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BIOTECHNOLOGY IN PEST MANAGEMENT: IMPROVING RESISTANCE IN SORGHUM TO INSECT PESTS

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ABSTRACT

Annual losses in grain production attributed to four major insect pests (shootfly, stem borers, midge and head bugs) are estimated at $1,098 million in Africa and Asia alone. Integrated pest management (IPM) strategies for these insects have been poorly focused. There is little scope for chemical insecticides in sorghum production in sub-Saharan Africa. Various cultural and biological methods, including recommended intercropping configurations and biocontrol have either not been adopted by farmers or have not shown lasting success. Although much effort has gone into the identification and development of insect resistant sorghums, apart from sorghum midge, conventional breeding techniques have not yielded agronomically desirable products. Several biotechnological approaches for achieving higher levels of resistance in sorghum are discussed. Marker-assisted selection can speed up the breeding process and lead to gene pyramiding from diverse sources. The transfer of resistance genes from wild relatives of sorghum is of particular relevance to shootfly. With recent advances in genetic engineering, the standardization of protocols for routine transformation is being pursued at ICRISAT. Three techniques are discussed. Biosafety concerns are briefly mentioned.

Key Words: ICRISAT, integrated pest management, marker-genes, selection, resistance

RESUME

Les pertes annuelles en production de graines attribuees a 4 insectes majeurs (mouches de pousses, foreuses de tiges, midge et headbugs) sont estimees a 1098 millions de dollars en Afrique et en Asie. Les strategies de lutte integree contre les insectes n'ont pas ete bien ciblees. Il y a, par ailleurs, peu d'avenir pour les insecticides a base chimique dans la production du sorgho en Afrique au sud du Sahara. Des methodes culturales et biologiques diverses y compris les configurations d'associations culturales recommendees et le controle biologique n'ont pas ete adoptees par les agriculteurs ou du moins, n'ont pas eu des succees viables. Bienque beaucoup d'efforts aient ete consacres a l'identification et au developement des sorgho resistants aux insectes, les techniques d,amelioration conventionelles n'ont pas donne les produits agronomiquement interessants, a l'exception do sorgho resistant au midge. Plusieurs approches biotechnologiques sont examinees en vue d'atteindre des niveaux eleves de resistance chez le sorgho. La selection faite eu utilisant des marqueurs peut accelerer le processus d 'amelioration et aboutir a l'accumulation de genes provenant de diverses sources. Pour le cas de la resistance aux mouches de

pousses, le transfert de genes resistants en provenance de parents sauvages de sorgho peut etre d'une grande utilite. A l'aide des progres recent de genie genetique, l'ICRISAT developpe des protocoles standards a utiliser pour les transformations de routine. Trois de ces techniques sont examinees et les problemes de biosecurite sont brievement analyses.

Mots Cles: ICRISAT, lutte integree, genes marqueurs, selection, resistance

INTRODUCTION

Almost 30% (13.36 million ha) of the harvested world sorghum area was in sub-Saharan Africa (SSA) in 1993 (FAO, 1993). Because this estimate discounts the actual area planted, the region accounted for a biased 25% (14.31 million t) of the world sorghum grain production in 1993. However, in terms of productivity, average yield ha^-1 was just over 50% of the world average. The sorghum belt in SSA can be equated with the seasonally dry semi-arid tropics (SAT) of Africa which stretch across West Africa and covers much of Southern and Eastern Africa. This region is of primary interest to ICRISAT whose mission is to contribute to sustainable improvements in the productivity of the world's SAT which also include most of India, parts of southeast Asia and parts of Latin America. Sorghum is one of ICRISAT's five mandate crops that are basic to life in the SAT and, with pearl millet, it constitutes the main food source for over 100 million of a very vulnerable sector of the world's population in SSA This sector will be most hard hit by the year 2000 when the shortfall in food production is forecast to rise to 100 million t. Next to marginal agricultural land and drought, arthropod pests are a primary constraint in sorghum production and are associated with the crop from the time of planting, through harvest to storage. The majority are insect species of world-wide economic importance. Some mite species are seldom reported and their damage is noted to be only occasionally serious.

