PN-ACD-011
98422

FINAL REPORT

1 Cover Sheet

Covering Period September 8, 1992 to September 7, 1997

Submitted to the Office of Agri & Food Security, Bureau for Global Programs, Field Support, U S Agency for International Development

Use of Biotechnology for improving Hessian Fly Resistance in Wheat

Principal Investigator Hari C Sharma
Grantee Institution Purdue University

Collaborator Ouafae Benlhabib
Institution Institut Agronomique et Veterinaire, Hassan II, Rabat, Morocco

Project Number 10 276
Grant Number HRN-5600-G-00-2032-00

USAID Grant Project Officer Judy Chambers

Project Duration September 8, 1992 to September 7, 1997

2 Table of Content

	Page
Executive summary	3
Research Objectives	6
Methods and Results	7
Impact, Relevance and Technology Transfer	11
Project Activities and Output	12
Project Productivity	17
Future Work	18
Literature Cited	18

3 Executive Summary

Wheat is a key food crop for people of the world Insect pests and diseases cause losses and instability in global wheat production Hessian fly is a pest of wheat that causes major losses in wheat production in Morocco and other countries including the US Genetic resistance is the only effective, economical and environmentally friendly means to control damage to wheat in countries with Mediterranean climate, such as Morocco, and new sources of genetic resistance to this biotic stress are needed Barley yellow dwarf virus (BYDV) is the most serious viral disease of wheat worldwide, including Morocco and the US, for which no resistance genes have been found in cultivated wheat thus necessitating wide crossing Genera and species related to cultivated wheat are reservoirs of variability for useful traits for unique germplasm enhancement Much of the world's genetic information is carried in species found in developing nations and wild species are the subject of choice for disease and pest resistance but the scientists need to learn to apply biotechnology to exploit these reservoirs of genetic variability Rapid advances in technology can make it difficult for the professionals from developing countries to keep abreast of change Development of biotechnology and the enlargement of wheat gene pool through it are often beyond the expertise of developing countries and little has been accomplished to integrate the tools of biotechnology in plant breeding programs in Morocco This PSTC project was between Institut Agronomique Veterinaire (IAV) Hassan II, Morocco, and Purdue University, USA The USPI was Dr Hari Sharma and the Moroccan collaborator was Dr Ouafae Benlhabib The goal of our research was to enlarge the gene pool of wheat for resistance to Hessian fly and possibly to other biotic stresses by wide crosses between wheat and wheat relatives using tissue culture and cytogenetic techniques, and to transfer technology in these areas of agricultural sciences to Morocco We wanted to obtain and characterize plants from wheat x Agropyron (Thinopyrum = wheatgrass) crosses, through anther (pollen) culture and backcrossing, that contained the wheat chromosome complement plus one or two Agropyron chromosomes We wanted to determine the genetic control of resistance to Hessian fly in primitive diploid progenitor (Triticum monococcum) of wheat We wanted to evaluate the germplasm of wild and primitive wheats and that from wheat x Agropyron crosses to Hessian fly in Morocco to identify resistant lines and hybridize them with wheat for gene transfer The grant was to provide support for the grantee's program and enhance collaboration and technology transfer between Purdue University, USA and IAV Hassan II, Morocco We wanted to establish a germplasm enhancement program through biotechnology in Morocco involving evaluation of diverse genetic resources of wheat relatives for useful genes, and gene transfer through interspecific crosses employing embryo rescue, anther culture and chromosome analyses The research involved both lab and field work

Overall we consider the project a 'success' We made considerable progress in accomplishing our goals Results were published in refereed journals, presented in professional meetings or manuscripts submitted/being prepared in a collaborative manner in the areas of anther culture, embryo rescue, gene introgression in wide crosses, inheritance of resistance to Hessian fly and other traits in diploid wheats, reaction of wide cross derivatives to BYDV, cytogenetic analysis of interspecific crosses, isolation of single chromosomes or genomes of Agropyron species in wheat, development and characterization of alien addition and substitution lines, release of improved germplasm, development and characterization of putative translocations from wide crosses, chromosome elimination in anther culture of wide crosses, identification of wheat relatives as sources of new genes for resistance to Hessian fly and crossing of some of them to Moroccan wheat for germplasm enhancement (Please see Publication list in Project activities/Output section of this report)

Anther culture technology was fine-tuned, and applied to Agropyron species and their intergeneric hybrids with wheat The wide hybrids were completely male sterile and almost female sterile Sterility of the intergeneric hybrids and non-culturability of their wheatgrass parents resulted in low or no response to anther culture The callus induction ability of wheat genotypes expressed in the wide hybrids and was influenced by the wheatgrass species Anther culturability improved as the number of Agropyron chromosomes decreased and fertility improved in backcrosses Large variation was found for chromosome numbers and chromosome doubling occurred in the callus roots 24% of the regenerants and callus roots had 22 or 44 chromosomes indicating that chromosome number of wide hybrid derivatives can be reduced and alien addition lines can be isolated 43-chromosome plants could be obtained in BC3 generation by backcrossing method also but one more generation of selfing was needed to isolate disomic alien addition lines compared to anther culture method The wheat x wheatgrass hybrids were advanced through backcrossing, and single wheatgrass chromosomes were added/substituted in wheat genome Lines with 44 chromosomes were characterized to be addition lines and those with 42 chromosomes substitution lines Excellent levels of resistance to BYDV were found in these lines and a substitution line from wheat x A intermedium cross was released as germplasm for breeding Putative translocation lines were developed from substitution lines and some of them were characterized and evaluated for reaction to BYDV To determine if the addition lines and the substitution lines isolated involved the same chromosome, intercrosses were made and analyzed cytogenetically which showed that the two substitution lines that were used to develop translocation lines contained the same Agropyron chromosome that replaced chromosome 7D of wheat Similarly, two alien addition lines were found to involve the same alien chromosome Four substitution and two addition lines were found resistant to Hessian fly in Morocco 11 putative translocations are being tested in Morocco All this material will be useful for increasing Hessian fly resistance in Moroccan wheat 358 accessions of wheat relatives were evaluated against field populations of Hessian fly in Morocco Some of the 42 accessions found resistant were successfully crossed to wheat following Purdue University protocols for crossing and embryo rescue for enlarging the gene pool of Moroccan wheat Genetic analysis of resistance to Hessian fly in diploid wheat was carried out which showed simple genetic control and hence feasibility of gene transfer to cultivated wheat Backcrossing of the interspecific hybrids to wheat for germplasm development in Morocco is continued

