Development of SSR markers for diversity studies on Yam (Dioscorea) genetic resources

Yam (*Dioscorea* spp.) is a tuber crop widely cultivated in Africa, Asia, and South America. This traditional staple crop is very important for regional food security and income generation especially in West Africa (Fig. 1). However, yam breeding is constrained by the crop's inherent attributes, including a long growth cycle, being inconsistent or non-flowering, polyploidy, and high heterozygosity. The use of advanced genetic tools and genomic resources in this crop has remained slow and difficult mainly because very little is known about yam genetics. Moreover, the available yam genetic resources are poorly characterized, limiting the utility of the existing diversity in yam improvement programs. In this study, effective Simple Sequence Repeat markers (SSRs) were developed to demonstrate their effectiveness in estimating genetic diversity and in understanding the phylogenetic relationships of the genetic resources. The results of the study will serve as guide for implementing their use in yam breeding for the region.

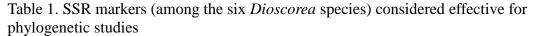
A total of 90 SSRs were developed from an enriched genomic library of yellow Guinea yam (*D. cayenensis*). The number of SSRs developed in this study exceeds the total number of SSRs developed in the previous studies (67 SSRs in the seven reports). Cross-amplification revealed that 85 (94.4%) and 51 (56.7%) of these SSRs could be successfully transferred to the other two major cultivated species of *D. rotundata* and *D. alata*, respectively. A total of 30 markers were selected based on stable amplification in six important species, i.e., *D. cayanensis*, *D. rotundata*, *D. alata*, *D. dumetorum*, *D. esculenta* and *D. bulbifera*, for the analysis of phylogenetic relationships among *Dioscorea* species (Table 1). Among these 30, the six marked with * were considered to be useful marker sets for phylogenetic studies for their polymorphisms and high level of transferability over five species. Clustering based on these six SSRs among *D. cayenensis*, *D. rotundata*, *D. alata*, and *D. bulbifera* were clearly separated, and *D. rotundata* and *D. cayenensis*, were closely related to each other (Fig. 2).

These developed SSR markers can be used as handy markers not only for genetic diversity analysis but also for cultivar identification, gene/QTL mapping, and authentication of progenies obtained from genetic crosses particularly for the modestly equipped national agricultural research systems located in major yam growing regions.

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Fig. 1. Yam germplasm field (left) and tubers sold in the market (right)



Marker	D. cayenensis	D. rotundata	D. alata	D. dumetorum	D. esculenta	D. bulbifera
YM002	++	++	++	-	-	+
YM003	++	++	<mark>++</mark>	-	-	-
YM005	++	++	++	-	-	-
YM006	++	++	-	-	-	-
YM009	++	++	++	-	-	-
YM010	++	++	++	-	-	-
YM011	++	++	-	-	+	-
YM012	++	++	++	-	_	-
YM013*	++	++	++	++	+	<mark>++</mark>
YM021	++	++	++	-	-	+
YM023	++	++	<mark>++</mark>	-	-	-
YM024	++	++	<mark>++</mark>	++	-	-
YM032	++	++	<mark>++</mark>	-	-	+
YM033	++	++	++	-	-	=
YM036	++	++	++	-	-	-
YM037	++	++	++	-	-	-
YM044	++	++	++	-	-	-
YM045	++	++	<mark>++</mark>	-	+	-
YM053*	++	++	<mark>++</mark>	+	+	+
YM055*	++	++	<mark>++</mark>	++	+	=
YM065	++	++	<mark>++</mark>	+	-	-
YM066*	++	++	<mark>++</mark>	++	-	+
YM071	++	++	<mark>++</mark>	++	-	-
YM074*	++	++	<mark>++</mark>	+	+	+
YM075	++	++	<mark>++</mark>	=	-	=
YM078	++	<mark>++</mark>	<mark>++</mark>	-	+	-
YM080*	++	++	++	++	+	++
YM084	++	++	-	-	_	-
YM087	++	++	++	-	-	-
YM089	++	++	++	-	-	-

++: amplified (polymorphic), +: amplified (monomorphic), -: no amplification

Symbols highlighted in yellow: showed species-specific polymorphisms *: Markers showed amplification in > 5 species

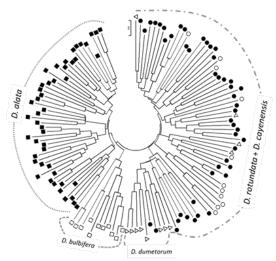


Fig. 2. Dendrogram of yam genetic resources generated based on the genetic distances of six cross-amplified SSR markers. \blacksquare : *D. alata*, \Box : *D. bulbifera*, $\textcircled{\bullet}$: *D. rotundata*, \bigcirc : *D. cayenensis*, \triangle : *D.* dumetorum