PEST SPECIES AND ASSOCIATED LOSSES

Approximately 150 insect species are reported to infest sorghum in different parts of the world (Jotwani *et al.,* 1980) but the species of economic importance are much fewer. Sharma (1993)listed 43 insect and mite species as important arthropod pests of sorghum. The major ones include shootfly, *Atherigona soccata* (Rondani); stem borers. *Chilo partellus* (Swinhoe), *Busseola fusca* (Fuller), *Diatraea saccharalis* (Fabricus), *Sessamia calamistis* (Hampson) and *Eldana saccharina* (Walker); armyworms, *Mythimna separata* (Walker), *Spodoptera frugiperda* (J.E. Smith) and *S. exempta* (Walker); aphids, *Schizapis graminum* (Rondani) and *Melanapis sacchari* (Zehntner); the chinch bug, *Blissus leucopterus leucopterus* (Say); the sorghum midge, *Contarinia sorghicola* (Coquillet); head bugs, *Calocoris angustatus* (Lethiery), *Eurystylus immaculatus* (Odhiambo) and several species of head caterpillars, grasshoppers, locusts and storage insects.

The incidence of most of these species has been reported in almost all sorghum growing areas stretching from Mauritania and eastwards through West Africa to Sudan and Ethiopia, then southwards through Kenya and Tanzania into Botswana and embracing the SADC countries (ICRISAT, 1985, 1989a; Nwanze, 1988; Nwanze *et al.,* 1991). Crop damage ranges from seedling death (shootfly and stem borers), defoliation (stem borers, army worms and grasshoppers), vascular tissue destruction (stem borers) to grain shriveling and chaffy florets (midge and head bugs). Unfortunately, actual data on grain yield losses are inadequate and often non-existent; and when they do exist, they are only simple estimations that are based on on-station experiments.

None-the-less, based on these estimations, grain yield losses in sorghum attributed to infestation by the main species of shootfly, stem borers, midge and/or head bugs, are placed at nearly 32% in India, 9% in the USA. and over 20% in Africa. In Africa and Asia alone, these losses are estimated to result in an annual loss of 8.9 million t of food grain which, at today's prices, is valued at over $1,098 million (ICRISAT, 1992). These losses will further aggravate the predicted shortfall in food production by the year 2000 if successful pest management strategies are not implemented at the farm level by this period.

PEST MANAGEMENT STRATEGIES

Cultural and biological methods. The concept of integrated pest management (IPM) emphasizes the optimization of farmer-oriented control options in as compatible a manner as possible and in the context of the associated environment. In SSA, three maj or elements, cultural methods, biological control and varietal resistance are, the primary loci of research by ICRISAT, ICIPE, INTSORMIL, National Agricultural Research Systems (NARS) and regional networks. It should. however, be added that apart from work at ICIPE in its study villages in western Kenya (Saxena *et al.,* 1990), there has not been a concerted effort at developing well-focused IPM strategies for farmers but rather one of experimentation of individual components the results of which have been put together in publications as parts of an IPM strategy

(Nwanze and Youm, In press).

The prospects for chemical insecticides in sorghum production in SSA will remain low as long as these crops continue to fetch low market values compared to maize and rice. There also exist good scientific data to support the potential contributions of several cultural practices in IPM (planting date, crop residue destruction, tillage, soil water and fertilizer management), but these are usually classified as impractical because they conflict with socio-economic values: traditional uses of crop residues, often labour-demanding and the lack of adequate financial resources. The effects of intercropping on pest populations and crop damage are well documented and are believed, among other factors, to be due to increased diversity in the agro-ecosytem, increased fertilization, and non-host effects. However, farmers have rarely adopted any of our recommendations on improved intercropping configurations (Nwanze and Youm, In press).

The published lists on natural enemies which include parasitoids, predators and insect pathogens are impressive but no lasting successes have been reported on sorghum (Nwanze and Youm, in press). Recent work at ICIPE, IITA and CAB's International Institute of Biological Control indicate that there are prospects in the near future for the use of insect pathogens in the control of locusts and grasshoppers (IITA, 1991; ICIPE, 1993). Apart from the well-developed pheromone trap network for *Helicoverpa armigera* (Hubner) which constitutes an integral part in the management of this cotton and pigeonpea pest in India (Srivastava *et al.,* 1992), there are very few cases where this element has been successfully used in the direct reduction of pest populations and crop damage. A recent exception relates to the pearl millet stem borer, *Coniesta ignefusalis* (Hampson) which holds immediate promise in the Sahel (Youm and Beevor, 1995).