The project led to technology transfer and its application to Morocco It helped Morocco acquire plant biotechnology training to scientists and students The US and Moroccan collaborators remained in constant touch regarding working with techniques, results, supplies, literature and joint publications During the tenure of this project, we evolved from training to active partnership and collaboration to independent research by the Moroccan collaborator The project enhanced the capacity of Morocco to conduct innovative research and contributed to the introduction of biotechnological tools in the breeding program in Morocco which was still using the conventional methods It fulfilled distinct need for a biotechnological collaboration that encouraged the developing country's researchers to focus on local problems, namely, germplasm evaluation and enhancement for pest resistance through the use of wide crosses in this project The project enabled Morocco to integrate biotechnology with conventional research for wheat improvement Dr Benlhabib came to Purdue in 1993 and received the necessary training in embryo culture, anther culture, and chromosome analysis of wide crosses, Hessian fly testing, and use of materials, reagents and equipment to do similar research in Morocco The necessary equipment and supplies were purchased for Ouafae's lab The USPI visited IAV Hassan II in 1994 and imparted hands-on training and guidance to her and her students, planned experiments, presented a seminar, and visited INRA's wheat breeding program at Settat in relation to integrating Ouafae's research with wheat breeding

This package of technical training and consultation, literature and discussion, conducting experiments together, reciprocal visits, and equipment and supplies constituted the major phase of technology transfer to Morocco for wheat improvement and achieved the level of collaboration and success in technology transfer This enabled Ouafae to actively participate in the project, and develop wheat germplasm program through biotechnology impacting wheat production in her country The germplasm enhancement program through wide crosses set up in Morocco extends from collection of wild species all the way to incorporation of useful genes from these species into wheat varieties that farmers grow in Morocco The project encouraged her to collect the biodiversity of wheat relatives in her country which will be useful in germplasm enhancement for Morocoo Interspecific hybridization, embryo rescue, chromosome analysis and anther culture techniques were used in local research program in Morocco 239 seeds of wheat x Agropyron hybrid derivatives from Purdue were analyzed successfully for chromosome numbers, and 7888 anthers from backcross plants were cultured and a callus induction frequency of 3% was obtained in Morocco Dr Benlhabib is extensively applying the cytogenetic and tissue culture procedures to produce interspecific hybrids and backcrosses of wheat with T monococcum and Aegilops species identified to be resistant to Hessian fly to develop germplasm, including that of durum wheat which is grown widely in Morocco but of which there are no Hessian fly resistant cultivars New germplasm produced through the interspecific hybridization is being prepared for further testing and use to breeders and scientists nationwide Thus, the project established a sound wide crossing program for germplasm enhancement involving lab, greenhouse and field research to augment the national breeding program The project enhanced the research capabilities of Moroccan investigator As a result of the project, Ouafae's lab was transformed and the research facilities in her lab became reasonably adequate as a result of equipment and supplies procured and put to use through this project which now remain in Morocco Five students worked in the project related research and defended their theses This attests to the success of our efforts in technology transfer Dr Benlhabib visited Purdue University during 1996 regarding consultation and discussion on results and her experience with the use of techniques in Morocco, and she confidently participated in lab and field activities From my visit to Ouafae's lab in April, 1997, I can say with confidence that the project will leave a permanent effect to carry out independent research of this kind for wheat improvement in Morocco The technology transferred to Morocco will also be useful in teaching The project will continue to impact creation of new knowledge in Morocco by research and use of that in agricultural development As the research program established at IAV and integrated into the breeding program in Morocco moves forward, it will impact this important food crop

The grant award has been very encouraging for international cooperation and development and for the USPI's research We are very pleased with our accomplishments and collaboration We are very thankful to AID/BOSTID for approving our project and providing assistance throughout its execution We would like to avail ourselves of the opportunity in future for continuation of the linkage that has been established, and a Moroccan molecular biologist and a virologist are ready to be a part of a future collaborative research We would appreciate receiving AID announcements soliciting research grant proposals in future

4 Research Objectives

Human population growth places increasing demand on the world's food crops Wheat provides more nourishment for people of the world than any other food source and it is a key food crop both in Morocco and the U S Insect pests and diseases cause losses and instability in global wheat production Hessian fly (Mayetiola destructor) is wide spread in North Africa as well as in the US (Maxwell and Jennings 1980, Lhaloui et al 1992, Ohm et al 1995) This pest constitutes a major constraint to wheat production in Morocco where national surveys find more than 80% infestation each year (Amri et al 1992) Genetic resistance is the only effective, economical and environmentally friendly way to control Hessian fly damage to wheat in Mediterranean climate of Morocco where cultural practices like fly-free planting date have limited use However, Moroccan wheats lack resistance to this pest Of the several resistant genes in the U S , only five have been found to be effective against Moroccan population of the fly (El Bouhssini et al 1985, Gallagher et al 1987, Obanni 1989) Whereas these genes are being used in wheat breeding since a few years, there is only one common wheat (T aestivum, 2n=AABBDD=42) cultivar carrying the H5 gene and no durum wheat (T durum, 2n=AABB=28) resistant cultivar is available to Moroccan growers Furthermore, genes for resistance in wheat are frequently overcome by new biotypes e g biotype L has become the most prevalent biotype in Indiana Thus new sources of resistance must be sought in wheat and its relatives to stay ahead of fly biotype evolution Genes derived from wheat relatives could be different and more durable Barley yellow dwarf virus (BYDV) is the most serious viral disease of wheat worldwide, including Morocco and the US, for which no resistance genes have been found in cultivated wheat thus necessitating wide crossing In Morocco, BYDV causes 11-26% yield losses in wheat (Yamani and Hill 1991) Gene pools of related species of cultivated wheat are reservoirs of variability for useful traits for unique germplasm enhancement (Knott and Dvorak 1976, Kerber and Dyck 1978, Sharma et al 1981, 1984, 1992, Sharma 1995a, and others) They are going to be more and more in need for crop improvement, especially for genes that are not found in our crop species The concerted use of traditional and more recent methods of genetic manipulations should contributor to wheat improvement through wide crosses between wheat and its related species However, development of biotechnology and its use in enlarging the gene pool are often beyond the current expertise of developing countries where little has been accomplished by way of integrating the tools of biotechnology in breeding programs and production of agricultural commodities (Tzotzos 1996) The overall objective of our project was to enlarge the gene pool of wheat, particularly for resistance to Hessian fly and also to BYDV by wide crosses between wheat and its related alien species employing tissue culture and cytogenetic techniques, and to transfer technology to Morocco in these areas Specifically, we wanted to obtain and characterize plants, from wheat x Agropyron (Thinopyrum = wheatgrass) crosses through anther (pollen) culture and backcrossing, with wheat chromosome complement plus one or two Agropyron chromosomes We wanted to determine the genetic control of resistance to Hessian fly in primitive diploid wheat Triticum monococcum We wanted to evaluate wild and primitive wheats, and wheat x Agropyron derived lines to Hessian fly in Morocco to identify resistant lines, and hybridize them with wheat for germplasm development The grant was to provide support for the grantee's program and enhance collaboration and technology transfer between Purdue University, USA and IAV Hassan II, Morocco

