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Review

Application of biotechnology for the improvement of Nigerian indigenous leaf vegetables

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Nigeria is endowed with many indigenous leaf vegetables (ILVs) species, which spread across the estimated cultivable land area of 71.2 million hectares. These ILVs provide food, income, employment and herbal medicine to the population. Uncollected and uncharacterized germplasm, pests, diseases, anti-nutritional factors, recalcitrant seed, seed dormancy and perishable produce militate against the realization of potentials of the ILVs. This paper discusses biotechnological applications such as meristem culture, *in vitro* selection, zygotic embryo culture, somatic embryo genesis, protoplast culture, anther culture and genetic engineering that can solve improvement and production problems associated with some selected ILVs. Among the problems envisaged in the application of these biotechnological techniques are lacks of resources, selections of crop for research, attitude of government and weather conditions.

Key words: Indigenous leaf vegetables, biotechnology, production problems.

INTRODUCTION

Nigeria is an agricultural giant nation in Africa, with a total land area of 93.7 million square kilometers out of which cultivable land area is about 71.2 million hectares. This land area accommodates several species of indigenous leaf vegetables (ILVs). The rich diversity of the ILVs of Nigeria has been documented by several researchers including Okigbo (1977), Okafor (1979, 1983) and Adebooye et al. (2003). A major problem with the ILVs of Nigeria is that they have not been selected for desirable traits and no serious genetic/biotechnological research has been done on them. Adebooye et al. (2003) reported that most of the ILVs are not easily available as farmers now gather them with great drudgery and difficulty from the few stands that are left in the wild.

Biotechnology is a continuum of technologies, ranging from traditional biotechnology to modern biotechnology. Biotechnology is defined as any technique that uses living organisms or substances from those organisms, to make or modify a product, to improve plants or animals or to develop microorganisms from specific uses (Persley, 1992). Some of the aspects of biotechnology that are currently being used in crop production are: meristem and bud culture, zygotic embryo culture, cell and tissue culture, genetic engineering, molecular markers and monoclonal antibodies (Monti, 1992).

The Federal Government of Nigeria has put in place a Nigerian Biotechnology policy, and has also established a Nigerian Biotechnology Development Agency to formulate policies towards accelerating the acquisition of biotechnology in the country. The International Institute of Tropical Agriculture (IITA), Ibadan is using biotechnology tools on its mandate crops like: maize, soybean, cassava, yam and plantain/banana (Brink et al., 1998; Kuta, 2004). In some national research institutes and universities with tissue culture laboratories, research efforts are concentrated on staple crops. Biotechnological applications have not been extended to the ILVs of Nigeria. Thus there is a need to include the ILVs in Nigerian biotechnological programmes and policy. This paper discusses the status of biotechnological application on indigenous vegetables, potential for its application on the species and envisaged constraints.

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Table 1. Biological constraint associated with some indigenous vegetables.

Indigenous vegetable	Biological problems	References
Corchorus olitorius L.	Numerous morphotypes, defoliator, grasshopper , nematodes, short shelf life	Akoroda (1985), Akinlosotu (1977)
Celosia argentea L.	Numerous morphotypes, black beetle, crown blight Nematodes,	Badra (1991)
Vernonia amygdalina Del	Leaves curl virus, thrips, cotton aphids, white fly, bitter factor.	Misari (1992)
Telfairia occidentalis Hook. f.	Uncharacterized germplasm, recalcitrant seed, mosaic virus	Esiaba (1982), Akoroda (1990), Ajayi et al. (2004), Odiaka and Schippers(2004)
Talinum triangulare Willd.	Highly perishable, high soluble oxalate, hydrocyanic acid	Akachuku and Fawusi (1995), Schippers (2000)
Solanecio biafrae (Olive & Hiern) C. Jeffery	Genetic erosion, shade and support requirements, low seed viability	Schippers (2000), Adebooye 2004)
Launea taraxicifolia (Willd) Amin Ex C.Jeffrey	Seed dormancy, bitter factor	Chweya and Eyzaguirre (1999)