Varietal resistance. Insect management through host plant resistance is recognized as a long-term control measure. Its success is highly dependent upon access to the world germplasm for systematic screening using insect bioassays that permit easy identification of resistant material and which guarantee reliable and consistent results. Screening techniques and resistance identification parameters have been developed and standardized at ICRISAT for the major pests (Sharma *et al.,* 1992). These methods have been used to screen over 30,000 accessions from the world sorghum collection to identify shootfly (60), stem borer (72), midge (3()), and head bug (18) resistant sorghums (Table 1).

In a recent review, Sharma (1993) provided information on the use of resistant sorghum cultivars in IPM in different ecosystems (Table 1). Apart from sorghum midge, where there has been remarkable success in India, Australia and the USA, in developing high yielding midge resistant sorghums, and stem borer tolerant selections such as Maldandi and Serena which are widely cultivated by farmers in India and Eastern Africa, respectively, sorghum insect pests have not yielded to successful conventional resistance breeding approaches.

TABLE 1. Sources of resistance to major sorghum insect pests (PRIVATE)

Insect group	Total Accessions screened ^1	Number of released Selected for resistance^2	germplasm and improved cultivars^3
Shoot fly	31,000	60	134
Stem borers	30,000	72	182
Midge	18,000	30	70
Head bugs	18,000	18	24
Aphids		-	128
Leaf feeding insects		-	45

1 At lCRISAT
2 At ICRISAT
3 World-wide (Sharma, 1993)

Over 99% of the genotypes listed by Shanna (1993) are described as "highly promising", have"good potential"or are "superior to susceptible checks" but have not gone beyond research stations onto farmers' fields. Basically, resistance levels are either too low to result in significant genetic improvement when transferred into agronomically improved cultivars or when high, conventional breeding techniques have not yielded agronomically desirable products.

In almost all cases, our knowledge of resistance mechanisms and factors and the bases of gene action and inheritance is not lacking. The range of morphological, physiological and chemical factors or traits clearly indicates an area that has been extensively studied (Tables 2 and 3). In spite of th~s, these traits present problems for traditional breeding approaches. Apart from the fact that cultivated sorghums lack sufficient levels of resistance to major insect pests, resistance traits are quantitatively inherited and have been difficult to manipulate (Stenhouse, 1991). An immediate question is: can existing knowledge and material be exploited in ways other than traditional breeding methods'?

PROSPECTS FOR BIOTECHNOLOGY IN SORGHUM RESISTANCE TO INSECTS

Possibilities for achieving higher levels of resistance in sorghum need to be explored because host plant resistance approach to insect management has very good potential both in terms of environmental sustainability and acceptance by small farmers. Wild germplasm resources have been exploited for developing resistant varieties of major crops.

TABLE 2. Factors/traits associated with resistance in sorghum to shoot pests

Factors/Traits	Selected reference	Shoot pest^1
Seedling vigor	Maiti *et al.*, 1994	SF, SB
Internode elongation	Taneja & Woodhead, 1989	SF, SB
Leaf glossiness	Maiti & Bidinger, 1979	SF
Leaf surface wetness	Nwanze, *et al.*, 1990	SF
Epicuticular wax	Nwanze. *et al.*, 1990; Bernays *et al.*, 1983	SF, SB
Trichomes	Blum, 1968	SF
Silica bodies	Blum, 1968	SF
Ligular hairs	Bernays *et al.*, 1983, 85	SB
Total phenols, lignins	Khurana & Verma, 1982	SB
Sugar content	Swarup & Chaugale, 1962	SB

SF = Shoot fly
SB = Stem borer

TABLE 3. Factors/traits associated with resistance in sorghum to panicle feeding insects

Factors/traits	Selected reference	Panicle pest^1
Glume characters	Sharma, 1993 Sharma, *et al.*, 1990	SM, HB
Rate of grain development	Sharma *et al.*, 1990 Sharma *et al.*, 1993	SM, HB
Tannin content	Santos & Carmo, 1974	SM, HB
Panicle compactness	Sharma *et al.*, 1993	HB
Water: Carbohydrate ratio	Tour *et al.*, 1992	HB

SM = Sorghum midge HB = Head bug

These have not been easy because many interspecific barriers are encountered in the traditional process of gene transfer from wild to cultivated species. Various biotechnological tools are now available and are being explored with respect to shootfly. Also the availability of RFLP markers for genes controlling resistance traits would assist in making screening for them more precise, thereby speeding up the breeding process in cases such as sorghum midge. Borer resistance involves more complex traits whose mechanisms are less understood. Therefore, genetic engineering with novel genes would be a key component for incorporating borer resistance in sorghum.