Agropyron species are useful sources of alien variation for wheat improvement (Wienhues 1973, Wang et al 1980, Soliman et al 1980, Sharma and Gill 1983, Sharma et al 1984, Whelan and Conner 1989, Jiang et al 1994, Sharma 1995a, and others) and our results showed several

Agropyron species to be resistant to the most virulent biotype of Hessian fly (Sharma et al 1992) and to the most potent and prevalent isolate of BYDV (Sharma et al 1989) Based on genomic affinities, whereas wild and primitive wheats have the same genomes as cultivated wheat, Agropyron species are distantly related to wheat and their chromosomes do not pair with wheat chromosomes due to lack of homology Thus, individual chromosomes from Agropyron have to be added into wheat before gene transfer can be accomplished Producing alien addition lines for gene transfer by conventional method is very time taking and in some instances an alien addition line may not be recovered (Islam and Shepard 1981, Lukaszewski 1988, Sharma 1996) Thus improvement of wheat by wide crossing from wheat-Agropyron crosses is a slow process and efficient methodologies are needed to hasten this process Anther culture holds potential to produce addition lines and somaclonal variation from wide crosses However, information is lacking on anther culture response and cytogenetic nature of pollen derived calli and regenerants of wide crosses We produced intergeneric hybrids between wheat and Agropyron species (Sharma et al 1989) We identified responsive genotypes of wheat for anther culture (Lu et al 1991) some of which were parents in wheat x Agropyron hybrids Zhuang et al (1985) and Wang and Hu (1985) had reported callus induction and regeneration from anther culture of intergeneric hybrids in the wheat group We applied anther culture technology to wheat x Agropyron wide crosses to investigate their response and the feasibility of inclusion of alien chromosomes in their pollen such that plants with a haploid set of wheat chromosomes plus one or more Agropyron chromosomes could be recovered In the event we could not recover addition lines via anther culture, the same were to be obtained by backcrossing We had identified three accessions of T monococcum for resistance to biotype L of the Hessian fly (Sharma et al 1992) Genetic basis of this resistance which has a bearing on the use of this resistance for germplasm development for resistance to Hessian fly was unknown Inheritance studies otherwise also have been very limited in diploid wheats (Kuspira and Bhanbhani 1986, Sharma and Waines 1994) and inheritance of Hessian fly resistance had not been investigated in diploid wheat before We investigated the genetics of this resistance Evaluation of germplasm in Morocco provided a different environment and fly populations and for starting a similar research program of germplasm enhancement by biotechnology to impact wheat production in Morocco

5 Methods and Results

For anther culture, anther donors were A elongatum (Th ponticum) cv Jose, A trichophorum (Th trichophorum) cv Luna, A intermedium (Th intermedium) cv Oahe, their F1 hybrids with wheat (female), and BC1, BC2, BC2F2 derivatives (Sharma et al 1989 1995) BC1 plants of A caninum x wheat (Sharma and Baenziger 1986) were also used Anther culture procedure, in general, was the same as followed for wheat (Jones and Petolino 1987, Metz et al 1988, Lu et al 1991) One-two spikes from each plant were used for backcrossing to wheat Chromosome numbers were counted in root-tip cells of not only donor plants but also of regenerated plants and calli, and of plants derived by backcrossing the anther donor plants Since different wheat cultivars were used in backcrossing depending on the availability for the irregularly flowering wide crosses, morphology was not useful in grouping the alien addition lines and the alien substitution lines that were isolated The addition lines were intercrossed and chromosome pairing behavior of the hybrids was studied in pollen mother cells If the hybrids showed 22 bivalents (22") of chromosomes as the predominant configuration at meiotic metaphase-I, the alien addition lines were classified as having the same Agropyron chromosome Plants having 21", Agropyron trait (BYDV resistance) and chromatin were crossed to wheat A chromosome pairing of 20"+2' (2 univalents) in the hybrids indicated that substitution has occurred Substitution lines were intercrossed Substitution lines whose hybrids showed 21" were

classified to be the same These results were further confirmed by RFLP analysis (Sharma et al 1995) and in situ hybridization using wheatgrass genomic DNA as probe (Sharma et al submitted)

To determine the genetics of resistance to Hessian fly in T monococcum, hybrids were produced between homozygous resistant accessions of T monococcum and homozygous susceptible accessions of T monococcum and T boeoticum which are subspecies of the same biological species (Sharma and Waines 1994) From the hybrids, F3 and TC1F2 progenies were produced and tested for resistance in controlled environment against biotype L of the fly at Purdue University according to the method described by Foster et al (1988) and Sharma et al (1992)

To evaluate of germplasm for Hessian fly resistance in Morocco, accessions of Triticum species and Aegilops species, and material developed at Purdue from wheat x Agropyron crosses were grown in field at Jemaat Shim which is known for natural infestation of the fly Each entry was planted in two rows, 3 ft long, 1 ft apart During April, the plants were uprooted while they were still in vegetative stage Plants with live larvae and stunting were declared susceptible Plants with dead larvae and no stunting were declared resistant Absence of larvae indicated 'escapes' For gene transfer of Hessian fly resistance, crosses were made between wheat and resistant accessions of T monococcum and Aegilops species A backcross program was followed using wheat as the recurrent parent Hybridization, embryo rescue and cytogenetic techniques were employed by the Moroccan investigator as learned by her during her visits to Purdue and as assisted by the USPI through correspondence and during his visits to Morocco

The research was coordinated in such a way that the Moroccan scientist/students got hands-on experience/training of different aspects of the research During the whole period of the project, the US and Moroccan collaborators remained in constant touch through letters, faxes, and recently through e-mail also, regarding execution of techniques, research results and progress, joint publications, travel arrangements, literature, functioning of equipment, shipping and receipt of equipment, spare parts, reagents and supplies Consultation, training and travels were utilized to achieve the level of collaboration in the project and success in technology transfer

