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Review

Potential role of biotechnology tools for genetic improvement of "lost crops of Africa": the case of fonio (*Digitaria exilis* and *Digitaria iburua*)

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Fonio (*Digitaria spp*), considered as one of the lost crops of Africa, remains an important food crop for millions of people in Africa. The intimidating challenge today is to produce enough fonio to meet the growing demand for its products. Research has an important role to play in enhancing fonio production in Africa. This paper discusses the innovative research techniques of agricultural biotechnology that are particularly relevant to facilitating the genetic improvement of fonio for higher productivity. The paper considers the potential role of biotechnology applications like DNA markers in understanding the evolution, origin, distribution and diversity of fonio in Africa; somaclonal variation in generating genetic variability in fonio; and genetic transformation in circumventing fonio breeding barriers to introduce alien genes of agronomic importance into fonio.

Key words: Fonio, Digitaria exilis, Digitaria iburua, lost crops.

INTRODUCTION

Food insecurity remains one of the major problems of modern Africa, where famine continues to threaten peace and stability in the mostly agrarian continent. This appears to be the price modern Africa must pay for abandoning most of its native crops that sustained Africa for thousands of years. It is reported (Vietmeyer et al., 1996) that among the more than 2000 crops native to Africa, grains like sorghum, pearl millet, finger millet, tef, African rice, and fonio could be the effective weapon against hunger in the continent.

Fonio, in particular, is sometimes regarded as "grain of life" as it provides food early in the farming season, when other crops are yet to mature for harvest (Ibrahim 2001).

Fonio grains are also considered as the best tasting and nutritious of all grains (Vietmeyer et al., 1996), with about 7% crude protein that is high in leucine (9.8%), methionine (5.6%) and valine (5.8%) (Temple and Bassa, 1991). The grains are also reported to have high brewing and malting potentials (Nzelibe and Nwasike, 1995). The enormous traditional and technological uses of fonio have earlier been reviewed elsewhere (Misari et al., 1996; Jideani, 1999).

Fonio [*Digitaria exilis* (Kippist) Stapf, and *Digitaria iburua* Stapf, Poaceae] is an interesting cereal crop with many names. Some of its popular African names include *fonio, findi,* and *fundi*. The english name, "Hungry Rice", believed to have been coined by Europeans, is considered misleading by some authors (Kwon -Ndung and Misari, 1999; Ibrahim, 2001; Anonymous, 2003). Fonio was suggested to have been once the major food

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Item	Farmers (percentage)				
	Bauchi	Kaduna	Kebbi	Plateau	Niger
Diversity of varieties under cultivation					
Growing different varieties of fonio	39.1	80.0	0.0	85.3	4.8
Only one or two major varieties	60.9	20	100	14.7	95.2
Average land area for fonio					
Cultivate below 1 ha	100.0	90.8	100.0	70.6	100.0
□ 2-3 ha	0.0	9.2	0.0	25.7	0.0
□ above 3 ha	0.0	0.0	0.0	3.7	0.0
Problem of pest and diseases	30.4	32.3	55.6	38.2	29.4
Grain yield per hectare					
Harvest below 800 kg/ha	43.5	73.8	55.6	76.4	28.6
□ 800 – 1000 kg/ha	3.5	15.4	38.8	11.8	71.4
Above 1000 kg/ha	13.0	10.8	5.6	11.8	0.0
Application of fertilizers	43.2	0	0	0	4.8
Chemical weed control	0	0	0	0	0
Manual weed control	100	100	100	100	100

Table 1. Survey of fonio husbandry activities of farmers in different fonio growing areas of Nigeria.

Source: Kwon-Ndung and Misari, 1999.

crop in West Africa, where it has been cultivated for thousands of years (Pulsegrove, 1975). This native African grain crop fits perfectly into the low-input farming systems of resource-poor African farmers because of its unique ability to withstand drought and tolerate poor and marginal soils (Vietnameyer et al., 1996; Aslafy, 2003).