ICRISAT and NARS can not undertake basic work on technology development because of the cost and complexity of biotechnological tools in sorghum research, They do, however, collaborate with advanced research institutions and laboratories for practical applications.

Interspecific hybridization. Over 340 accessions of wild relatives of sorghum belonging to sections *Chaeto, Hetero, Stipo, Para* and *Sorghum* were evaluated for resistance to shootfly at ICRISAT and seven accessions showed very high levels of resistance which in some was close to immunity (ICRISAT, 1988, 1989b; Prasada Rao *et al.,* 1991). One of these accessions, *S. dimidiatum* (IS 18945, 2n=10), was crossed with a cultivated type (IS 2146, 2n=20) with considerable difficulty. The distortion in segregation in the progeny continues to be high even in the F8 generation and the recovery of progenies with reliable resistance levels and good agronomic characters has been difficult. Fresh attempts are now being made for new crosses with *S. timorense (= S. australiensis)* (IS 18954, 2n=20). F2 plants will be protected and samples off F3 from progeny rows will be screened for resistance and gene mapping.

Should difficulties persist in direct hybridization, other techniques such as embryo rescue and protoplast fusion will be explored. Recently, new sorghum lines derived from tissue culture have been registered which carry such traits as insect resistance and acid soil tolerance (Godwin *et al.,* 1992).

Genetic transformation for borer resistance. The technology for the transfer of foreign genes to sorghum is advancing rapidly (see Bennetzen, 1995; and Kononowicz *et al.,* 1995). Work at ICRISAT is currently directed towards the standardization of protocols for routine transformation of a variety of materials. We are exploring at least three techniques in collaboration with advanced institutions: (a)direct gene transfer to protoplasts: plants can now be successfully generated from mesophyll protoplasts of sorghum and we are in the process of standardizing protocols for direct DNA uptake; (b) use of particle gun for transformation: initial attempts to transform sorghum by bombarding immature embryos (Casas *et al.,* 1993) have been successful. We have also successfully obtained transformants by bombarding embryonic calli derived from protoplast culture; (c) use of *Agrobacterium* for transformation (Gould *et al.,* 1991): this work is being done in collaboration with the University of Queensland, Australia, where direct injection of plasmids into shoot tips is in progress.

More than one method may be used for gene transfer to a single genotype because this can increase the frequency of transformation as shown for canola (Chen and Beversdorf, 1994).

Lack of access to gene constructs can hinder progress towards finding the right gene with the potential for incorporation of resistance into improved sorghums. The IRRI-led consortium for acquiring synthetic CryA (b) genes from *Bacillus thuringiensis* subspecies *kurstaki* from the Plantech Research Institute, Tokyo, is a new dimension for the IARCs. This offers ICRISAT access to plasmid pBT1291 containing the Bt. gene under the control of cauliflower mosaic virus 35S promoter, the castorbean catalase intron, and nopaline synthease terminator.

BIOSAFETY CONCERNS

Various aspects of biosafety and biosafety regulations have been extensively covered in this workshop and no attempt will be made to duplicate that effort in this paper. Few countries in SSA have specific guidelines pertaining to safety regulations for biotechnological products basically because modern biotechnology is in its infancy in most, if not all countries of SSA. This, however, does not reflect a lack of awareness. The immediate concerns associated with biotechnologically engineered sorghum are: (i) fear of genetic erosion arising from random crossing with wild sorghums, (ii) environmental impact of genetically engineered sorghum whose center of origin is Africa, and (iii) lack of containment facilities and the necessary personnel and therefore the ability of NARS to test and evaluate such products without compromising their national priorities.

Other concerns not directly related to biosafety and the environment but are of relevance to the African situation consist of: (i) the fear that biotech products could replace traditional export products, (ii) biotech products would fall in the hands of powerful agribusiness who can influence government decision making process, and (iii) biosafety guidelines may be over stringent in many countries and difficult to implement.

In conclusion, biotechnologically engineered sorghum should be viewed as an at tempt to increase the level of resistance in sorghum to insect pests and, therefore, is a component in IPM. Our primary concern is that extremely high levels of resistance introduced by the incorporation of a single gene may not be sustainable as is known from conventional breeding in other crops. It will, therefore, be necessary to rotate resistant genes, or at least deploy crystal proteins with new properties.

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