The data on wheat anther culture given in the grant proposal was published, and wheat genotypes responsive to anther culture were identified (Lu et al 1991) Anthers of Jose, Luna and Oahe of Agropyron species were not responsive From 2836, 755 and 300 anthers of hybrids of these genotypes with wheat, mean callus induction was 0%, 0 26% and 0%, respectively (Sharma et al 1994a) The F1 plants were completely male sterile giving backcross seed set as low as one seed per 200 florets pollinated Sterility of the intergeneric hybrids and non-culturability of their wheatgrass parents could largely be responsible for the low or no response Due to a very high female sterility of the hybrids, only a few backcross (BC)1 seeds were obtained and these were used to produce BC2 seed sexually instead of using them for anther culture and running the risk of dead end situation Plants with chromosome numbers similar to those of BC1 were available in BC2 population for anther culture A caninum x wheat BC1F2 anthers gave 4 8% callus induction but did not produce any plantlets Pioneer 2550 (wheat) x Luna F1 had the highest (2%) callus induction Pioneer 2550 was the best responder to anther culture (Lu et al 1991) Thus, anther culturability expressed in the wide hybrids Of 9294 anthers from 190 BC2 plants cultured, 1 6% formed calli, and 1 3% calli regenerated into plantlets These were mixoploid (2n =42, 45) or haploid (2n = 22) From 2160 anthers of BC3 plants derived from the BC2's that had shown response to culture, 1 2% formed calli and 8% calli regenerated into plantlets Anther culturability improved as the number of Agropyron chromosomes decreased and fertility improved by backcrossing Wheat x A trichophorum cross was

the best responder giving up to 37% callus induction from some of the BC2 plants Large variation was found in the callus roots for chromosome numbers and chromosome doubling was occurring as cells with double and half chromosome numbers were found 24 1% of the regenerants and callus roots had 22 or 44 chromosomes 43-chromosome plants were obtained in BC3 generation by backcrossing method but one more generation of selfing was needed to isolate disomic addition lines This provided an evidence that the chromosome number of wide hybrid derivatives can be reduced efficiently to a desirable level by anther culture, and by spontaneous chromosome doubling or colchicine treatment of 22-chromosome plants, alien addition lines from wide crosses can be isolated for germplasm enhancement by wide crossing (Sharma et al 1996a, Sharma et al in prep a)

Seedlings from 239 seeds of wheat x Agropyron hybrid derivatives produced at Purdue were analyzed successfully in Morocco for chromosome numbers, and 7888 anthers from BC1, BC2 and BC3 plants with higher than 44 chromosomes were cultured A callus induction frequency of 3% was obtained Experience for this was acquired by Dr Ouafae Benlhabib during her 1993 visit to USPI's lab She set up, fine-tuned, and carried out this work and published the results (Benlhabib et al 1996a) Additionally, Aegilops species were used and 0-8% callus induction response was obtained This attests to technology transfer success

The anther culture response was low but it is typical of cereals There has been little success in anther culture of wheatgrasses Marburger and Wang (1988) obtained only albino plants from Th ponticum Wang et al (1991) and Niu et al (1991) obtained some green haploids from only two of the several species Wang et al (1975) obtained mostly albino or partly green plants from a 56-chromosome hybrid of T vulgare x Th trichophorum Recently, Jia et al (1993) obtained up to 5% regeneration from anther culture of wheat x Th intermedium hybrids According to Wei et al (1993), it was difficult to induce callus from anthers of wheat x Leymus multicaulis derived plants grown in greenhouse and callus induction frequency from field grown plants reached 5% In an effort to improve anther culturability of wheat x Agropyron hybrids, we studied co-culture of anthers with their ovules in five BC populations Overall pollen callus induction was 1 6% without and 3% with co-culture indicating some beneficial effect of ovules on anther culturability (Sharma et al 1996b, Sharma et al in prep b)

Wheat x wheatgrass hybrids were advanced through backcrossing Our results of these wide crosses underscored the significance of paternal genotypes of alien species and cold treatment of rescued embryos (Sharma and Ohm 1990) BC1 seed set was only 0 5-10% depending on species Comparison of chromosome numbers of the BC1 and BC2 seedlings indicated wheatgrass chromosome elimination A significant improvement in fertility was achieved in BC2 By coupling backcrossing, cytology and BYDV resistance testing, fertile backcross derivatives with one or two wheatgrass chromosomes were isolated Disomic alien addition lines (2n=44) were obtained by selfing the monosomic alien additions In addition to whole chromosome addition lines, some telocentric addition lines were also isolated In the process of backcrossing/selfing, chromosome substitution also occurred and some alien substitution lines were recovered Alien addition and alien substitution lines developed from wheat x Th intermedium cross were characterized They had regular meiotic behavior as expected Resistance to BYDV from Agropyron and a chromosome pairing of 20" + 2' in the hybrids of substitutions with wheat provided evidence of substitution wherein the wheatgrass chromosome replaced chromosome 7D of wheat This was based not only on chromosome pairing but also on DNA analysis (Sharma et al 1995) and in situ hybridization (Sharma et al submitted) Characterization and evaluation of these lines lead to a germplasm line P29 release for wheat improvement (Sharma et al 1997a) Cytogenetic analysis of the intercrosses

between two substitution lines showed that they contained the same Agropyron chromosome which replaced chromosome 7D of wheat Similarly two of the addition lines showed to have the same Agropyron chromosome (Sharma et al submitted)

The substitution and addition lines resistant to BYDV were irradiated to induce translocations Based on the segregation pattern in M2 generation, resistant plants from families that represented the putative translocations were followed Some of the translocation lines were characterized (Crasta et al 1996, Anderson et al 1997, Francki et al 1997, Sharma et al Submitted, Crasta et al In prep) Four substitution and 2 addition lines were tested in Morocco for Hessian fly All were found to be resistant Results of evaluation for BYDV resistance at Purdue showed that not only addition and substitution lines but also some translocation lines represented valuable germplasm with excellent levels of resistance to BYDV (Sharma et al 1997a, Sharma et al submitted) for which there is no other resistance source found so far 11 putative translocation lines were evaluated in Morocco for the fly resistance All were resistant but this needs to be confirmed because the fly attack in the field was not homogeneous

Evaluation of wheat relatives for reaction to Hessian fly at Purdue University that was in progress at the time of submitting the grant proposal was completed and the results were published (Sharma et al 1992) In addition to T monococcum, 13 accessions of Agropyron species and 13 accessions of Aegilops species resistant to the most virulent biotype (biotype L) of Hessian fly were identified For the genetic analysis of resistance to Hessian fly in diploid wheat T monococcum, in all, 4996 plants of 435 progenies from F2-derived and TC1-derived families involving resistant and susceptible lines were tested for their reaction to biotype L of Hessian fly The resistance was inherited as a simple Mendelian trait (Sharma et al 1997b) Simple genetic control for resistance to Hessian fly indicated thefeasibility of transfer of this resistance to wheat

