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Collection of wild rice (*Oryza* L.) in east and southern Africa in response to genetic erosion

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Introduction

Collecting germplasm is the first step in *ex situ* conservation and clearly an important prerequisite for the use of the material by breeders. The reasons for collecting germplasm include danger of genetic erosion or extinction, users at national and international level have expressed a clear need for the germplasm, the genetic diversity is missing or insufficiently represented in existing *ex situ* germplasm conservation, and that more needs to be known about it (Engels et al. 1995).

Floristic studies, plant inventories and herbarium specimens indicate that the east and southern Africa region is home to five wild species of *Oryza*. These are *Oryza barthii*, *O. brachyantha*, *O. eichingeri*, *O. longistaminata* and *O. punctata* (Fernandes et al. 1971; FAO 1987; Vaughan 1989, 1994; Leistner 1990). Clayton (1968, 1970) clarified the species names for the African wild relatives of cultivated rice by distinguishing *O. barthii* as the annual relative of *O. glaberrima* and *O. longistaminata* as the rhizomatous perennial relative. Studies on the origin and evolution of twin microsatellites in the genus *Oryza* have now clearly revealed that *O. barthii* is the ancestor of the cultivated African rice, *O. glaberrima* (Akagi et al. 1998), primarily found in west Africa but has now spread to parts of east Africa, Zanzibar in particular (Vaughan 1994; WARDA 1997). Geographically, the species are widely distributed in different countries of this region. The highest inter-specific diversity is found in Zambia and Tanzania, in each of which at least four out of the five species occur (Clayton 1968, 1970; Fernandes et al. 1971; FAO 1987; Leistner 1990; Vaughan 1994; Phillips 1995).

The wild relatives of rice are an important source of agronomically useful traits that have been extensively used in rice improvement programmes (IRRI 1970; Khush 1977; Lin and Yuan, 1980; Agueirro et al. 1984; Heinrichs et al. 1985; Jena and Khush 1990; Khush et al. 1990; Brar et al. 1991; Amante et al. 1992; Ishii et al. 1994; Multani et al. 1994; Brar and Khush 1995). In particular, there are reports of the invaluable contribution that the germplasm of the wild relatives of cultivated rice found in east and southern Africa has made in rice breeding programmes. For example, *O. longistaminata* A. Chev. et Roehr. has been reported to have genes for bacterial blight resistance, high pollen production, long stigmas and drought tolerance (Second et al. 1977; Taillebois 1983; Khush et al. 1990; WARDA 1997). Resistance to *Meloidogyne graminicola*, the nematode species causing significant damage to rice in west Africa, was found in an *O. longistaminata* accession originating from Botswana (Jones et al. 1996). Genes for

resistance to brown plant hopper and bacterial blight have been transferred from *O. brachyantha* A. Chev. et Roehr. to *O. sativa* L. (Khush et al. 1990). *Oryza brachyantha* also has traits for adaptation to lateritic soils, and resistance to yellow stemborer, leaf folder and whorl maggot (Khush 1977; Heinrichs et al. 1985; IRRI 1990; Brar and Khush 1995). *Oryza barthii* A. Chev. has resistance to green leaf hopper, sheath blight and bacterial blight, and shows characteristics for drought resistance (Henrichs et al. 1985; Amante et al. 1990). Ou (1985) has reported that genes for tolerance to yellow mottle occur in *O. eichingeri* A. Peter, while salt tolerance was found in *O. punctata* Kotschy ex Steud. by Farooq et al. (1994). The tetraploid race of this last species, reported in Tanzania, was found to have resistance to zigzag leaf hopper (Heinrichs et al. 1985).

Marshall and Brown (1975) and Brown and Marshall (1995) suggested that the objective of any germplasm sampling strategy is to include in the sample at least one copy of 95% of the alleles that occur in the target population at frequencies greater than 0.05, i.e. the so-called 'common' alleles. However, Allard (1970) recognized that collectors might have little time and resources at their disposal. Therefore, the problem is to define a sampling procedure that yields the maximum amount of useful genetic variation within a specified and limited number of samples (Marshall and Brown 1983).

The diversity of the wild rice species is gradually being eroded for a multiplicity of reasons. These include destruction of natural wild rice habitats to pave the way for expanding agricultural activities resulting from increasing population pressure, overgrazing and changes in land use (Reid et al. 1988; NWCMP 1996; Emerton and Muramira 1999). The need to assess the socio-economic activities that are causing genetic erosion, as well as collect and conserve this diversity, is therefore high.

In the past there have been some collecting missions in Kenya, Tanzania, Madagascar and Zambia by D. Vaughan of the International Rice Research Institute (IRRI) and others, as reported in Bezançon and Second (1979), Miezan and Second (1979) and Katayama (1987, 1990). However both the species' and geographical coverage were narrow. Consequently, the wild rice gene pool of the region was poorly represented in gene bank collections. According to Vaughan et al. (1991) and Vaughan (1994), prior to the collection missions reported in this paper, only 52 accessions of the wild rice relatives from the region were conserved in the International Rice Genebank at IRRI. This represented only 2.75% of the total world wild rice collection and 21% of the total wild rice accessions from Africa, despite their widespread distribution and occurrence in the continent. Moreover, before the collection missions reported in this paper, there were no wild rice germplasm collections conserved in the National Plant Genetic Resources Centres of the countries that participated in the work because, at the time of earlier collections, these countries didn't have appropriate conservation facilities. This made collection and conservation of the germplasm urgent in order to expediently respond to the erosion pressures facing the wild rice species.

This paper reports some of the indicators of genetic erosion of wild rice diversity, and the systematic collection and conservation of this germplasm in nine countries of the east and southern Africa region. The collecting missions were undertaken under the auspices of a

collaborative programme involving the national PGR programmes of the region, the IRRI, SADC Plant Genetic Resources Centre (SPGRC), and the International Plant Genetic Resources Centre (IPGRI). The collaborators from the national programmes were: Joseph Kemei (Kenya National Gene Bank); Charles Mhazo and A. Mafa (Department of Research and Seed Services, Zimbabwe); Paulino Munisse (Instituto du Investigacao Agraria, Mozambique); John Wasswa (National Agricultural Research Organization of Uganda; Edwin Chiwona(Chitedze Agricultural Research Station, Malawi); M. Nawa (National Plant Genetic Resources Centre, Zambia); Herta Kolberg (National Biodiversity Institute, Namibia) and Margaret Nkya (Tanzania NPGRC). The objective was to sample the diversity of wild rice species in all the geographic and ecological areas where they have been recorded. The target taxa were *O. barthii*, *O. brachyantha*, *O. eichingeri*, *O. longistaminata* and *O. punctata*. The species are all inbreeding, with the exception of *O. longistaminata*, which is outcrossing. This paper also reports the field observations and the overall results of collecting missions co-ordinated by the first author and highlights observations made in Kenya, Uganda, Zimbabwe, Mozambique and Malawi where the author participated in the collecting missions.

Materials and methods

Ecogeographic survey

The collecting programme was initiated and agreed upon in a planning meeting held in Lusaka, Zambia in November 1996 and attended by all the collaborators. In the meeting, the sampling strategy was agreed upon and the target areas identified based on ecogeographic surveys and information provided by the national programme staff. In addition, the range of distribution of the species was determined through ecogeographic surveys carried out at the Royal Botanic Gardens, Kew, UK; the East African Herbarium (EA) of National Museums of Kenya, that holds a good east African collection and some collections from southern Africa; the National Herbarium and Botanic Garden (SGRH) in Harare, Zimbabwe, that holds a southern Africa regional collection, and the Botany Department Herbarium (LMA) of Instituto Nacional de Investigacao Agronomica (INIA) in Mozambique. Information was also obtained from herbarium specimens held at the Makerere University Herbarium (MHU) in Uganda, the National Botanic Institute (WIND) in Namibia, the National Herbarium and Botanic Gardens (MAL) in Malawi, the National Herbarium of Tanzania (NHT) and the Botany Department Herbarium (DSM) of the University of Dar-es-Salaam, both in Tanzania. Other sources of information included available literature on occurrence and distribution of the wild species in the region (Clayton 1970; Fernandes et al. 1971; FAO 1987; Katayama 1987, 1990; Vaughan 1989; 1994; Leistner 1990). This provided useful information on where the species have been recorded in the past. Information on flowering and fruiting periods was also recorded from herbarium specimens and it was used to guide the timing of collecting missions.