Though fonio was once considered as one of the "Lost Crops of Africa" (Vietnameyer et al., 1996) and was completely neglected by agricultural scientists (Kwon-Ndung and Misari, 1999), it is now gradually being 'rediscovered' and considered for improvement as a cultivated species (Ibrahim, 2001; Morales-Payan et al., 2002). This paper reviews the status and challenges of research for development of fonio and discusses the potentials of biotechnology tools in enhancing genetic improvement of this "grain of life."

FONIO IMPROVEMENT: STATUS, CONSTRAINTS AND PROSPECTS

The increasing problem of food insecurity in Africa and the recognition of fonio as a potential buffer against famine is expected to stimulate the expansion of land area devoted to fonio cultivation in the continent. However, available statistics demonstrates the reduction of fonio harvest area in several countries (Figure 1), except in Nigeria, Cote D'Ivoire, and Guinea. In year 2002, a total area of 347,380 hectares was devoted to fonio production in Africa (FAOSTAT, 2003), with Nigeria

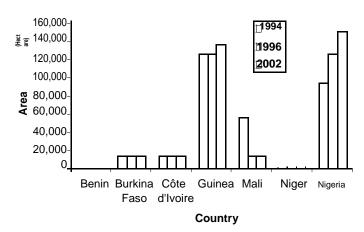


Figure 1. Land area devoted to fonio cultivation across the years in different countries of West Africa (Source:FAOSTAT, 2003).

alone providing almost half of that area (150,000 ha).

Several factors are responsible for the general decline in fonio production. Some of the major disadvantages of fonio are lodging, small grain size, lower yields than other cereals, and shattering (Vietnameyer et al., 1996; Misari et al., 1996; Kwon-Ndung and Misari, 1999; Anonymous 2003). A survey of farmers' fonio husbandry activities in Nigeria (Table 1) demonstrates the lack of improved agronomic practices in fonio production, especially in the area of weed control. Apart from the general poor husbandry, the husking process of fonio grains is very tedius and time-consuming (Vietnameyer et al., 1996; Kwon-Ndung and Misari, 1999), constituting a major bottleneck in its processing and utilization.

Years of research by NGOs and research institutions have contributed immensely in addressing the husking problem of fonio (Aslafy, 2003; Diakite, 2003). The breakthrough in fonio processing may enhance fonio production to meet local demands in Africa and even for export (Aslafy, 2003). This might be responsible for the increased interest in fonio production in Africa in recent years (Figure 2). The scientific challenge now is to develop new improved high-yielding and non-shattering varieties of fonio with larger grain size (Vietnameyer et al., 1996), shorter and stronger culms (Kwon-Ndung and Misari, 1999) and with good grain quality.

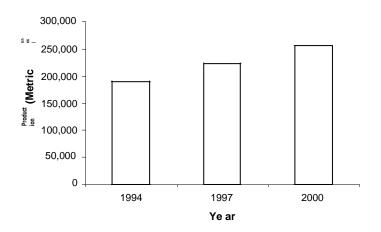


Figure 2. Fonio production across the years in Africa (Source: FAOSTAT 2003).

Currently, the genetic improvement of fonio centers on germplasm collection generally and their morphological characterization with the objective of understanding and broadening the fonio gene pool. In Nigeria, it is reported that about 240 different accessions of fonio have been collected and are being evaluated to identify those that could be utilized in breeding programmes (Kwon -Ndung and Misari, 1999). However, morphological the conventional characterization technique alone might not provide the much needed information on the evolution, origin, distribution and diversity of fonio in Africa. Again, the development of improved fonio cultivars by the traditional method of hybridization appears unfeasible due to the miniature size of fonio floral organs and the general dearth of information on fonio floral biology.

There is the need, therefore, to exploit other alternative techniques, like biotechnology, in elucidating the genetic

diversity in fonio, broadening the fonio gene pool and facilitating the development of improved fonio varieties. The recent progress in the application of biotechnology tools for genetic improvement of cereals (Repellin et al., 2001) constitutes the major prospect for fonio genetic improvement in Africa.