BIOLOGICAL CONSTRAINTS ASSOCIATED WITH ILVS PRODUCTION

For the purpose of this paper, seven ILVs previously described by Adebooye et al. (2003) as high premium species across Nigeria were selected. Table 1 consists of a list of problems on production, improvement and biological characteristics of the selected common ILVs of Nigeria. The constraints range from germplasm collection to pests problems. Because ILVs suffer neglect from research and development process, many of them have not had their germplasm collected and characterized in Nigeria. Only a few like *Corchorus olitorius,, Solanecio biafrae* and *Telfairia occidentalis* have received some research attention. However molecular technology has not be used to characterize their germplasm. Field genebanks are features of some few ILVs and there are fears that much of the genetic diversity could disappear because of pressures exerted by mankind.

High perishability of produce is a common problem of ILVs. They do not keep for long. Recalcitrant seed is a major production constraint of *T. occidentalis (*Ajayi et al., 2004*)*. For seeds of *T. occidentalis*, germination capacity declines when seed moisture is less than 40% and is completely lost when moisture content falls below 30% (Odiaka and Schippers, 2004). The common practice is to keep seeds inside the fruit but the seeds, being viviparous, often begin to germinate before onset of planting season. Insect pests and nematodes are common pest. Insect pest such as *Acrae eponina*, defoliate leaves of *C. olitorius* thereby reducing the quality and market value and if severe, render them unfit for human consumption (Akinlosotu, 1977; Akoroda, 1985; Schippers, 2000). Nematodes attack roots making plants produce yellow and unattractive leaves. *T. triangulare* is the only ILV with high content of antinutritional factors (Akachukwu and Fawusi, 1995).

BIOTECHNOLOGY CAPACITY BUILDING IN NIGERIA

Recently, the interest of Nigerian Government in the acquisition of biotechnology capability was demonstrated. A national Biotechnology Policy has been formulated and relevant biotechnology agencies and laboratory are being established to promote the exploitation of biotechnology for the benefit of farmers in Nigeria. A list of such programmes is contained in Table 2.

FUTURE ROLES OF BIOTECHNOLOGY ON SEVEN SELECTED INDIGENOUS VEGETABLES

More than any other groups of crop plants, ILVs require the urgent application of biotechnology. This is because there are many unsolved practical problems and unanswered research questions that limit the efforts towards exploiting the nutritional and production potentials of these multi-purpose crops. These problems range from germplasm conservation to sustainable production strategies.

Corchorus olitorius L.

Jute mallow (*Corchorus olitorius)* is one of the few ILVs that received research attention in Nigeria. National Horticultural Research Institute (NIHORT), Ibadan, Nigeria maintains a large germplasm collection of local landraces that have been characterized. NIHORT established genetic improvement programme for the vegetable that resulted in the production of improved and pure/varieties of the four major morphotypes known in Nigeria. Despite this research attention, there are rooms for biotechnological applications. On the few improved varieties developed by NIHORT, micropropagation is

Table 2. Biotechnology programmes in Nigeria and contact persons.

Biotechnology programmes	Major goal	Contact person
National Biotechnology Development Agency (NABD) Abuja	Development of appropriate policies and programmes for building the required	Prof. C.P.E. Omaliko
SHESTCO National Biotechnology Advanced Laboratory, Abuja	Central Laboratory for advance biotechnology research in Nigeria.	Prof. G.H. Ogbadu
National Center for Genetic Resources and Biotechnology, NAGRAB, Ibadan	Biotechnology in the conservation Nigerian genetic resources	Mr. M.B. Sarumi
National Biosafety Frameworks, Federal Ministry of Environment, Abuja	Capacity building and effective regulation of genetically modified products in Nigeria	Mr. M.P.O. Dore
Nigeria Agriculture and Biotechnology Project (NABP), USAID-IITA-NABDA, IITA-Ibadan.	Promote the adoption of biotechnology for enhanced agricultural	Dr C.A. Fatokun