Geographic locations of collection

Collecting missions were organized in collaboration with the National Programmes of the nine countries; Malawi, Kenya, Mozambique, Namibia, Zimbabwe, Tanzania, Uganda, Zambia and Zanzibar, covering a geographical range of latitude 18 36'S in Zimbabwe to 1 43'N in Uganda, longitude 40 54'E in Mozambique to 18 36'E in Namibia. The climatic conditions in these geographic locations are very diverse. Kenya, Uganda and Tanzania fall within the tropics, while the rest of the countries are sub-tropical. The tropical countries experience two rainfall maxima, with dry spells between June and September and December and March. The average annual rainfall ranges from 400 mm to 2500 mm. In the sub-tropical countries, viz. Malawi, Zambia, Mozambique and Zimbabwe, there is only one rainy season between December and March followed by a long dry spell. Rainfall in the target areas ranges from 50 mm in Namibia to 3000 mm in some highland areas of Mozambique.

Species identification

The taxonomic key modified by Vaughan (1994) from Chang (1976, 1988) and Vaughan (1989) was used for species identification. In addition, herbarium specimens were collected in either triplicate or quadruplicate with a set each for the national herbaria of the countries where populations were sampled, viz. IRRI and the SPGRC.

Collecting routes

The routes followed were defined prior to the collecting expeditions. Vegetation, road, geo-political and relief maps with scales ranging from 1:250 000 to 1:1 350 000 were used. In general, collecting followed the major roads but in several instances the routes diverged from[2] to 30 km from the major roads into the wild rice habitats by either driving or walking. Collecting from riverbanks was made using boats and canoes.

Sampling strategy

The overall sampling strategy was to collect from as many sites as possible representing diverse environments and to collect enough material to represent maximum diversity in a minimum number of samples in a particular site. Within a specific site, sampling was carried out by collecting single panicles randomly from 10 to 15 individuals per cluster, this being a group of plants forming a tuft. The sampling technique aimed at the random selection of >5 clusters within a population so that >50 individual plants were sampled per population (Brown and Marshall 1995). Where populations were large and close-knit with no apparent clusters, about 50 plants were sampled randomly in a zigzag pattern through the field. This was particularly the case for *O. longistaminata*, where the populations were mostly widespread because of the vegetative propagation system through rhizomes. The seeds were then bulked to represent one sample. This procedure was followed as long as the population was large enough. Many populations were small and in such cases sampling was made from as many plants as were available to obtain at least 600 seeds. For the purpose of this study, a population was defined as all the individuals of the target taxon found in a particular more or less ecologically homogeneous site at a given time. The number of sites was determined by environmental

diversity, with sampling being made in as many different diverse environments as possible using altitude, vegetation, topography and microenvironments as indicators. Where the environment was homogeneous, sampling was made every 15 km. Where populations were large and seed fertility high, samples were collected in triplicate, one set for the National Programme, another for safety duplication at IRRI and the other for genetic diversity studies.

Documentation of collecting sites and information handling

Passport data were collected and the specific locality or the geographical location specifying the latitude, longitude and altitude of collecting site recorded using a global positioning system (GPS) receiver Garmin 100. The IRRI wild rice collecting forms and IPGRI and SPGRC collecting forms were used in recording passport data, including sample labelling and identification data, sampling information, collecting site localization, collecting site context and description, population information and socio-economic indicators of erosion. In addition, comprehensive field notes were taken during the collection missions. Herbarium specimens were also collected, mostly in triplicate, from 172 populations for taxonomic verification. Photographs of 59 populations were taken, representing 31% of all the populations sampled. Documentation of collecting sites and a distribution map of the populations in the target region was made using ArchView □Version 3.1 GIS software. All the passport data and documentation of collecting routes are available from the following institutions: International Rice Research Institute (Los Banos, Phillipines); National Gene Bank of Kenya (Muguga, Kenya); National Plant Genetic Resources Centre of Tanzania (Arusha); Zambia National Plant Genetic Resources Centre (Mount Makulu); Namibia National Botanical Institute (Windhoek); Zimbabwe National Plant Genetic Resources Centre/Department of Research and Seeds Services (Lusaka); Mozambique National Plant Genetic Resources Centre/Instituto Nacionale de Investigacao Agronomica (Maputo); Malawi National Plant Genetic Resources Centre (Chitedze); and Uganda National Plant Genetic Resources Centre/National Agricultural Research Organization (Entebbe).

Genetic erosion

Threats to the wild rice species were assessed using human population growth, grazing pressure and observed agricultural activities in the proximity of the wild rice habitats as the genetic erosion indicators. Genetic erosion was taken to be the reduction of diversity at the genetic, population, inter- and intra-specific levels. For grazing pressure, a score available in the IRRI germplasm collecting form was given to each population depending on the visual assessment of the percentage of the plants in the population that showed evidence of grazing; grazing as a threat to diversity depends on the species and the extent to which the reproductive cycles are adversely affected. The threat of habitat loss was modelled based on human population growth. Those areas undergoing the highest rate of population growth were assumed to present the greatest risk of genetic erosion.

Results

A total of 17 collecting missions were undertaken in Kenya, Malawi, Mozambique, Namibia, Tanzania, Uganda, Zambia and Zimbabwe between April 1997 and April 1998 (**Table 1**). These missions depended on the time of the year when the wild species were ready for collection in different geographic locations. The flowering and maturity periods of the wild rice populations were very variable. In the east African region, where there are two clear rainfall maxima, there seem to be two distinct collecting periods, March–May and September–November, or 3–4 months after each rainy season. This was particularly observed in Kenya, where collections were made in April and observations made during the exploration missions held in September of the previous year revealed that many populations were ready for collection while others were at different stages of maturity. In the southern region, which experiences only one rainfall maximum, between December and March, the flowering and peak collecting period is March–May. These findings generally agree with reports made by Vaughan (1994).

The wild species populations were very scattered, far apart and often difficult to reach because of topographic conditions, impassable roads, and the nature of the habitats where they grow. Access was particularly difficult for populations of *O. longistaminata* and *O. barthii*, which were sometimes found growing in very deep ponds, swamps, or marshes. *Oryza longistaminata* was sometimes found growing on the riverbanks of large rivers with strong currents. During the collecting missions, a total of 211 populations were sampled for *ex situ* conservation of the five target species. The full results of the collecting missions giving the breakdown of number of accessions per taxon and country are summarized in **Table 2**.

Most of the populations sampled were *O. longistaminata*, which was found in all the countries explored and comprised 44.6% of all the samples collected. *Oryza barthii* was the second most common species sampled, comprising 25.1% of the total collections, mainly in Tanzania, Malawi, Zimbabwe and Zambia; more than 75.4% of the *O.barthii* samples were collected from Malawi. *Oryza punctata* comprised 24.6% of the total collections and was collected in Kenya, Tanzania, Mozambique, Malawi and Zanzibar with more than half of the samples collected in Kenya and Tanzania. *Oryza eichingeri* populations were collected in Uganda and Tanzania and comprised 3.3 % of all the samples collected. *Oryza brachyantha* comprised only 2.4% of the total populations sampled and all were collected in Zambia. It is reported to occur in Tanzania but no populations were found during the collection missions. The largest number of samples were collected in Malawi, followed by Kenya and Tanzania; the smallest number of samples were collected in Zimbabwe. Relatively few populations were collected in Namibia and Uganda. The highest number of taxa were collected in Tanzania, where four out of the five targeted species were collected, followed by Zambia and Zanzibar where three taxa were collected. Two taxa were collected in Kenya, Mozambique, Uganda and Zimbabwe, while only one was collected in Namibia (**Figure 1**).

