzyme activity (dosage) of this three-banded PGD phenotype depicted a heterozygous state expected of a dimeric enzyme, it was more evident in *P. ramosum* and to a lesser extent in *P. polystachion* and *P. pedicellatum*.

Analysis of three *P. ramosum* populations from Chad and two accessions from East Africa did not reveal any single-banded isozyme expected for a homozygous locus, but were consistently of the three-banded phenotype. This suggests that a case of fixed heterozygosity arising from a duplicated PGD locus occurs in *P. ramosum*, a diploid species. This phenomenon has been widely reported for a number of enzyme systems in diploid plants (Gottlieb 1982; Pascual et al. 1988).

A single-banded PGD phenotype of similar mobility to PGDb was observed in all the *P. squamulatum* accessions (Fig. 2B). The interspecific hybrid of pearl millet × *P. squamulatum* revealed the formation of heterodimeric bands between PGDb of *P. squamulatum* and the PGDa and c bands derived from the pearl millet parent (Fig. 2B).

However, the variable nature of PGDa (which appears to be developmentally regulated) became evident when other samples of the interspecific hybrid showed only the heterozygous state of a dimeric enzyme between PGDb and c.

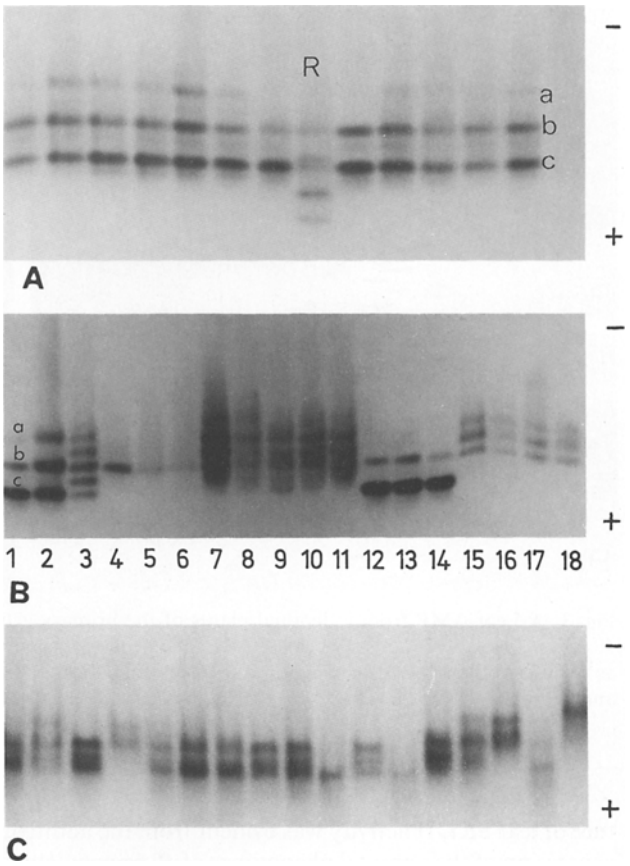

Fig. 2. **A** Leaf PGD isozyme patterns in the primary gene pool of pearl millet showing the monomorphic b and c bands and the variable PGDa band. R is the rare variant found in a *monodii* accession. **B** Leaf PGD isozymes of the primary gene pool (*lanes 1, 12, 13, 14*), *P. squamulatum* (*4–6*), *P. purpureum* (*7–11*), *P. polystachion* (*15–17*), *P. subangustum* (*18*) and a hybrid of *P. squamulatum* and pearl millet (*lane 3*) showing heterodimers. *Lane 2* is a backcross derivative of (*P. squamulatum* × pearl millet) × pearl millet (parent, *lane 1*). **C** Leaf SKDH isozyme patterns of the primary gene pool of pearl millet

Shikimate dehydrogenase (SKDH)

Five bands of SKDH activity were resolved in the primary gene pool. Combinations of either two or three bands were observed in individual plants (Fig. 2C). The two-banded phenotypes were designated as SKDHa, b, c and d (Table 2). The three-banded phenotypes were SKDHa plus the most cathodal band, combinations of SKDHa and b and SKDHb and d. Among the two-banded phenotypes, SKDHa and b phenotypes were the most common isozymes, whereas SKDHc occurred in only one plant of a ssp. *monodii* accession.