A total of 358 accessions were tested in Morocco, excluding those that failed to germinate or give a satisfactory number of seedlings, of wild and primitive wheat germplasm These collections were from Purdue University, ICARDA and Morocco Forty-two accessions belonging to the species T monococcum, T boeoticum, Ae squarrosa, Ae longissima, Ae variabilis, Ae triaristata, Ae caudata, Ae triuncialis, Ae cylindrica, Ae ventricosa and Ae ovata were identified as resistant (El Bouhssini et al submitted) Some of these were hybridized to common and durum wheats and interspecific hybrids were developed following Purdue University' methodologies for crossing and embryo rescue (El Khlifi et al 1996, El Khlifi et al in prep) These included T monococcum, Ae ovata, Ae squarrosa, Ae triaristata and Ae ventricosa Cultivar Baraka was identified to be the best bread wheat for crosses with T monococcum (20% seed set) In wheat x Aegilops species crosses, the best wheat was Jori that gave 19% hybrid seed set and 16% plants after embryo Ae ovata and Ae aristata were more compatible than the other two species Crosses with wheat as the pollen parent always had more seed set than reciprocal crosses Backcrossing was necessary to produce seed from hybrids involving Ae ovata and Ae ventricosa Some of the durum x Ae squarrosa hybrids produced synthetic hexaploids (El Khlifi et al in prep) Hybrids and BC progenies were scored for morphology, chromosome composition and fertility Some of these were further backcrossed to wheat for developing Hessian fly resistant germplasm in Morocco Fertile advanced backcrosses will be selfed and homologous gene transfer from the A or D genome of T monococcum and Ae squarrosa, respectively, to wheat will be accomplished in future as this material is processed and screened to isolate desirable recombinants

Additionally, results were obtained that showed that several genes conferring resistance to Hessain fly in the US appear to occupy a single linkage block on wheat chromosome 5A (Ohm et al 1995) The support of this project for the USPI's program also helped him in writing a paper on the limits of wide crossing (Sharma 1995a), a paper on the role of students in research (Sharma 1995b), an invited paper on embryo rescue following wide crosses (Sharma submitted), participating with students and other scientists in locating disease resistance genes on wheat chromosomes (Hu et al 1996), and evaluating the near relatives of wild and primitive wheats for BYDV (Sharma et al 1997c) Please see Project activities/Output section for publication of the results

6 Impact, Relevance and Technology Transfer

The project provided developmental assistance to Morocco During the tenure of this grant, a germplasm enhancement program was started and established at IAV Hassan II for enlargement of the gene pool of wheat for pest resistance, particularly for Hessian fly resistance, by wide crossing through the application of tissue culture and cytogenetic techniques This program extends from collection of wild species all the way to incorporation of useful genes from these species into wheat varieties that farmers grow in Morocco This would impact wheat production in Morocco from the eventual development of new wheat cultivars resistant to Hessian fly and other stresses The interaction due to this project has certainly had a positive impact and several of the results and techniques are being used and some new leads for future research were found Considering the tedious and time taking nature of interspecific gene transfer, the practical impact of this research will be realized in future, but we have made a significant headway in this direction during the tenure of this project as we had proposed The project led to the identification and utilization of resistance for Hessian fly in wild gene resources for wheat improvement, especially for durum wheat which is grown widely in Morocco but of which there are no resistant cultivars The program in Morocco was developed to the point that the Moroccan PI is now independently able to conduct this research and complement the institute's accomplishments for agricultural developments The model can be followed for other traits in wheat and on other crops as well

The project promoted human resource development and built institutional strength for future independent gains by Morocco Rapid advances in technology can make it difficult for the professionals, especially from developing countries to keep abreast of change (Lindsay et al 1977) The US has invested considerable resources into the improvement of Moroccan agriculture and recently there has been more emphasis on technology transfer (Shroyer et al 1992) The project accomplished the technology transfer component with full success It brought into play new capabilities and enhanced Morocco's scientific capacity to conduct innovative research with new techniques to solve their serious agriculture problems The IAV Hassan II is the center for agricultural training and research in Morocco The scientists at this institute understand the local problems and potential of biotechnology to their problems Biotechnology is a recent addition to international crop improvement and to be effective it must be integrated with conventional agricultural research and developing countries often cannot keep pace with biotechnological agricultural research (Cohen 1993) The project fulfilled distinct need for a biotechnological collaboration between Morocco and the US that encouraged the developing country's researchers to focus on critical areas of local problems (germplasm evaluation and enhancement for pest resistance through the use of wide crosses, embryo culture, anther culture and cytogenetic techniques in this project) It introduced new biotechnological tools in the breeding program which was still using mainly the conventional methods Thus, this kind of research is unique and complementary for IAV

and INRA Interspecific crosses were made and used for the first time by Moroccan team to transfer the Hessian fly resistance Much of the world's genetic information is carried in species found in developing nations Many staple foods have their origins in these areas and wild species are the subject of choice for disease and pest resistance but the scientists have a need to learn to apply biotechnology to exploit these reservoirs of genetic variability The project encouraged Ouafae to collect the biodiversity of wheat progenitor species in her country which will be useful in germplasm enhancement for Morocoo (Benlhabib et al 1996b) The research on food crops and their relatives offers prospects of increasing yield and nutritional quality by combining new genetic traits using hybridization and gene transfer technologies The project offered training to Moroccan scientist and students in these areas and enabled them to apply these methods in their scientific endeavor of developing pest resistant wheat Interspecific hybridization, embryo rescue, anther culture, chromosome analysis and interspecific hybridization were put into practice which are now being employed routinely at IAV The Moroccan collaborator actively participated in the project, obtained encouraging results in Morocco leading to publications with Moroccan researchers as the senior authors (Benlhabib et al 1996a, 1996b, El Bouhssini et al submitted, El Khlifi et al 1996, El Khlifi et al in prep , Fatima et a in prep), and exhibited a great potential of future research in this area of biotechnology She is aggressively conducting research through the use of biotechnology to impact wheat production in her country The information transferred to Morocco will also be useful in teaching The project will continue to impact creation of new knowledge in Morocco by research and use of that in agricultural development Ouafae is now collaborating with the molecular biologist of her institute to find molecular markers for useful traits in related species for wheat improvement Wide cross progenies were produced locally and are being evaluated before their incorporation in cultivar development As a result of this project, Ouafae's lab was transformed The research facilities in her lab became reasonably adequate due to the equipment and supplies purchased through this project The equipment to be left behind for the independent continuation of the research program includes a microscope with a camera, an incubator, a computer, and a dissecting microscope Additionally, IAV should have books, reagents and supplies These equipments and supplies will enable Ouafae to continue the research Faculty and students at IAV are also using these facilities

In addition to identifying and exploiting alien germplasm for Hessian fly resistance, we developed BYDV resistant germplasm through a wide cross of wheat with a wheatgrass Some of the alien addition and substitution lines from wheat x A intermedium cross were evaluated for fly resistance in Morocco They were resistant to Moroccan population of Hessian fly and are being used in national wheat breeding program in Morocco Furthermore, translocations developed from the substitution and addition lines will be even more refined material for breeding

7 Project Activities/Output

The following papers were published, presented in professional meetings, or are in the publication process