Most of the samples collected were seeds. The quantity of seed collected per sample was variable and ranged from <100 to >1000. Four vegetative samples of *O. longistaminata* were collected in Uganda and Tanzania. The number of plants sampled was recorded in 138 populations. The results indicate that the targeted sampling of at least 50 individuals

per population was achieved in 56 populations, or 40% of cases. At the species level, 44% of *O. longistaminata*, 44.8% of *O. punctata*, 40% of *O. eichingeri* and 35% of *O. barthii* populations were collected from at least 50 individual plants. Most of the populations were randomly sampled because their spatial occurrence was not clustered, as anticipated. This was particularly the case for *O. longistaminata* that mostly regenerates through rhizomes, and the individuals are uniformly spread within a given population. Where it occurred on riverbanks, the spread followed the moisture gradient leading to long narrow populations along the edges of the river. **Figure 2** shows the number of individual plants sampled from different populations of four out of the five species studied. *Oryza brachyantha* is not included as the data on number of plants sampled was not available. For *O. longistaminata*, 'number of plants' is an approximation because the extent of a single plant in the populations with rhizomatous propagation could not be determined.

Oryza longistaminata, *O. punctata* and *O. barthii* were collected both from the wild and in farm fields or field borders. Over 29% of these species populations were found in farm fields or farm borders, mostly in abandoned rice fields or growing together with *O. sativa* as weeds. In certain instances they were found growing in field borders between rice and other crops or in close proximity to cultivated rice on the fringes of the fields. This was particularly true in Kenya, Tanzania and Malawi where rice growing is quite widespread in the areas explored. In Mozambique, Zambia and Zimbabwe, the populations were predominantly sampled from the wild. *Oryza longistaminata* and *O. barthii* were also found growing in irrigation canals and trenches. All the populations from Namibia and several from Kenya were collected along riverbanks. Several populations of *O. brachyantha* and *O. eichingeri* were collected predominantly from the wild in natural habitats.

Population areas

From the 136 records available on the area covered by the populations that were visually estimated, it is clear that most of the populations of the five taxa were small in extent. More than 62% of the populations covered less than 1000 m^2 and 15.4% were between 1000 and 5000 m^2. Large populations, >5000 m^2, comprised 22.1%. The range of population areas was wide for all taxa, except for *O. brachyantha* where all the populations covered areas of less than 1000 m^2. The population areas for the other taxa ranged from 0.5 to 100 000 m^2. This range was found for *O. longistaminata*.

In Kenya, Tanzania, Namibia, Uganda, Mozambique and Malawi, large populations of various species were found occupying large tracts of land, predominantly of wild species, with ground coverage of 60–80%. This was the case with *O. longistaminata* in several countries where large populations of more than 10 000 m^2 were found growing in swamps, abandoned rice fields, and flood plains. On the whole, *O. longistaminata* had the largest populations with the largest population area being 100 000 m^2 along the floodplains of the Okavango River in Namibia. Several large *O. longistaminata* populations, ranging between 20 000 and 50 000 m^2, were also found in Kenya, Mozambique, and Tanzania. The largest *O. punctata* population was 40 000 m^2 and was

found in a swamp in Iringa, southern Tanzania. Large populations of *O. eichingeri* were found in Uganda with the biggest being 45 000 m^2 under shade in the Tome forest. Large *O. barthii* populations were only found in Malawi where five were >10 000 m^2, the largest being 50 000 m^2. *Oryzabrachyantha* populations were comparatively smaller than all the other species. Four of the five populations were between 5 and 10 m^2, while the fifth, which was the largest, was only 100 m^2. **Table 3** shows the populations sizes and ranges for the five taxa sampled.

Occurrence of spontaneous inter-specific hybrids

Some wild species growing in and along the borders of farmers' fields were found flowering almost at the same time as *O. sativa*, leading to the production of spontaneous inter-specific hybrids. Most of the hybrids and their derivatives were more vigorous than the wild forms with which they grew. Sometimes they formed separate colonies or populations. In some cases they were found growing together with the cultivated forms as mixed populations. Some characteristics of cultivated rice found in the hybrids include larger grain size, thicker stems, narrower and more compact panicles, higher fertility, and no shattering of panicles. However, they retained some of the strong wild characters such as awns and rhizomes in the case of *O. sativa–longistaminata* hybrids.

Examples of these spontaneous hybrids were found in Kenya, Mozambique and Malawi. In Kenya, a hybrid was found between *O. punctata* and an *O. sativa* landrace ('sindano bahari'). This was in a small farmer's field in a valley bottom. The five hybrid plants were growing as weeds in the cultivated rice field. Farmers do not eat the grains and they normally uproot plants from the field. The hybrids were characterized by vigorous vegetative growth, larger seeds than the wild forms, and short and strong whitish awns. The other type of hybrid was between *O. longistaminata* and a landrace ('kalulu'), found in a farmer's field in Malawi. Several immature populations of both *O. barthii* and *O. longistaminata* were found on the farm borders and irrigation canals. Two types of hybrids were observed. One had a non-shattering rachis, was rhizomatous and also vigorous with long, soft whitish awns, characteristics of *O. longistaminata*. It was found growing in small populations in 0.3m deep water in which the landrace would not normally survive. The other had these characteristics, but without rhizomes. This is consistent with results from the work on inter-specific hybridization between *O. longistaminata* and *O. sativa* carried out by West Africa Rice Development Association (WARDA) (1997). The seeds of these hybrids mature earlier than those of the wild species and later than cultivated rice, which was already being harvested. The farmers started noticing them three years ago and use them as food though they have a different scent from that of the pure landrace. Birds avoid them because of the awns. Herbarium specimens of these hybrids were collected.

Indicators of genetic erosion in wild rice populations

The populations of the wild rice species are currently under pressure and are faced with genetic erosion in many of the habitats where they were found. An analysis of passport data, field notes and observations on 189 populations of the five species revealed that 131

(or 69.3%) are affected by various human activities. The biggest threats are grazing and agricultural activities including opening up of habitats where wild rice is naturally found, to give way to subsistence farming and other socio-economic activities. On the whole, 60 of the populations sampled (or 31.7%) were adversely affected by agricultural activities because they were growing in farmers' fields or in areas where rice cultivation is rapidly increasing. Expansion of agriculture is leading to drainage of swamps and other wetlands where wild rice grows, to give way to cultivation of rice and other crops. Some 29% of the populations of *O. barthii*, *O. longistaminata* and *O. punctata* were found in farm borders and in the fields. They grow as weeds in the same fields as cultivated rice and are normally uprooted when farmers are preparing land for rice cultivation or in routine farm management. In most cases, the wild species colonize the fields after cultivated rice has been harvested and the land is left fallow. Farmers burn or weed them out when preparing land for the next growing season. In one field in Kilifi, Kenya, a farmer was found burning a large population of *O. punctata*, about 5000 m^2 with 90% ground coverage. This population was growing in his abandoned rice field, which he was preparing for the next rice-growing season.

In Malawi and Uganda, where cultivation of rice is increasing, farmers use the presence of wild species as an indicator of suitable conditions for growing rice. In the central region of Malawi, particularly Salima and Chitenche, population areas as small as 20–50 m^2 were found cleared and replaced with cultivated rice. These populations were in natural habitats in close proximity to farm fields, shallow ponds and water pools and in roadside ditches. In Uganda, the swamps where wild rice occurs naturally are being systematically drained to make way for settlements and cultivation of subsistence crops. It is estimated that 90% of the converted swamps in Uganda are being used for rice production (NWCMP 1996). This is posing a great threat to the wild species. Habitat destruction by human activity, especially around Lake Kyoga where populations in the fishing villages have increased rapidly of late, has greatly increased the threat to *O. longistaminata*. Many populations that had previously been known to occur in this area, and had been targeted for collection, have disappeared because of displacement by settlements and other disturbance factors including agriculture, fishing and brick making (Emerton and Muramira 1999).

However, grazing by livestock is the most widespread and serious threat affecting all the five species. Grazing pressures with scores ranging from 0 to 100% were recorded in various populations. These scores represent the visual estimate of the percentage of the plants that have been grazed in each population using a scale of 1–4, where 1 represents 0–25% of the plants while 4 represents 75–100% of the plants. Of the 189 populations studied, 95 (or 50.2%) are under pressure from grazing but the degree of threat varies from one population to another. **Figure 3** gives a breakdown of the number of populations affected by different degrees of grazing on a taxon-by-taxon basis.