All inbreds were characterized by two-banded phenotypes and, hence, were considered to be the common homozygous state for leaf SKDH activity in the primary gene pool. The three-banded phenotypes were heterozygous plants expected from monomeric enzymes, where a common band is present in both homozygous parental two-banded phenotypes. The monomeric struc-

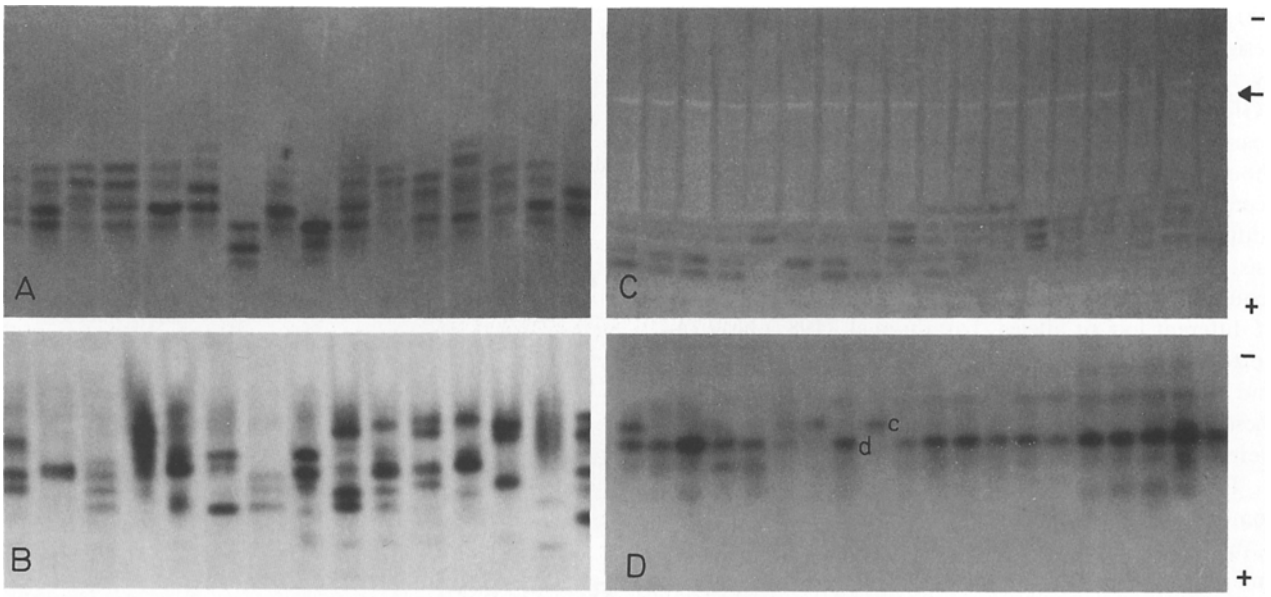

Fig. 3. **A** Leaf AMP from individual plants of the primary gene pool. **B** Polymorphic leaf AMP found within *P. pedicellatum* and *P. polystachion* accessions. **C** Leaf AMP of the primary gene pool showing the achromatic band (*arrowed*) using Fast Garnet GBC as the dilazonium substrate. **D** Leaf PGM isozyme patterns of the primary gene pool. The lettered bands refer to two alleles PGMc and PGMd

ture of leaf SKDH activity was evident from the additive expression of the parental phenotypes of *P. squamulatum* and pearl millet in the interspecific hybrid. At a higher pH of leaf enzyme extraction followed by separation on a starch gel system, the monomeric nature of SKDH was observed in hybrids of pearl millet; however, only single bands were observed with the parental phenotypes studied (Tostain and Riandey 1984).

In the secondary gene pool, bands corresponding to SKDHa were identified in all five accessions of *P. purpureum* studied for SKDH activity. Four of these accessions possessed a band of equivalent mobility to the common band of SKDHb and c (Table 2) of the primary gene pool and an additional anodic band. High SKDH activity in the more cathodal zone (relative to Rf 0.43 band) was prevalent in *P. purpureum*, but remained unresolved as discrete bands. The most anodal migrating zones of SKDH activity occurred in accessions of the primary gene pool, *P. purpureum*, *P. squamulatum* and *P. massaicum* compared to the other *Pennisetum* species. The most contrasting SKDH phenotypes within the sectional taxa of the tertiary gene pool were observed in species of the Gymnothrix section. Species belonging to each of the sections Heterostachya, Brevivalula and Eupennisetum had at least one SKDH band in common. *Pennisetum ramosum* possessed the most cathodal SKDH isozymes in the tertiary gene pool and had no overlapping zone of activity with *P. massaicum*; both species belong to the section Gymnothrix.

Leucine amino peptidase (AMP)

Eight bands of AMP activity were observed in the primary gene pool species (Table 2). They occurred in various combinations ranging from two- to five-banded phenotypes in individual plants within accessions from the entire Sahelian region (Fig. 3 A). Two-banded phenotypes were mainly composed of adjacent migrating bands. The most cathodal band (Rf 0.52) was prevalent in ssp. *monodii* accessions from Central Mauritania. The monomeric nature of leaf AMP was deduced from the additive pattern of parental bands observed in the interspecific hybrid of pearl millet × *P. squamulatum*.