APPLICATION OF DNA MARKERS FOR FONIO GERMPLASM CHARACTERIZATION

The major factors that reduce fonio harvest on resourcepoor farmers field are: inherent low yield of the crop, weeds, shattering, lodging, birds, insect pests and diseases (Vietnameyer et al., 1996: Kwon-Ndung and Misari, 1999). To develop new improved fonio varieties, breeders must have access to diverse fonio accessions with a broad range of agronomic traits like maturity, grain size, plant height, weed competitiveness, and yield potential. The collection and conservation of local and exotic germplasm of fonio in a gene bank is therefore the key to successful genetic improvement program. However, germplasm collections can only be exploited in breeding programs after they are properly analyzed.

Fonio germplasm are traditionally analyzed using several phenotypic traits that demand extensive observations. Moreover, phenotypic traits are often affected by environmental conditions. DNA markers, like RFLP, RAPD, AFLP, SSR, are now available to make gene pool analysis more precise. DNA markers generated by random amplified polymorphic DNA (RAPD) have been extensively used for genetic mapping, molecular taxonomy and molecular diagnostics (Williams et al., 1990). In rice (Oryza sativa L,), RAPD and AFLP (amplified Fragment length polymorphism) have been shown to be the effective DNA markers for characterization of diverse rice germplasm (Jackson, 1999). These markers might be effective for DNA fingerprinting of fonio accessions, collected from diverse fonio growing regions of Africa. The challenge now is to initiate a research project to screen several decamer primers for their ability to generate polymorphism in genomic DNA of fonio. The primers that generate polymorphic bands on gels could then be selected to analyze the whole fonio germplasm.

BROADENING FONIO GENE POOL THROUGH SOMACLONAL VARIATION

Genetic variability for traits of agronomic importance is often limited among cultivated germplasm. For example, agronomic traits like large grain size, strong culms and resitance to lodging appear to be absent in the gene pool of fonio accessions (Kwon-Ndung EH, unpublished data). Biotechnology tools, like somaclonal variation, may provide the opportunity to broaden the gene pool of fonio for such important traits. Somaclonal variation is a form of genetic variation that may occur during prolong in-vitro culture of somatic plant cells and tissues (Larkin and Scowcroft, 1981). Bouharmont and colleagues (1991) demonstrated the potential of somaclonal variation in rice improvement programs. Since then, breeding involving somaclones and mutants has been attempted in tissue culture systems of rice to select for tolerance to several stress factors, including salinity (Lutts et al., 1999), drought (Adkins et al., 1995) and panicle blast disease (Boonchitsirikul et al., 1998).

The general methodology of somaclonal breeding in rice involves the induction of embryogenic calli from excised embryos of mature caryopses and selection of somaclones in the induction or regeneration medium, or even in both the media. However, the genetic variation that arises during somaclonal variation is generally low but could be enhanced through the use of chemical mutagens or irradiation. A review on the integrated use of mutation induction and tissue culture for crop improvement is available elsewhere. A protocol for the application of irradiation to enhance the output of stress-tolerant somaclones has recently been perfected for rice (Pathirana et al., 2002). The new protocol involves irradiating mature caryopsis of rice prior to *in vitro* induction of calli.

These innovative approaches could be exploited for fonio genetic improvement. However, the prospect of using somaclonal variation in fonio genetic improvement depends greatly on the availability of an efficient protocol for induction of embryogenic calli of fonio. This challenge is currently being addressed by scientists at the Nigerian Biotechnology Advanced Laboratory, SHESTCO, Abuja (Prof. GH Ogbadu, personal communication). Thousands of fonio somaclonal variants can be produced using this biotechnology approach. Fonio breeders can then evaluate these somaclones to identify novel plants with desired agronomic traits. Results from such research activities are expected to demonstrate the importance of biotechnology for enhancing research on native crops of Africa.

A TARGET FOR GENETIC TRANSFORMATION

Among all the tools of biotechnology, genetic transformation offers the greatest opportunity to circumvent breeding barriers in the development of improved crop varieties. It has been argued (Wambugu, 1999; Wambugu, 2001; Machuka, 2001) that genetic transformation of food crops has the potential of eradicating hunger in Africa. That is correct, but the target crops should be carefully selected for any meaningful impact on food security in Africa. Currently, none of the crops native to Africa, and which hold the key to food security in Africa, has been marketed as transgenic crop.

Fonio could, therefore, be the right target to demonstrate the usefulness of genetic transformation for taming hunger in Africa.