The populations of *O. barthii* and *O.punctata* are the most affected by grazing pressure, with 33.4% and 17.7%, respectively, classified as scale 4. In Kenya an *O. punctata* population was found so overgrazed that it had been reduced to two individual plants, from which samples were taken. In Zimbabwe, a population of *O. barthii* from which a

herbarium specimen had been collected in 1991 was found so overgrazed that there were no plants surviving. Being a sexually propagated annual, the phenological cycle was adversely affected year after year, resulting in no seed production and eventually leading to population eradication. *Oryza longistaminata* populations are also facing considerable grazing pressure. One *O. longistaminata* population found in Zimbabwe was so heavily grazed from one season to another that the plants had been reduced to mere stumps. Consequently, no herbarium specimen or seed samples could be collected because there were no seeds or flowers on the plants.

In Zimbabwe National Parks, *O. longistaminata* populations are also under various degrees of grazing pressure from wild animals as they come to drink water in the wetlands where these populations are found.

Swamps and wetlands in Uganda have been singled out as important sources of dry season grazing for livestock (Emerton and Muramira 1999). This puts the wild rice populations under heavy grazing pressure. For example, sampling could not be made on three populations of *O. longistaminata* earlier found in swamps around the shores of Lake Kyoga because most of the plants had been overgrazed before reaching the seed production stage. **Table** 4 gives a summary of the percentage of the populations that are adversely affected by grazing and agricultural activities.

On the whole, *O. barthii* and *O. punctata* are the most affected by over-grazing or agricultural activities, with the percentage of populations threatened by either grazing pressure or agricultural activities as high as 91.6 and 87.9%, respectively. The *O. eichingeri* and *O. brachyantha* populations are not adversely affected by agricultural activities. **Figure 4** shows the percentage of threatened populations on a geographical basis. Tanzania has the highest number of threatened wild rice populations from both grazing pressure and agricultural activities. The percentage of threatened populations in Kenya and Malawi is also high. The percentage of populations under threat in Uganda and Namibia is low. Mozambique has the lowest number of populations under threat; fortunately not a single population has been affected by grazing and only three are threatened by agricultural activities.

Genetic erosion of the wild rice populations is exemplified by recorded evidence of their disappearance in specific localities where herbarium specimens and/or seed samples have been collected in the past. Three such sites where *O. longistaminata* and *O. punctata* could not be found were recorded in Kenya and three in Zimbabwe where *O. longistaminata*, *O. barthii* and *O. punctata* could not be found. In Uganda, several wetland sites around Lake Kyoga are now drained and built-up where *O. longistaminata* populations had been previously recorded. Consequently, these populations have now been displaced. One site where *O. eichingeri* populations were previously recorded but have since disappeared was noted in Uganda.

Seed fertility and production

Seed fertility, maturity and production varied among and within species. The variability in maturity dates was highest in *O. longistaminata*. All phenological stages of seed development were usually found in a single population, and even in panicles of individual plants within a population. On panicles of some plants, this variability ranged from dehisced seeds, fully mature seeds and flowers. This variability was highest where water was not a limiting factor, particularly on riverbanks and in deep-water ponds, marshes and swamps. In general, however, seed set was very poor and 92% of the *O. longistaminata* populations were recorded as having low seed production.

Sterility was also highest in *O. longistaminata*. Out of the 53 populations for which fertility was scored, 13.3% had no seeds at all. This included five populations of more than 4000 m 2, in which 1000–2500 m 2 of the population were sampled. These populations were found in Kenya, Uganda and Tanzania and had no seeds due to sterility; the spikelets were all empty. Some 66% of the populations had very few seeds and most of the spikelets were sterile. Nine percent of the populations had some sterile spikelets and 3.7% had very few sterile spikelets. In many populations, most of the panicles had fewer than 10 spikelets with viable seeds, while all the rest were empty. Spikelets containing mature seeds could easily be identified by holding the panicle against the light because they have a much darker appearance than the empty spikelets (**Figure 5**).

High uniformity in seed maturity was observed in *O. punctata*. Fertility was also highest in this species, and no infertility was recorded in any of the 53 populations sampled. The fertility levels in *O. barthii*, *O. eichingeri* and *O. brachyantha* were comparable but seed production in *O. eichingeri* was higher than in either *O. brachyantha* or *O. barthii*. A comparative analysis of fertility in the five wild species is shown in **Figure 6**.

Discussion

The observed optimal collecting periods generally agree with previous reports by Fernandes et al. (1971), FAO (1987) and Vaughan (1994). These seem to be correlated with the rainy seasons and occur 3–5 months after the rains, about the same time it takes for seeds to mature in a normal phenological cycle of the wild rice species. However, in east Africa (Kenya, Tanzania and Uganda) where there are two distinct rainy seasons in a year, there seems to be two peak seasons for collection of the wild rice species that follow this rainfall pattern. The ‘long rains’ normally fall between March and May. It is therefore reasonable to expect some wild rice populations to be mature by September. The second rainy season comes between November and December and similarly some populations would be expected to be mature by March. In southern Africa, there is only one rainfall season in the summer, between November and March. Consequently the peak collecting period is 4–5 months after the rains, i.e. March–May. A few panicles in some populations would still have seeds in June but most of them would have shattered by this time, as was observed during collecting missions in Namibia, Mozambique and Zimbabwe. This explains the relatively few populations that were sampled in these countries.

Though there was no clear pattern of population sizes, *O. longistaminata* populations were larger than in the other species. This is probably because it has two simultaneous modes of reproduction, rhizomes and seeds. It is also perennial and reproduction is continuous in both time and space. The rate of population increase would therefore be expected to be higher than in the other species, which are annuals. It also stands better chances of survival because the rhizomes make it difficult for the entire population to be eradicated by either overgrazing or weeding.

The inter-specific hybrids between *O. punctata* and *O. sativa* observed in a farmer's field in Kenya are difficult to explain as these two species belong to different complexes (Vaughan 1994). Hybridization between *O. longistaminata* and *O. sativa* would be expected, as observed in Malawi because they belong to the same complex and have the same genome, AA. This is consistent with similar reports by Bezançon et al. (1977), Ghesquiere (1987), Vaughan (1989) and WARDA (1997). However, more research work is needed on establishment of ploidy status of the *O. punctata* collections. In addition, detailed descriptions and laboratory confirmations on biological status of the hybrids observed might be required.

The wild rice genetic diversity is currently being eroded by grazing and agricultural activities in most countries. The increase in human population is leading to the draining of natural wetland habitats to make way for subsistence farming. In countries such as Malawi and Uganda, increased subsistence rice production is continually leading to destruction of habitats where wild rice grows (NWCMP 1996; Emerton and Muramira 1999). Farmers use the occurrence of wild species as an indicator of suitable environmental conditions for growing cultivated rice. Increased rice production has become necessary because of the diversification of the food base and the high costs of imported rice. In addition, the wild species are threatened by their eradication from farm fields because they are considered weeds. *Oryza longistaminata* is a particularly notorious weed because of its rhizomes, which are difficult to completely eradicate.

The level of threat varies across the region. The threat is highest in Tanzania, Kenya and Malawi probably because of the high population densities leading to expansion and intensification of the agricultural production systems, in addition to high livestock populations in the areas where wild rice occurs.

The level of threat is lowest in Mozambique probably because the country is still emerging from a long period of civil strife. The livestock population is therefore very low and agricultural activities are just picking up. This is confirmed by the observations made in the field. Farmers are also very cautious of clearing natural habitats for fear of the landmines. Most of the populations from Mozambique were collected from the coastal area and this diversity may be under threat from floods as recently witnessed.

In Uganda, *O. longistaminata* populations are threatened by settlements, increased socio-economic activities and draining of swamps for subsistence farming. The increasing settlements and agricultural activities could be attributed to the relative stability the country is now enjoying, after a prolonged period of civil unrest. The *O. eichingeri*

populations, however, seem to be relatively safe as they were mostly found in protected forest reserves.