Source: Kuta (2004)

desirable for further *in vitro* selection to obtain lines with desirable morphological traits and improved resistance to insect pest attack because a major production-limiting factor is the attack by leaf defoliator (*Acrae eponina*). Somaclonal variation from tissue cultured jute mallow could produce variants with improved nutritional qualities and shelf life. Considering the several morphotypes of jute mallow available, molecular characterization is desirable to establish the genetic relationships among them. This can be achieved through development and use of molecular markers like Restriction Fragment Length Polymorphism (RFPL), Random Amplified Polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and microsatellite markers. As a result of many close and wild relatives of *C. olitorius* like *C. tridens, C. tricularis, C.aetuans, C. aspenifolius* and *C. fascicularis*, inter-specific crossing appears to be a possible way of developing high yielding hybrids with acceptable nutritional qualities and long shelf life. Protoplast culture to achieve somatic hybridization can produce such hybrids. Where natural crossing is possible, zygotic embryo culture is recommended to prevent seed dormancy prevalent among *Corchoru*s. Furthermore, transformation system (such as *Agrobacterium tumefaciens*-mediated gene transfer) should be developed to drive novel genes into elite *Corchorus* genome.

Vernonia amygdalina Del.

The common bitterleaf (*Vernonia amygdalina)* received substantial research attention on nutritional status, but little work was conducted on agronomic practices or genetic enhancement.The studies on nutritional qualities of *V. amygdalina* were carried out in Nigeria and other African countries (Schippers, 2000). Micropropagation of common bitter-leaf is an urgent need to produce virus-free plantlet against leaf curl virus that is threatening the availability of high quality leaves. Interspecific crossing between *V. amygdalina* and its close relatives *V. colorata* and *V. hymenolepsis* could produce high yielding hybrids. Researchers should double their attention on this vegetable. This is because studies have revealed many uses of the crop plant (Adebooye et al., 2003). In view of this, biotechnological tools should be used to develop variety/lines for specific purposes. *In vitro* selection, somaclonal variation and somatic embryogenesis could produce line for these specific purposes. Anther and microspore culture for production of haploids for selection of somaclonal variant with desirable trait is a way of increasing genetic variability in *Vernonia*. Somatic hybridization through protoplast culture and embryo culture are possible techniques for inter-specific crosses among *Vernonia* variants to obtain superior hybrids.

Telfairia occidentalis Hook.f.

Fluted pumpkin (*Telfairia occidentalis)* has several production problems to which biotechnology can provide solutions. The natural diversity or genetic base is not very wide and available morphotypes have not been characterized. Molecular characterization would provide solution for establishment of genetic relationship among fluted pumpkin germplasm. Molecular markers like RFLP, RAPD, AFLP and micro satellites are potent tools in this direction. Similarly, sex-linked markers would help distinguish female plants from male ones at early stage of growth. New plants can be obtained through somatic embryogenesis from pedicels, stem, leaves roots and other explants and this can solve the problem of recalcitrant seed. This technique can make possible the production of artificial seeds in *T. occidentalis*. *In vitro* propagation of *T. occidentalis* should be encouraged to provide disease-free starting material for genetic improvements and cultivar breeding. *In vitro* conservation is a solution against genetic erosion of the crop genetic base. Biotechnologies to induce and express genetic variability like pollen and ovary culture, anther and microspore culture, meristem culture, somatic embryogenesis, somaclonal variation and embryo culture could produce cultivars with a higher proportion of female to male plants, capable of producing more seeds/fruits for consumption purposes, with stronger, more vigorous shoots and which are tolerant to water stress and resistant to *Telfairia* mosaic virus.

Celosia argentea L.

Lagos spinach (*Celosia argentea)* is a fast-growing crop, which is popular in southwest Nigeria owing to the soft texture of its leaves when compared with amaranth leaves. However, its production is being threatened by nematodes, as it is highly susceptible to both migratory and sedentary nematode species. Unfortunately, *C argentea* has not received much research attention in Nigeria compared to its close relative *C. cristata* in South Africa (Badra, 1991). Considering its numerous close relatives like *C. isertii*, *C. cristata* and *C. trigyna*, inter-specific crossing among these relatives could produce hybrids with high degree of nematode resistance. This can be achieved by the technology of protoplast culture and chromosome engineering. Where possible, molecular markers like RAPD, RFLP and AFLP should be use to establish genetic relationship among the species. Furthermore, developing *in vitro* propagation protocol for the plant could widen genetic variability of *C. argentea*. It will be followed by *in vitro* selection for desirable agronomic traits among the plantlets. Occurrence of somaclonal variant could produce lines with resistant to nematode and other desirable traits. *In vitro* conservation is an option for arresting the eroding genetic diversity of the plant. Transformation systems for *C. argentea* need to be developed to facilitate genetically engineered nematode resistance.