Fonio, being a cereal crop, may benefit from the existing routine protocols for genetic transformation of cereals (Repellin et al., 2001). Mass production of fonio in Africa is currently hampered by the inherent low yielding potentials of existing unimproved landraces, susceptibility to insect pests and diseases (Kwon-Ndung and Misari, 1999) and lack of tolerance to available herbicides (Bakare et al., 1995). Genetic engineering of fonio for shorter plants, higher photosynthetic efficiency and better carbon partitioning could lead to larger grain size and high yield. Introduction of herbicide-tolerant genes into fonio could enhance the use of herbicides for effective weed control in fonio, thereby circumventing the laborious and time-consuming manual weed control method. Genetic transformation of fonio could also lead to the development of transgenic fonio that are resistant to insect pests and diseases. Fortunately, gene constructs for all these traits are available and routinely used for cereal transformation. The challenge for the scientific community is to adapt the existing cereals regeneration and transformation protocols for fonio genetic transformation.

CONCLUSION

For thousands of years, Africans have depended mostly on their native fruits, vegetables, root/tuber and cereal crops for their subsistence. Today, most of these crops, which have the potential of providing enough food for the increasing population of poor people in the continent, are now regarded as "lost crops of Africa", due to the decline and total neglect of their production in the continent. The result is food insecurity in Africa, and, as Jimmy Carter rightly mentioned, "there can be no peace until people have enough to eat...." (Carter, 1999).

Several factors are responsible for the steady decline in the production of food crops native to Africa, ranging from inherent low yield potential to laborious unimproved husbandry practices. Fonio, for example, has the problems of very small grain size, weak culms, low yield, and susceptibility to insect pests and diseases (Vietnameyer et al., 1996; Kwon-Ndung and Misari, 1999). Genetic improvement of fonio may require the assembling in a genebank of available fonio accessions from all the fonio growing regions of Africa for evaluation of its evolution, origin and diversity. For this type of evaluation, the conventional approach of germplasm characterization, which relies sorely on morphological features, has to be complemented with the biotechnology tool of molecular markers. The DNA markers like RAPD and AFLP, used for rice diversity study (Jackson, 1999), could also be suitable for fonio germplasm evaluation. The results of such investigations will provide the

necessary information to crop improvement specialists to identify appropriate fonio accessions for used in hybridization programs (Kwon-Ndung and Misari, 1999).

However, dearth of information on floral biology of fonio and the extraordinary miniature nature of its floral organs hamper fonio hybridization program. Alternative techniques are therefore required to create genetic variability in fonio for important agronomic traits. Somaclonal variation, especially in combination with mutation induction, holds a lot of prospect in the development of genetic variations in fonio. The available protocols for cereal tissue culture could be exploited for the production of fonio somaclones. Another powerful biotechnology tool that could circumvent the fonio breeding barriers is genetic transformation. This tool involves the use of 'gene-gun' or Agrobacterium tumefaciens to transfer foreign gene from one organism to the other. Genetic transformation approach could be exploited to create transgenic fonio with high efficiency of photosynthesis and carbon partitioning (which may translate to larger grain size and high yield), resistance to insect pests, diseases and herbicides. The gene constructs for such traits are readily available and have been used for several cereal crops (Repellin et al., 2001), which makes the genetic transformation of fonio a feasible project.

Fonio, among other grains native to Africa, is selected as a target for biotechnology because of its exceptional culinary and nutritional properties. In Nigeria, fonio product is currently recommendable as a choice carbohydrate for diabetic patients. Today, fonio is the most expensive grain crop in Nigeria, providing resourcepoor farmers with enough income to alleviate their poverty. The recent news of fonio being "groomed to conquer the European market" (Aslafy, 2003), is another indication of the importance of fonio for food security and economy revival of Africa. That is why fonio production in Africa has to be stimulated by initiating research activities towards addressing the existing biological constraints that currently discourage its expanded production. Agricultural biotechnology is not everything, but it is the only tool that can rapidly bring out fonio from the list of "lost crops of Africa" to "African grain of life and prosperity". We have shown, in this paper, that biotechnology tools have the potential of enhancing genetic improvement of fonio, thereby encouraging its expanded production in Africa.

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