Sharma HC and Ohm HW 1990 Crossability and embryo rescue enhancement in wide crosses between wheat and three Agropyron species Euphytica 49 209-214

Lu CS, Sharma HC and Ohm HW 1991 Wheat anther culture effect of genotype and environmental conditions Plant Cell, Tissue and Organ Culture 24 233-236

Sharma HC, Foster JE, Ohm HW and Patterson FL 1992 A note on resistance to Hessian fly (Mayetiola destructor) [Diptera Cecidomyiidae] biotype L in the tribe Triticeae Phytoprotection 73 79-82

Ohm HW, Sharma HC, Dweikat I, Mackenzie S, McFatridge D, Patterson FL, Shaner G, Lister R, Huber D, Buechley G, Ratcliffe R, Shukle R, Safranski G and Cambron S 1993 Indiana, Purdue University Annual Wheat Newsletter 39 269-277

Sharma HC, Benlhabib O, Ohm HW and Lu CS 1994a Anther culture response of wheat and wheat x wheatgrass hybrids 1994 Congress on Cell and Tissue Culture (Abstract P-1090 PO)

Sharma HC, Ohm HW, Lister RM and Benlhabib O 1994b Reaction of wheat -Agropyron hybrids derivatives to BYDV Barley Yellow Dwarf Newsletter 5 10

Sharma HC, Ohm HW, Goulart L, Lister R and Appels R 1994c Introgression of BYDV resistance from a wheatgrass into wheat 2nd Intl Conf, Plant Genome II, p 61

Sharma HC and Waines JG 1994 Inheritance of leaf pubescence in diploid wheat J Hered 85 286-288

Ohm HW, Sharma HC, Goulart L, and Lister R 1994 Introgression of barley yellow dwarf virus resistance from wheatgrass into wheat Indiana Crop Improvement Association Conf, Indianapolis, Feb 3-4 (Poster presentation)

Bentika A 1994 Evaluation of durum and wild wheats for resistance to Hessian fly MS Thesis IAV Hassan II, Morocco

Hmoud R 1994 Analysis of chromosome numbers and plant morphology of backcross derivatives from wide crosses between wheat and Agropyron species MS Thesis, IAV Hassan II, Morocco

Sharma HC 1995a How wide can a wide cross be? Euphytica 82 43-64

Sharma HC 1995b Publication trends in Crop Science journal and role of students in research publications J Natural Resources and Life Sci Education 24 197-199

Sharma HC, Ohm HW, Goulart L, Lister R, Appels R and Benlhabib O 1995 Introgression and characterization of barley yellow dwarf virus resistance from Thinopyrum intermedium into wheat Genome 38 406-413

El Ouafi I 1995 Caracterisation et evaluation d'une collection Marocaine d'Aegilops spp Et de certaines accessions T monococcum, croisements interspecifiques ble (T durum, T aestivum) x T monococcum These de 3eme cycle, IAV Hassan II

Houari A 1995 Evaluation de la resistance a la Cecidomyiidae d'un germplasme de ble et d'especes apparentees et etude des mecanismes de resistance de la varietes Massira These de 3eme cycle, IAV Hassan II

Rafiqi K 1995 Analyse d'hybrides intergeneriques T durum x Aegilops spp et tests preliminaire de production de plantes haploides chez le ble dur et les Aegilops These de 3eme cycle, IAV Hassan II

Ohm HW, Sharma HC, Patterson FL, Ratcliffe R and Obanni M 1995 Linkage relationships among genes on wheat chromosome 5A that condition resistance to Hessian fly Crop Sc 35 1603-1607

Ohm H, Anderson J, Berzonsky W, Dweikat I, Mackenzie S, McFatridge D, Patterson FL, Sharma HC, Buechley G, Huber D, Lister R, Perry K, Shaner G, Maas F, Ratcliffe R, Shukle R, Safranski G, Cambron S and Stuart J 1995 Indiana, Purdue University Annual Wheat Newsletter 41 225-232

Sharma HC and Ohm HW 1995 Wheatgrass based BYDV resistance in wheat USDA-ARS BYDV Workshop St Louis, Mo, May 10-11 p 7

Sharma HC, Benlhabib O, Jekkel Z and Ohm HW 1996a Anther culture and concomitant ovule culture in wheat x wheatgrass hybrids and wheat Am Soc Agronomy 88 160

Sharma HC, Ohm HW, Jekkel Z and Benlhabib O 1996b Effect of concomitant ovule culture on anther culturability of wheat and wheatgrasses 1996 World Congress on In Vitro Biology, San Francisco, CA, p 84

Benlhabib O, Hmoud R and Sharma HC 1996a Analyse cytogenetique de descendants intergenerique entre Triticum aestivum et Agropyron spp Actes IAV, Maroc 16 27-32

Benlhabib O, Nachit M and El Bouhssini M 1996b Collecte et utilisation des especes Aegilops apparentes dse cerels In Proc SEWAN Network Meetings, IAV Hassan II, Rabat, Dec 6-7

Sharma HC 1996 Maintenance of haploid genome of Agropyron junceum in wheat Wheat Information Service 83 21-24

Hu X, Bostwick D, Sharma HC, Ohm HW and Shaner G 1996 Chromosome and chromosomal arm locations of genes for Septoria glume blotch in wheat cultivar Cotipora Euphytica 91 251-257

El Khlifi O, Benlhabib O, Nachit M and Sharma H 1996 Croisement interspecifique entre le ble dur (Triticum durum) et differentes especes d' Aegilops In Proc SEWAN Network Meetings, IAV Hassan II, Rabat, Morocco, Dec 6-7

Crasta O, Francki M, Greene A, Bucholtz D, Sharma HC, Ohm HW and Anderson J 1996 Molecular characterization of Thinopyrum chromatin in wheat toward targeted mapping 2nd Int Crop Sci Congress, Nov 17-24, New Delhi

Sharma HC, Ohm HW and Perry K 1997a Registration of barley yellow dwarf virus resistant wheat germplasm line P29 Crop Sci 37 1032-1033

Sharma HC, Ohm HW, Patterson F, Benlhabib O and Cambron S 1997b Genetics of resistance to Hessian fly (Mayetiola destructor) biotype L in diploid wheats Phytoprotection 78 61-65

Sharma HC, Ohm HW and Lister R 1997c Reaction of near relatives of wheat to P-PAV isolate of BYDV BYD Newslett 6 7-8

Anderson J, Crasta O, Francki M, Bucholtz D, Sharma HC and Ohm HW 1997 Molecular and cytogenetic analysis of potential translocation lines containing Thinopyrum intermedium chromosomal segments Plant and Animal Genome 5 90