Bands corresponding to Rf 0.54, 0.56 and 0.61 of the primary gene pool were present in all accessions of *P. purpureum* (Table 2). Differences between accessions were revealed by the presence or absence of the Rf 0.59 band. The zones of AMP activity were about the same for both the primary and secondary gene pools. The assay used for AMP (where fast Garnet GBC was the diazonium salt) showed very little to no activity in almost all the tertiary gene pool species. In a few species such as *P. ramosum*, *P. schweinfurthii* and *P. villosum*, where little activity was observed, clear differences existed between them, but were monomorphic for most of the bands. *P. ramosum* possessed bands of mobility equivalent to Rf 0.56 and 0.65 of the primary gene pool. Increased activity and a better resolution was obtained through the subsequent use of the diazonium salt Fast

Black K in the AMP assay for some of the tertiary gene pool species. In *P. pedicellatum* and *P. polystachion* (all within the section Brevivaluvula), a high degree of polymorphism was observed. Although some bands were common in some accessions, no two accessions possessed the same zymogram pattern (Fig. 3 B). In *P. orientale* and *P. squamulatum,* as many as five and eight bands were resolved, respectively.

A discrete achromatic cathodal band (Rf 0.38) against the orange-stained gel background characterized all pearl millet inbreds, landraces and primary gene pool species (particularly with Fast Garnet GBC in the assay) (Fig. 3 C). It is not clear whether this is an artifact of enzyme extraction and separation in the electrophoretic system used, since the achromatic band appears to be genetically controlled. It is absent in the secondary and tertiary gene pool, the F_1 interspecific hybrid of pearl millet × *P. squamulatum* and suppressed in backcrosses to pearl millet, thus implicating the existence of an intergenomic suppression mechanism for the achromatic band.

Phosphoglucomutase (PGM) and malate dehydrogenase (MDH)

Consistent PGM activity was mainly observed in the primary gene pool species using the system employed in the present study. Six bands of PGM activity were resolved, of which the two central migrating bands (RF 0.65 and 0.68) were usually of much higher activity compared to the two relatively anodal (Rf 0.71, 0.75) and two cathodal migrating bands (Rf 0.59, 0.62) (Fig. 3 D). The PGM patterns observed ranged from single-banded phenotypes designated as PGMc and d, the presence of both bands and three- to five-banded phenotypes in individual plants (Fig. 3 D). PGMc and d were of frequent occurrence in the landraces, whereas PGMd was prevalent in the ssp. *monodii* (particularly those from Senegal). The absence of hybrid bands between PGMc and d is in accordance with the monomeric structure of this enzyme reported in other plants (Goodman and Stuber 1983) and the *Pgm A* locus in cultivated pearl millet controlling single-banded alleles (Tostain 1985).

Two species of the tertiary gene pool, *P. orientale* and *P. villosum,* with consistent PGM activity (but different from the primary gene pool) were characterized by two- and three-banded phenotypes, respectively. However, the relative mobilities of *P. orientale* revealed differences between the Australian and Pakistani accessions, whereas the South African and Israeli *P. villosum* accessions were the same.

Regions of high MDH activity were observed in the polyacrylamide gels for all *Pennisetum* species, but were poorly resolved into discrete bands. However, species-specific zymograms were evident in *P. schweinfurthii, P. nervosum, P. ramosum, P. massaicum, P. villosum, P. divisum, P. polystachion* and *P. pedicellatum.* Within the sectional taxa, species of the Brevivalvula section were identical in their MDH patterns in contrast to the Heterostachya and, to a lesser extent, species of the Eupennisetum and Gymnothrix sections.

Isozyme analysis of the *Pennisetum* gene pool reveals highly polymorphic zymograms of EST, SKDH, AMP and MDH. However, PGD appears to be highly conserved in most species. A study of 8 wild and 74 cultivated samples (from West Africa) of the primary gene pool revealed three distinct groups – wild, early and late maturing cultivars – on the basis of eight enzymes (Tostain et al. 1987). In addition, enzyme diversity was higher in the early maturing cultivars than in the wild millets. In this study, highly variable isozyme phenotypes were observed, using wild millet samples from Mauritania, Mali, Senegal, Niger and Chad; the wild millet analyzed by Tostain et al. (1987) was restricted to Mali, Niger and Burkina Faso. The distinct isozyme phenotypes of some of the tertiary gene pool species can be explored for possible linkage with desirable traits to be introgressed into pearl millet. The results of this study epitomize how the choice of an enzyme system may lead to variable deductions on phyletic relationships of species in the *Pennisetum* gene pool. For example, species of the Brevivalvula section are shown to be closely related on the basis of monomorphic PGD zymograms and α-esterases but seem highly divergent based on AMP.

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