Talinium triangulare(Jacq) Willd.

Water leaf (*Talinium triangulare)* is considered a cheap crop and can easily be collected from the wild as vegetable. To date, no research priority has been accorded this crop. Improvement of its nutritional qualities should be the first objective of biotechnological application on *T. triangulare*. Somaclonal variants could emerge as a result of manipulation of *in vitro* propagation conditions and medium. This could widen genetic variability of the crop plant. Protoplast fusion could be used to achieve inter-specific crossing among its wild relatives like *T. paniculata* and *T. cuneifolium*. From the crosses, hybrid with desirable agronomic, nutritional and post-harvest qualities could emerge. Molecular characterization and *in vitro* conservation of its germplasm are areas of research in the near future.

Solanecio biafrae (Olive&Heirne) C. Jeffery

Solanecio biafrae is a commonly consumed ILV in southwest Nigeria. *S. biafrae* is being replaced by other vegetables that do not require support and shade and thus becoming rare and gathering for consumption is done with great difficulty (Adebooye 2004). Therefore, the area of urgent biotechnological application on this crop plant is both short and long term *in vitro* conservation of its germplasm. Before this could be achieved, micropropagation protocol for *S. biafrae* must be developed. However, to date there is no attempt to develop one. When conservation strategies are put in place, *in vitro* selection techniques to develop varieties/lines that are less dependent on shade and support for productivity should be a priority. Genetic variability of the crop could be widened by anther culture, embryo culture, somatic embryogenesis and protoplast culture.

Launea taraxacifolia (Willd) Amin Ex. C. Jeffery

Launea taraxacifolia is mainly collected from the wild in Nigeria and other African countries. Seed dormancy and propagation difficulty are hindrance to production of this crop plants. Micropropagation is a reliable solution to this problem. Therefore, *in vitro* propagation protocol for this crop should be established. Embryo rescue technique can solve seed dormancy problem of the crop. Selection of line with less bitter leaves would raise people interest in the crop. Inter-specific crossing between *L. taraxacifolia* and its wild relatives like *L. sativa*, *L. serriola* and *L. cornita* could result in hybrid with desirable traits

PROBLEMS AND CONCERNS

Constraints to the application of biotechnology to the ILVs of Nigeria are many. Among these constraints are:

Lack of resources for plant biotechnology

A serious deficit of skilled human resources in the plant sciences and biotechnology is evident in Nigeria. The building up of such knowledge and development of human resources capacity is necessary to produce improved varieties through use of biotechnology. Another serious constraint is the loss of skilled personnel who have received training and accepted jobs in developed countries. Working opportunities in Nigeria are often inadequate. Funds to pay salaries and absorb running costs of projects are either limiting or inadequate thus contributing to reduced availability of personnel. Even specific focal research points that were created in Nigeria at high donor expenses lack the critical mass of skilled personnel. Furthermore, training gained abroad is often not attuned to local needs because of the different research and infrastructure environment in Nigeria. As a result the demand and opportunities present in the home countries are often not met or remain unanswered.

Selection of crops for research

The research institutes in Nigeria focus mainly on the routinely cultivated species. It has been reported by Adebooye et al. (2003) that ILVs often do not receive attention. Therefore, the research priorities of Nigerian plant research institutes contribute substantially to the under-utilization and extinction of some ILVs. Even for the food staples, researches are not adequately funded and where funds are made available, little or nothing could be achieved because of the insufficiency of the funds.

CONCLUSION

Presently, little consideration is given to the use of biotechnological techniques on the ILVs of Nigeria. As Nigerian government is demonstrating political will for increasing biotechnology capacity and application through the National Biotechnology policy and programme on staple crops, the same gesture should be extended to ILVs. The benefits accruable from such gesture are enormous. It could be done in phases; from low-tech tissue culture to sophisticated DNA manipulation.

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