Rice is not cultivated in Zimbabwe and grazing pressure from both livestock and wild animals is the only threat to the wild rice populations. The populations were mostly found in communal lands with no grazing control.

The populations at the highest risk are those that are under pressure from the combined effects of both agricultural activities and varying degrees of grazing pressure. Populations of *O. punctata* and *O. barthii* are the most vulnerable to genetic erosion. This is probably because they are annuals and the populations become highly threatened once the growing cycle is adversely affected, particularly by grazing, in more than two consecutive seasons. Many populations are also found in farm fields and are therefore weeded out. *Oryza longistaminata* is less threatened than either *O. punctata* or *O. barthii* because it is perennial and has rhizomes. It can therefore withstand grazing and other disturbance factors such as weeding. The rate of regeneration is also faster because it is both sexual and asexual. Many populations in Kenya and Namibia were collected from the banks of large rivers, such as the Kunene, Tana and Okavango. Threats from grazing and agricultural activities are minimal because most of the populations were found in deep water.

The reason for the observed high level of sterility in *O. longistaminata* is not obvious although populations growing in habitats with high water levels were more fertile. The relatively longer phenological cycle and variability in maturity of seeds that was observed in *O. longistaminata* may be caused by its perenniality. It is also an out-breeder and the variability could be attributed to this factor.

In response to genetic erosion concerns, this paper reports the collection and conservation of 211 accessions of different wild rice species. The germplasm is currently conserved in the gene banks of the National Plant Genetic Resources Centres and Institutes that participated in the programme. In addition, duplicates have been sent to the International Rice Research Institute for long-term storage. A significant contribution in arresting the erosion of wild rice germplasm in the region has consequently been made. However, the systematic characterization and evaluation of the germplasm is now required in order to recover genetic traits of immediate value to rice breeding and improvement programmes. Studies on the relationships between genetic variability and the ecology of the collecting sites are also necessary, especially in *O. barthi*, which is a complex species with wild and weedy forms of various evolutionary origins.

Conclusion

The genetic diversity of wild rice populations in east and southern Africa is being eroded by the expansion of agricultural activities and over-grazing. Concerted efforts are therefore required to collect and conserve this diversity for future use in crop improvement programmes. Although the reported work has made a significant contribution to this enormous task, many targeted areas were not sampled because of

logistical problems, inaccurate timing of collection in some countries, long distances that needed to be covered and insufficient seed because of sterility and small population sizes. These gaps include the Yala swamp and the shores of Lake Victoria in western Kenya, parts of Lake Kyoga and the western districts in Uganda, the Zambezi River valley in Zimbabwe, the Okavango delta in Botswana and the Cunene River valley in Angola. Further sampling is required in most of the countries in order to capture the maximum inter- and intra-specific variability.

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References

Aguierro VM, Cabauatan PQ, Hibino H. 1984. A possible source of resistance to rice grassy stunt virus (GSV). International Rice Research Newsletter 9(3):11–12.

Akagi H, Yokozeki Y, Inagaki A, Fujimura, T. 1998. Origin and evolution of twin microsatellites in the genus *Oryza*. Heredity 81: 187-197.

Allard RW. 1970. Population structure and sampling methods. In: Frankel OH, Bennet E, editors. Genetic Resources in Plants—Their Exploration and Conservation. Blackwell Scientific Publications, Oxford, UK, pp. 97–107.

Amante AD, de la Pena R, Sitch LA, Leung H, Mew TW. 1990. Sheath blight (ShB) resistance in wild rices. International Rice Research Newsletter 15(3):5–6.

Amante AD, Sitch LA, Nelson R, Dalmacio RD, Oliva NP, Aswinoor H, Leung H. 1992. Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*. Theoretical and Applied Genetics 84:345–354.

Bezançon G, Second, G.1979. Report of wild rice collection mission in Zambia. International Rice Research Institute, Manilla, Philippines.

Bezançon G, Bozza JL, Second G. 1977. Variability of *Oryza longistaminata* and the sativa complex of *Oryza* in Africa. Ecological and evolutive aspects. In: Meeting on African Rice Species, 25–26 January 1977, IRAT-ORSTOM, Paris.

Brar DS, Khush GS. 1995. Wide hybridization for enhancing resistance to biotic and abiotic stresses in rainfed lowland rice. In: Fragile Lives in Fragile Ecosystems.

Proceedings of the International Rice Research Conference, 13–17 February 1995, Manilla, Philippines, pp. 901–910.

Brar DS, Elloran R, Khush GS. 1991. Interspecific hybrids produced through embryo rescue between cultivated and eight wild species of rice. Rice Genetics Newsletter 8:91–93.

Brown AHD, Marshall DR. 1995. A basic sampling strategy: theory and practice In: Guarino L, Rao VR, Reid R, editors. Collecting Plant Genetic Diversity: Technical Guidelines. CAB International, UK.

Chang TT. 1976. The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. Euphytica 25:425–441.

Chang TT. 1988. Taxonomic key for identifying the 22 species in the genus *Oryza*. International Rice Research Institute, Los Banos, Philippines. IRRI Newsletter 13:5.

Clayton WD. 1968. Studies in *Gramineae*xVII, West African wild rice. Kew Bulletin 21:487–488.

Clayton WD. 1970. *Gramineae* (Part I). In: Milne-Redhead E, Pohill RM, editors. Flora of Tropical East Africa. Kew Botanical Gardens, UK.

Emerton L, Muramira E. 1999. Uganda biodiversity: economic assessment. Paper prepared for the Uganda National Biodiversity Strategy and Action Plan. IUCN–World Conservation Union/National Environmental Authority, Uganda.

Engels JMM, Arora RK, Guarino L. 1995. An introduction to germplasm exploration and collecting: planning, methods and procedures, follow-up. In: Guarino L, Rao VR, Reid R, editors. Collecting Plant Genetic Diversity: Technical Guidelines. CAB International, UK.

FAO. 1987. An Illustrated Manual of Kenyan Grasses. Food and Agricultural Organisation of the United Nations, Rome, Italy.

Farooq S, Shah TM, Asghar M, Askari E, Arif M, Iqbal N. 1994. Rapid identification of rice genotypes through RAPDs. Rice Biotechnology Quarterly 19:14–15.

Fernandes A, Launert E, Wild H, editors. 1971. Flora Zambesiaca. Vol. 10. Part I. Crown Agents for Overseas Governments and Administration, London, UK.

Ghesquiere A. 1987. Evolution of *Oryza longistaminata*. ORSTOM, Paris, France.

Heinrichs EA, Medrano FG, Rapusas HR. 1985. Genetic evaluation for insect resistance in rice. International Rice Research Institute, Manilla, Philippines.

IRRI. 1970. Annual report for 1969. International Rice Research Institute, Manilla, Philippines.

IRRI. 1990. Program report for 1989. International Rice Research Institute, Manilla, Philippines.

Ishii T, Brar DS, Multani DS, Khush GS. 1994. Molecular tagging of genes for brown plant hopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *O. sativa*. Genome 37:217–221.

Jena KK, Khush GS. 1990. Introgression of genes from *Oryza officinalis* Well ext Watt. to cultivated rice, *O. sativa* L. Theoretical and Applied Genetics 80:737–745.

Jones MP, Dingkuhn M, Johnson DE, Fagade SO. 1996. Inter-specific hybridization: progress and prospects. Proceedings of Workshop on: Africa/Asia Joint Research on Inter-specific Hybridization between the African and Asian Rice Species (*O. glaberrima* and *O. sativa*) pp. 44-49. West Africa Rice Development Association, Bouake, Cote D'Ivoire .

Katayama TC. 1987. Studies on distribution and ecotypic differentiation of wild and cultivated rice species in Africa. Occasional Paper No. 10. Kagoshima University Research Centre for the South Pacific.

Katayama TC. 1990. Distribution and ecotypic differentiation of wild and cultivated rice species in Africa. Occasional Paper No. 18. Kagoshima University Research Centre for the South Pacific.

Khush GS. 1977. Disease and insect resistance in rice. Advances in Agronomy 29:265–341.