Ohm H, Anderson J, Berzonsky W, Dweikat I, McFatridge D, Patterson FL, Sharma HC, Buechley G, Goodwin S, Huber D, Perry K, Shaner G, Cambron S, Maas F, Ratcliffe R, Shukle R, Stuart J and Williams C 1997 Indiana, Purdue University Annual Wheat Newsletter 43 http //www ksu edu/wheatpage/

Francki M, Crasta O, Sharma HC, Ohm HW and Anderson J 1997 Structural organization of an alien group 7 Thinopyrum intermedium chromosome in US soft winter wheat Genome 40 716-722

Sharma HC Embryo rescue following wide crosses In Plant cell culture protocols, Molecular bilogy series In press (submitted by invitation of the editor)

Anderson J, D Bucholtz, A Greene, M Francki, S Gary, H Sharma, H Ohm and K Perry Characterization of wheat-derived barley yellow dwarf virus resistance in wheat alien chromosome substitution line Phytopath Accepted for publication

El Bouhssini M, Benlhabib O, Bentika A, Sharma HC and Lhaloui S Sources of resistance in Triticum and Aegilops species to Hessian fly (Dipter Cecidomyiidae) in Moroccco Arab J Plant Protection Submitted

Sharma HC, Francki M, Crasta O, Gyulai G, Bucholtz D, Ohm HW, Anderson J, Perry K, and Patterson FL Cytological and molecular characterization wheat lines with Thinopyrum intermedium chromosome additions, substitutions and translocations resistant to barley yellow dwarf virus Euphytica Submitted

Sharma HC, Benlhabib O and Ohm HW Anther culture and chromosome reduction in wheat x Thinopyrum wide crosses In preparation a

Sharma HC, Z Jekkel, O Benlhabib and Ohm HW Effect of concomitant ovule culture on anther culturability in wheat and wheat x wheatgrass backcrosses In preparation b

Crasta O, M Francki, D Bucholtz, G Zhu, HC Sharma, H Ohm and J Anderson Identification and molecular characterization wheat-wheatgrass translocation lines and localization of BYDV resistance on the alien chromosome In preparation

Fatima et al Androgenic potential comparison in durum wheat effect of ovaries and relationship with proteins and peroxidases Plant Cell, Tissue and Organ Culture In preparation

El Khlifi O, Benlhabib O, Sharma HC and Mailki M Reciprocal differences in interspecific cross between durum wheat and Aegilops ovata to transfer resistance to Hessian fly In preparation

Also, an article on an overview of this USAID-supported international collaborative project between Morocco and the US was published in a national journal in Morocco (Agro Vet Magazine, 1996) As will be seen from the publications, the overseas collaborators secured fair credit for their

participation in the research The funding promoted several additional aspects of our research and some of the above publications show the on-going research where this project fitted and provided assistance to the research program of the USPI in addition to enhance collaboration and technology transfer to Morocco Reprints of some of the publications have been sent with the annual reports Those of some others are enclosed with this report

During the tenure of this project, the USPI attended Intl Pl Path Meeting in Montreal, Indiana Crop Improvement Association Conference in Indianapolis, American Soc of Agronomy meetings in Indianapolis, BYDV Workshop in St Louis, Stadler Genetics Symp in Columbia (Missouri), and World Congress on In Vitro Biology in San Francisco and Washington Moroccan PI attended Wheat Workshop in ICARDA She organized third SEWANA (South Europe West Asia North Africa) network meetings in IAV Hassan II We presented some of our work in these meetings

Two visits by Ouafae to the US and two visits by Hari to Morocco were undertaken With these visits, the Moroccan scientist learned the techniques for technology transfer The training/skills were imparted to conduct on-station and on-farm experimentation Considerable time was devoted on consultation and training, discussion and planning the experiments, conducting some of the experiments together, buying equipment, reagents and supplies for Dr Benlhabib's lab This training in technique development, technique use and literature search in the area of tissue culture and cytogenetics was necessary for Moroccan counterpart to be able to actively participate in the project and to initiate similar work in Morocco The techniques she learned included preparation of cells and slides for chromosome analysis of wide crosses, preparation of various stocks and working solutions for embryo culture and anther culture, reagents formulations for cytology and tissue culture media, culturing anthers of wide hybrid derivatives, and handling research data and report files on computer Ouafae mastered these techniques in the US and set up and fine-tuned these procedures in Morocco As she worked on each technique, she kept making list of the reagents and supplies required and then purchased these Subsequently she trained her staff and students in these techniques Arranging exchanged visits, synchronizing experiments with the visits, imparting hands-on training/consultation, purchasing and shipping equipment and supplies were significant investment accomplishments of this project in strengthening IAV Hassan II in Morocco The training enabled the IAV scientists to evaluate and apply new techniques in their program These will get extended to other programs in Morocco through the trained students and Dr Benlhabib's interaction with other researchers Training such as this is a way to acquire and diffuse biotechnology by a developing country

The training in technology was acquired by the Moroccan scientist during the first year (1993) at Purdue University The USPI visited Morocco in 1994 to facilitate technology establishment and to consult on hurdles in this process He worked with Ouafae and her students in preparing stains, scoring callus, culturing wide cross embryos and anthers, preparing slides for chromosome counts from callus and seed-derived plants, performing wide hybridization between wheat and alien species in the nursery, transplanting Hessian fly resistant plants from field to greenhouse for seed increase and hybridization, imparting needed guidance, and answer to their problems The various supplies and equipment along with technical training and reciprocal visits by the Moroccan and US investigators constituted the major component of technology transfer enabling the Moroccan scientist to start actively participate in the project, enhance her research capabilities and start developing similar program of her own During the USPI's visit to Morocco in 1994, we also solved the problems related to photography and stain preparation for cytogenetics During this visit, Sharma also met with Ouafae's four students (Hmoud, Oum, Ahmed, El Khlifi), as well as A Ouassou (wheat and barley breeder), M'hmed Sedrati (Director of IAV), N Assali (molecular

biologist/physiologist), M El Bouhssini (Entomologist), M El Yamani (virologist), Nasralla (durum wheat breeder), M'hmed Hanafi (USAID mission advisor), C Uphaus (USAID mission chief) to accomplish project goals, apprise about the project, learn about their activities and/or build linkages for future collaboration During 1996, Oufae visited Purdue University to consult and discuss field and lab results, analyze data and prepare manuscripts and reports During this visit, she also mastered the chromosome pairing technique and interpretation of the data gathered by this technique She also met with Purdue University's budget office to review the expenditure reports, purchased research supplies and consulted literature in library Finally, the USPI during early April, 1997 visited Moroccan investigator's lab in IAV Hassan II, her collaborative work with INRA (Settat), and field plots This provided an opportunity to carry out interspecific crosses in Morocco, work on manuscrips, further ensure technology transfer to run the program on relatively more solid and independent basis, make demonstration on chromosome banding that Dr Ouafae and her students (El Khlifi, Daria, Hislander) would need for alien chromatin identification as they move forward in their research Wide hybridization in nursery and chromosome banding in lab were also attended by other students/staff (Fatima, Sadia and Bushnell) These techniques and interactions would further enhance Morocco's research capabilities and future collaboration probability with the US scientists During this visit, we also started working on our final report