Khush GS, Balacangco E, Ogawa T. 1990. A new gene for resistance to bacterial blight from *Oryza longistaminata*. Rice Genetics Newsletter 7:121–122.

Leistner OA, editor. 1990. Grasses of Southern Africa: Memoirs of Botanical Survey of South Africa, No. 58. National Botanical Gardens, South Africa.

Lin SC, Yuan LP. 1980. Hybrid rice breeding in China. In: Innovative Approaches to Rice Breeding. International Rice Research Institute, Manilla, Philippines, pp. 35–51.

Marshall DR, Brown AHD. 1975. Optimum sampling strategies in genetic conservation. In: Frankel OH, Hawkes JG, editors. Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge, UK, pp. 53–80.

Marshall DR, Brown AHD. 1983. Theory of forage plant collection. In: McIivor JG, Bray RA, editors. Genetic Resources of Forage Plants. CSIRO, Melbourne, Australia, pp. 135–148.

Miezan K, Second G. 1979. Collecting wild rice species in Zambia. International Rice Research Institute, Manilla, Philippines.

Multani DS, Jena KK, Brar DS, de los Reyes BG, Angeles E, Khush GS. 1994. Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice, *O. sativa* L. Theoretical and Applied Genetics 88:102–109.

NWCMP. 1996. Environmental assessment of rice production in Western Uganda. National Wetlands Conservation and Management Programme, Ministry of Natural Resources, Kampala.

Ou, SH .1985. Rice diseases. Commonwealth Mycological Institute, Surrey, UK.

Phillips S. 1995. Poaceae (*Gramineae*). In: Hedberg I, Edwards S, editors. Flora of Ethiopia and Eritrea, Volume 7. Upsala, Sweden.

Reid R, Attere F, Toll J. 1988. Germplasm collection and conservation activities in Africa: the role of the International Board for Plant Genetic Resources. In: Ng NQ, Perrino P, Attere F, Zedan H, editors. Crop Genetic Resources of Africa, Vol. II. Proceedings of an International Conference on Crop Genetic Resources of Africa, 17–20 October 1988, Ibadan, Nigeria.

Second G, Bezançon G, Bozza J. 1977. Variabilite d'*O. longistaminata* et du complexe sativa des *Oryza* en Afrique. Aspects ecologiques et evolutifs. In: Reunion sur les especes africaines de riz. 25–26 Janvier, ORSTOM, Paris, France, pp. 47–55. ORSTOM, Paris, France.

Taillebois J. 1983. Une nouvelle perspective pour la production des semences hybrides F1: le transfert des caracters d'allogamie de l'espece *O. longistaminata* A. Chev. a l'espece *O. sativa* L., Agronomie Tropicale 38(4):303–307.

Vaughan DA. 1989. The genus *Oryza* L. Current status of taxonomy. IRRI research paper series No. 138. International Rice Research Institute, Los Baños, Philippines.

Vaughan DA. 1994. The wild relatives of rice: a genetic resources handbook. International Rice Research Institute, Los Banos, Philippines.

Vaughan DA, Almazan MaS, Oliva M, Naredo maE. 1991. Wild relatives of rice conserved at the International Rice Germplasm Centre, IRRI. Rice Genetics Newsletter 8:38–44.

WARDA. 1997. Inter-specific hybridization: progress and prospects. Proceedings of Workshop on: Africa/Asia Joint Research on Inter-specific Hybridization between the African and Asian Rice Species (*O. glaberrima* and *O. sativa*). West Africa Rice Development Association, Bouake, Cote D'Ivoire, December 16–18, 1996.

Table 1. Summary of collection missions carried out

Country	Institution	Number of missions	Target areas covered
Kenya†	NGBK	1	Coast province
Malawi†	MPGRC	3	Northern, Central and Southern regions
Mozambique†	INIA	1	Nampula, Zambezia
Namibia	NPGRC	3	Okavango, Kunene river, Ruacana
Tanzania	NPGRC	2	Morogoro, Mbeya, Iringa, Tanga, Pwani, Singida
Uganda†	NARO	1	Nakasongora, Masindi, Mukono, Rabai
Zambia	NPGRC	3	Eastern, Southern, Western, Northern, Luapula provinces
Zimbabwe†	DRSS	3	Manicaland, Midlands, Masvingo, East Mashonaland, Matabele

†Countries in which the first author participated in collecting missions.

Table 2. Number of populations sampled per taxon in different geographile locations

Country	*Oryza longistaminata*	*Oryza punctata*	*Oryza eichingeri*	*Oryza brachyantha*	*Oryza barthii*	Total
Kenya	22	17	0	0	0	39
Malawi	23	3	0	0	40	66
Mozambique	14	1	0	0	0	15
Namibia	10	0	0	0	0	10
Tanzania	3	17	2	0	8	30
Uganda	3	0	4	0	0	7
Zambia	6	0	0	5	3	14
Zanzibar	8	14	1	0	0	23
Zimbabwe	5	0	0	0	2	7
Total	94	52	7	5	53	211

Table 3. Number of accessions of the taxa collected by population class†

Species	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6

O. longistaminata	45	14	8	3	3	3
O. punctata	17	4	1	0	0	1
O. barthii	26	2	2	0	2	3
O. eichingeri	0	1	1	0	1	1
O. brachyantha	5	0	0	0	0	0
Total	93	21	12	3	6	8

†Key to classes of population sizes: Class 1=>1–1000 m^2; Class 2=1001–5000 m^2; Class 3=5001–10 000 m^2; Class 4=10 001–15 000 m^2; Class 5=15001–20 000 m^2; Class 6=

Table 4. Percentage of populations affected by grazing and agricultural activities

Species	Grazing only	Agriculture only	Agriculture and grazing
O. longistaminata	42	23	12.5
O. punctata	40	37.9	32.6
O. barthii	50	41.6	25
O. brachyantha	20	0	0
O. eichingeri	20	0	0

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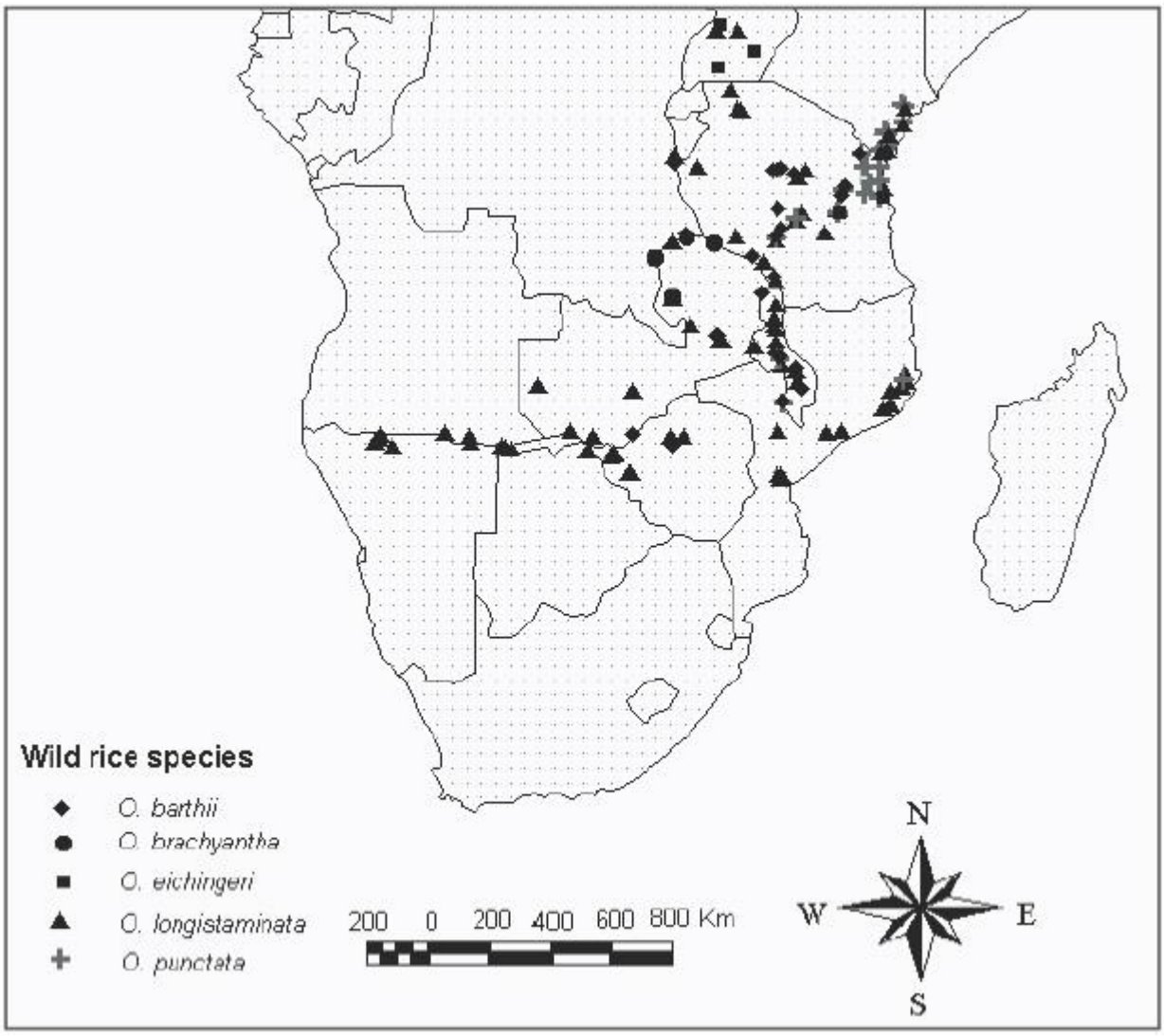

Figure 1. The distribution of collecting localities of wild rice species.

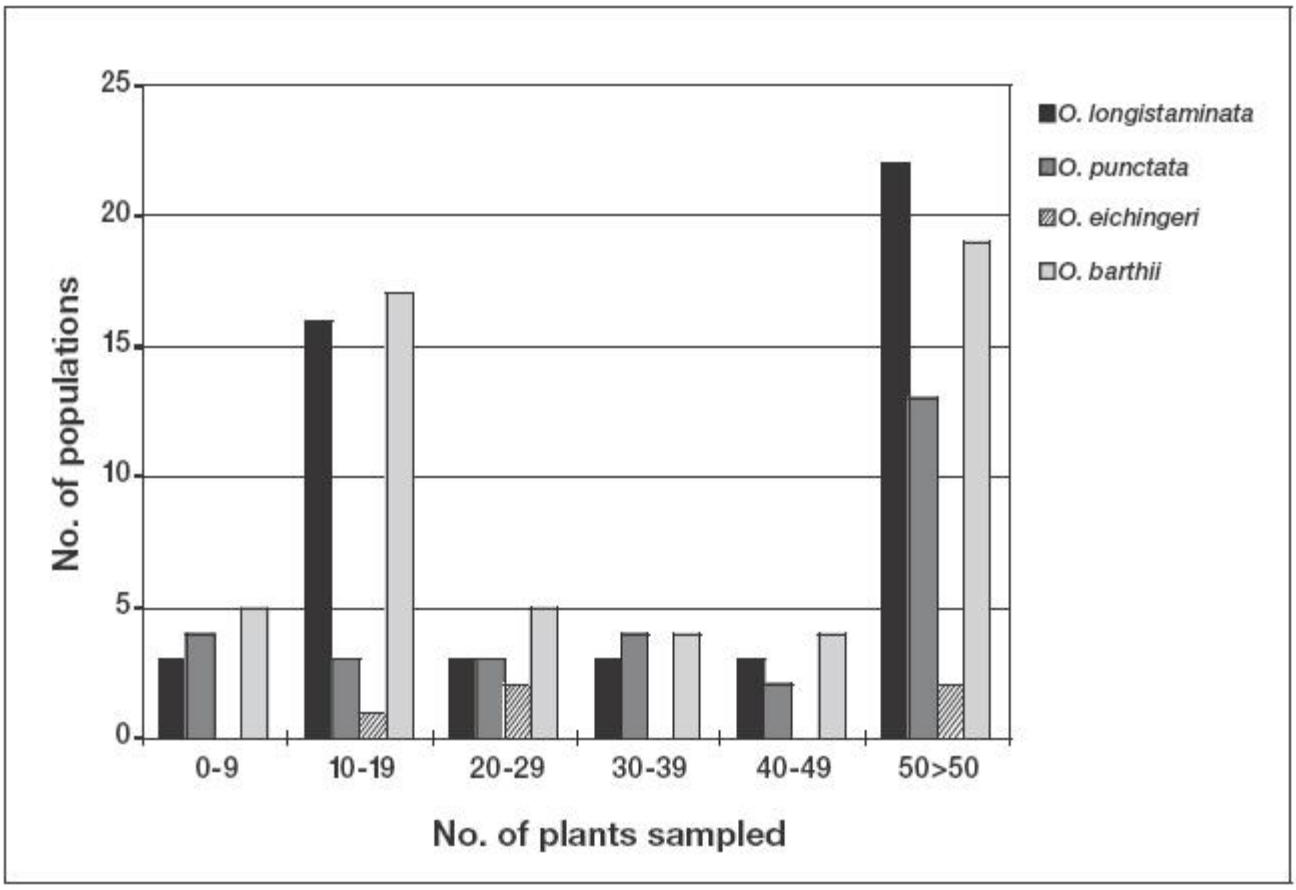

Figure 2. Number of plants sampled in populations of the wild species.

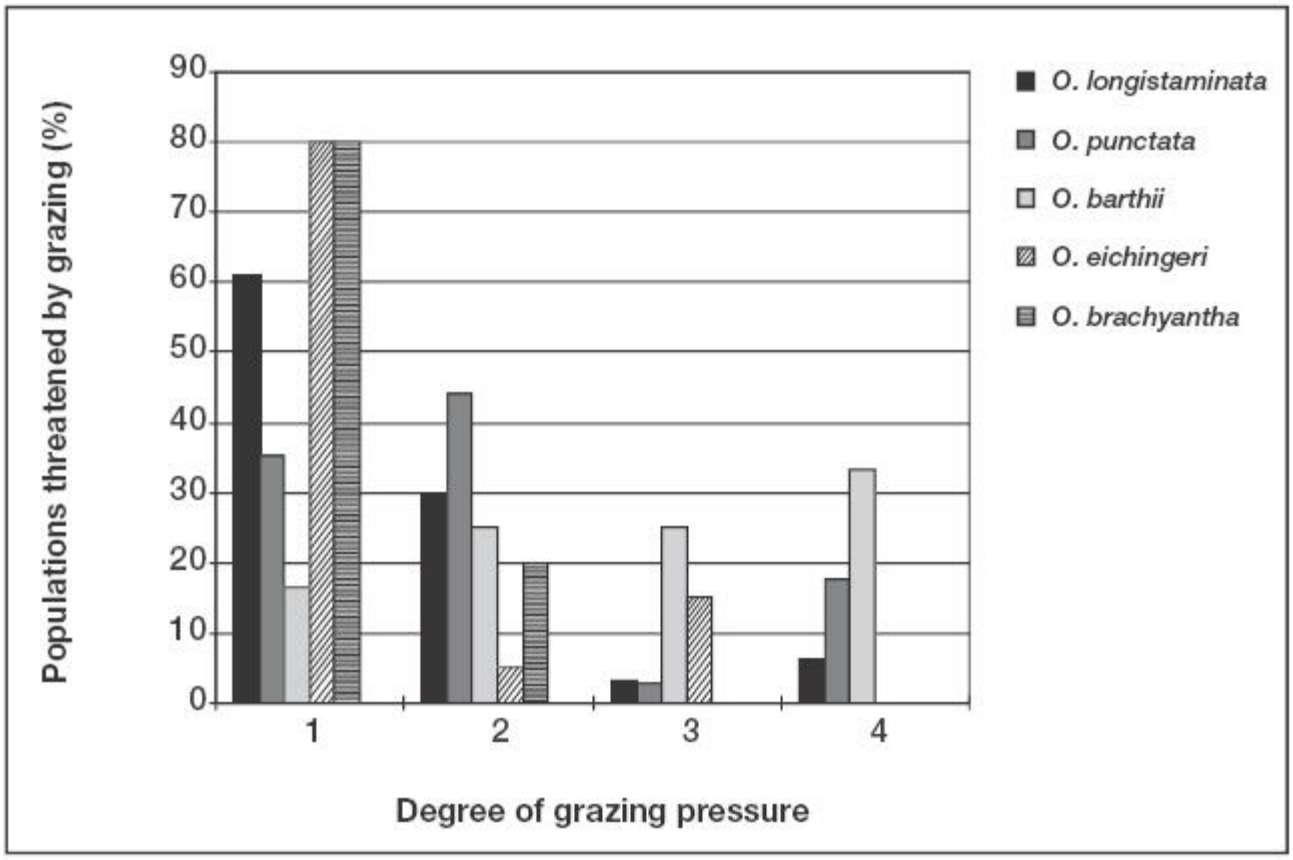

Figure 3. The incidences of grazing on wild rice populations.