Five students in Dr Benlhabib's lab worked on this project related research and prepared and defended their dissertations Two more students are working toward their degrees, respectively, on biotechnological research initiated during the tenure of this project This is a proof of success of our efforts on technology transfer

8 Project Productivity

Several of the goals of the project were accomplished along with much beyond the goals in certain areas As will be evident from our publications, we, identified alien sources of resistance to Hessian fly and diseases in wheat relatives, understood the genetic and chromosomal basis of some, and exploited techniques to transfer these traits for germplasm enhancement of wheat We evaluated and utilized techniques in wide crosses leading to the incorporation of alien chromosomes carrying new genes into wheat for resistance to Hessian fly, BYDV, and possibly to other stresses as will be found in future The project established and enhanced collaboration between Purdue University and IAV and accomplished the technology transfer component successfully

Some evidence was obtained through this project that isolation of individual chromosomes of alien species in the form of alien chromosome addition lines from wide crosses through anther culture will be feasible and more efficient than conventional backcrossing The response of pollen to form callus and calli to form plantlets was low due to pollen sterility of intergeneric hybrids and poor anther culturability of their wheatgrass parents in addition to imprecise meiotic stage at culture, less than perfect culture media and environmental conditions, and these being grasses Anticipating low anther culture response, we had proposed to obtain alien addition lines through backcrossing for germplasm development This provided comparison with the anther culture work, and alien addition/substitution lines were developed and characterized that are resistant to BYDV and Moroccan Hessian fly, and one of these was released as germplasm Germplasm evaluation for Hessian fly in Morocco was not promising during the third year (1994-95) due to drought However, we already had identified a number of resistant accessions for interspecific crossing work Establishing crop was somewhat difficult in Morocco during the fourth year (1995-96) Gene

transfer from wheat x T monococcum did not go as fast as we had anticipated That backcrossing wheat x T monococcum to wheat is very hard has been observed by Cox et al (1991) also who got only 0 5-1 5% seed set and that too with one genotype

9 Future Work

We would like to follow some of the research aspects that remain in progress We would like to submit our remaining manuscripts for publications After a detailed characterization of wheat x wheatgrass-derived translocations, we will identify and use those in breeding that will have the trait and the smallest amount of chromatin from the wheatgrass In the germplasm enhancement program initiated in Morocco, several activities are being previewed for the few comming years Screening the Aegilops collection for other insects, diseases and abiotic stresses, advancing and testing the progenies of wheat x Aegilops species crosses and wheat x T monococcum crosses, studying the genetic control resistance, developing addition/substitution lines, effecting gene transfer, and incorporating newer molecular cytogenetic techniques for detection of alien gene introgression

The grant award was very encouraging for our research, and international cooperation/development We are very pleased with our progress, results and collaboration We are very thankful to AID/BOSTID for approving our project and providing assistance and guidance throughout its execution We are very much interested in maintaining the collaboration and a molecular biologist and a virologist in Morocco are ready to be a part of a future collaborative research We would appreciate receiving AID's future announcements soliciting research grant proposals

10 Literature Cited

For our own references for the period of this project, please see 'Project activities/outputs section'

Amri A, M El Bouhssini, S Lhaloui, T Cox and J Hatchett 1992 Al Awamia 77 75
Cohen J 1993 Crop Sci 33 913
Cox T, L Harrell, P Chen and B Gill 1991 Pl Breed 107 105
El Bouhssini M, A Amri and J Hatchett 1985 Rachis 5 23
Foster J, J Araya, G Safranski, S Cambron and P Taylor 1988 Purdue Univ AES Bull 536 1
Gallagher L, A Benyassine, O Benlhabib and M Obanni 1987 Euphytica 36 591
Islam A and K Shepherd 1981 Genet Res Cambridge 37 215
Jia X, S Hu, D Nie and J Zhuang 1993 Int Wheat Genet Symp 8 635-637
Jiang J, B Friebe and B Gill 1994 Euphytica 73 199-212
Jones A and J Petolino 1987 Pl Cell, Tissue and Organ Cul 8 215
Kerber E and P Dyck 1978 Int Wheat Genet Symp 5 358
Knott D and J Dvorak 1976 Annu Rev Phytopath 14 211
Kuspira J and R Bhambhani 1986 Can J Genet Cytol 27 51
Lhaloui S, L Buschman, M El Bouhssini, A Amri, J Hatchett, D Keith, K Starks and K Houssaini 1992 Al Awamia 77 21
Lindsay C, J Morrison and E Kelly 1977 Adult Edu 15 3
Lukaszewski A 1988 Int Wheat Genet Symp 7 363
Marburger J and R Wang 1988 Pl Cell Reports 7 313
Maxwell F and P Jennings 1980 Breeding plants resistant to insects John Wiley & Sons, 683 p
Metz S, H Sharma, T Armstrong and P Mascia 1988 Genome 30 177

Niu Y, J Scoles and K Kao 1991 Agron Abst P 199
Obanni M 1989 Ph D Thesis Purdue Univ , 72p
Sharma H and P Baenziger 1986 Theor Appl Genet 71 750
Sharma H and B Gill 1983 Euphytica 32 17
Sharma H, B Gill and J Uyemoto 1984 Phytopath Z 110 143
Sharma H, H Ohm, R Lister, J Foster and R Shukle 1989 Theor Appl Genet 77 369
Sharma H, J Waines and K Foster 1981 Crop Sci 21 555
Shroyer M, J Shroyer and D Hicks 1992 J Natural Resources & Life Sci Edu 21 175
Soliman K, J Bernardin and C Qualset 1980 Biochem Genet 18 465
Tzotzos G 1996 In, World Congress on In Vitro Biol , San Francisco, CA, June 22-27, p 24
Wang C, Z Chu, C Sun, C Hsu, K Yin and F Bi 1975 Acta Genetica Sinica2 72
Wang R, E Barnes and L Cook 1980 Cereal Res Commun 8 335
Wang R, J Marburger and C Hu 1991 Theor Appl Genet 81 151
Wang X and H Hu 1985 Theor Appl Genet 70 92
Wei J, R Zhou and Y Dong 1993 Int Wheat Genet Symp 8 651-655
Whelan E and R Conner 1989 Crop Sci 29 838
Wienhues A 1973 Int Wheat Genet Symp 4 201
Yamani M and J Hill 1991 Phytopath Medit 30 93
Zhuang J, J Xu, G Chen and S Sun 1985 Theor Appl Genet 70 294