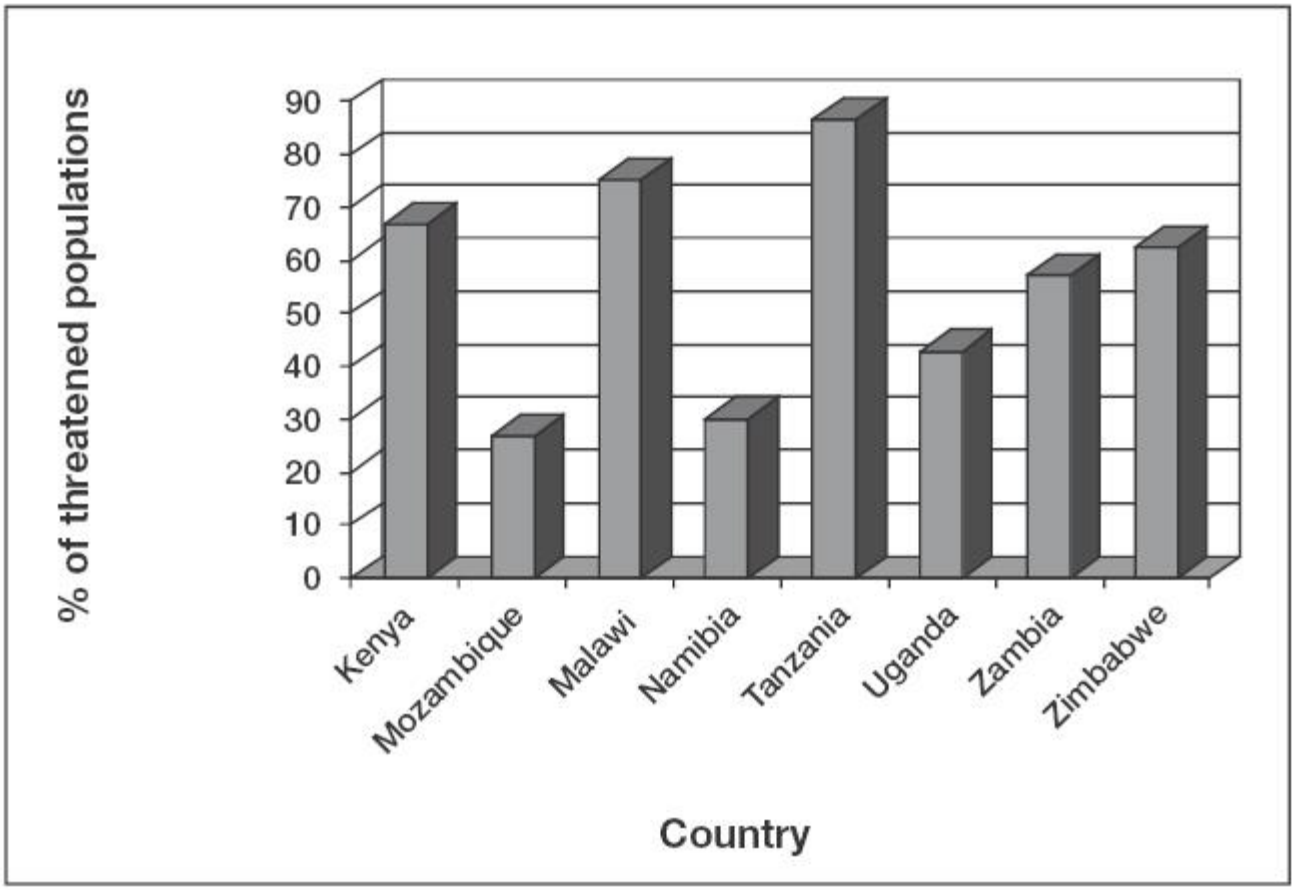

Figure 4. Percentage of threatened populations on a geographical basis.

Figure 5. A panicle of *O. longistaminata* with only one fertile seed (encircled).

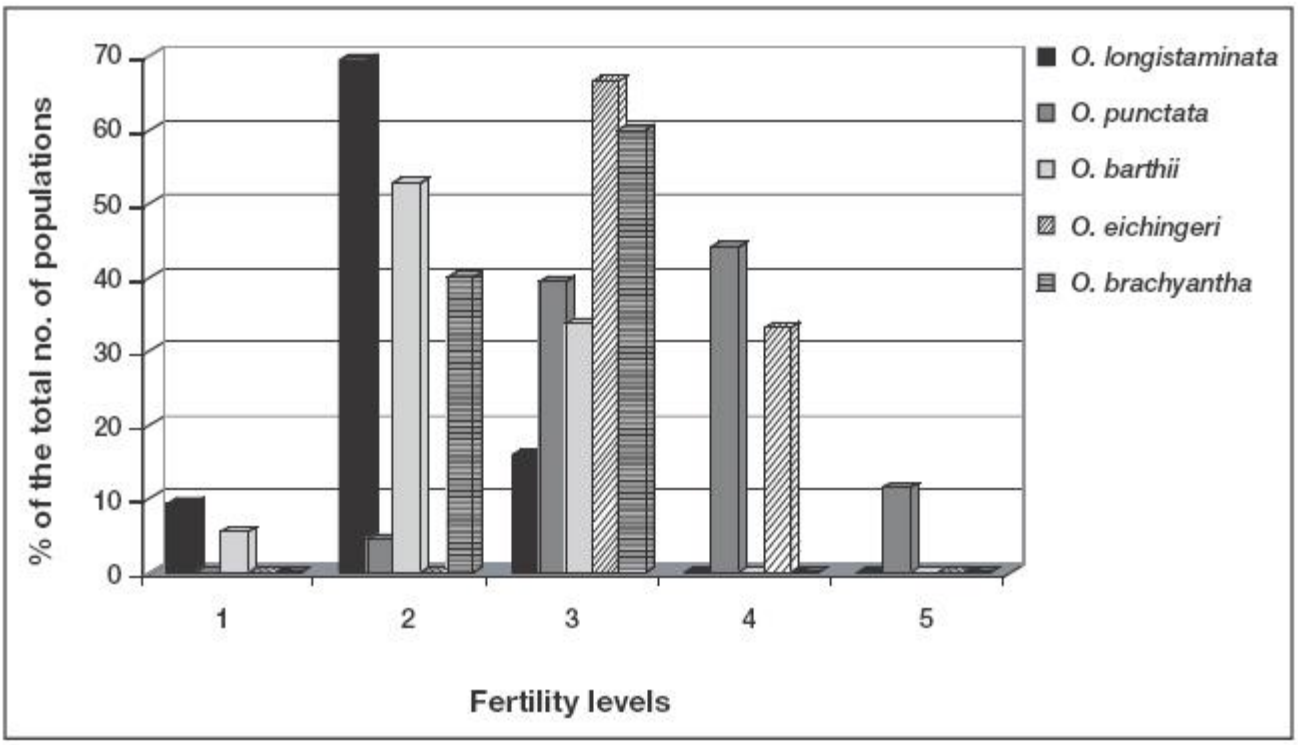

Figure 6. Levels of fertility in populations of the five wild rice species.