A doubled haploid, bread wheat-transformed cultivar: its use in conventional transfer to elite spring wheats and development of germ plasm for detecting the location of the transgene.

A. Mujeeb-Kazi, S. McLean, A. Pellegrineschi, R. Delgado, and S. Cano.

Wheat transformation has been limited by the inability to transform and regenerate the proper cells in the target tissue and also by problems related to gene expression, plus stability of expression after several cycles of selfing the transformant. We were provided a *Bar-Gus*-transformed bread wheat winter/facultative cultivar (K-39) by Dr. Richard Brettell after his sabbatical stay at CIMMYT, El-Batan, Mexico. Our objectives were as follows:

1. To produce doubled haploids on the K-39 transformed stock and test the DHs for expression. If the DHs were positive for expression, a seed increase would be made for further studies. Selfed progeny could be analyzed for gene expression and compared with the selfed derivatives of the original stock, which might indicate if gene silencing occurred was the DH material more stable. Theoretically, this must be the case, because all alleles will be homozygous and if the original C_0 DH expressed resistance, then this should be expressed permanently.

2. To conventionally transfer the transgene to three elite, spring-habit, bread wheat cultivars (Attila, Kauz, and Luan) using the backcross protocol coupled with diagnostic assays to detect the positive plants at each BC generation.

3. To use the original K-39 DH germ plasm to develop stocks that might enable us to locate the transgene. This attempt would use the conventional monosomic procedure.

Our progress for these three steps is provided. All experiments were conducted in biosafety greenhouses and were monitored stringently by the CIMMYT Biosafety Committee.

Results. 1. Our doubled-haploid protocol with spring bread wheat cultivars generally allows us to obtain at least five embryos per spike, of which four generally differentiate, and all double. Hence, obtaining four DHs per spike is considered normal for bread wheat. The spikes of the K-39 plants were weak in growth and small. This cultivar has a winter habit, which may explain why we obtained only 11 embryos, from which seven differentiated into plants, and six were doubled successfully. All DH plant seed were germinated, given limited vernalization, tested for the transgenes expression, and found to be positive. The DH germ plasm now can continue to be tested alongside its parental K-39 cultivar for studying the stability of gene expression after continuous selfing.

2. During DH production with the K-39 winter-habit cultivar, we also pollinated six spikes by three elite spring bread wheat cultivars (two spikes/cultivar of Attila, Kauz, and Luan). Three F_1 progenies were formed, and the F_1s were grown, tested positive for transgene expression, advanced to the F_2, and also used for producing BC_1 seed from each combination by pollinating each F_1 by Attila, Kauz, and Luan. The F_2 generation was planted and tested for the transgene presence. The population tested for each of the F_2s studied showed a perfect 3:1 (resistant : susceptible) ratio. The BC_1 similarly tested gave the expected (resistant : susceptible) 1:1 ratio. The BC_1 plants have been advanced to the BC_3, and one more BC is planned for making the derivative phenotypes akin to Attila, Kauz, and Luan. From the transgene-positive, BC_4 plants, DHs will be produced and after testing for the transgene, these DHs will represent the culmination of this study. This result will demonstrate one option for transferring an initial stable event to elite cultivars, in this case, from K-39 to Attila, Kauz, and Luan.

3. Transgene location. Using FISH is a swift procedure to locate transgenes on wheat chromosomes. The procedure requires chromosome banding as an indicator to identify the wheat chromosomes. So far, we have not been successful with this approach. Simultaneously, the conventional monosomic analysis route utilized the Chinese Spring monosomic stocks has been pursued. The 41-chromosome monosomes of each of the 21 wheat chromosomes have been hybridized with the stable DH K-39 transformed cultivar. F_1 seed from each monosome have been obtained, and hence the germ plasm necessary to locate the transgene has been produced. Our approach will follow a protocol that identifies at least five F_1 plants with 41 chromosomes for each of the 21 combinations. From these 41-chromosome, F_1 plants, haploids will be produced and will be tested for gene expression. Segregating and nonsegregating haploids will indicate the transgene locations.

Our contention is that once the desired transgene or the futuristic 'clean' transgene events have been obtained, the practical utilization of the material can be integrated with conventional breeding procedures mediated by the homozygosity DH protocol. Inheritance studies considered crucial for basic information can be pursued independently.

Performance of advanced bread wheat x synthetic hexaploid derivatives under reduced irrigation.

R. Trethowan, M. Van Ginkel, and A. Mujeeb-Kazi.

The annual increase in genetic potential in drought environments is only about half (0.3–0.5 %) of that obtained in irrigated, optimum conditions. Attempts by many researchers to produce wheat adapted to semiarid environments have had limited success. At CIMMYT, we follow a system for drought tolerance in which yield responsiveness is combined with adaptation to drought conditions.

The T1BL·1RS translocation wheats have a demonstrated advantage in dryland wheat areas, and the search for other diverse sources to exploit continues. One such unique gene pool resides in the primary Triticeae diploid *Ae. tauschii*. We have combined this diploid grass with elite durum cultivars to produce synthetic hexaploids. Field testing under reduced irrigation over the past several years has led to the identification of some synthetics classified as drought tolerant. The best five of these SHs have been crossed with a drought susceptible cultivar Opata, and the resulting F_1s are being used to develop doubled haploid mapping populations.

Utilizing a few drought-tolerant synthetics, some crosses with Opata were advanced beyond the F_1, and the performance of these advanced derivatives was studied in Obregon, Mexico. Very little rainfall was recorded during the 1998–99 crop cycle, resulting in good evaluation for drought tolerance (Table 11). A number of synthetic derivatives yielded more than Baviacora, the long-term check used in drought trials sown in Obregon. These synthetic derivatives are free threshing with large, bold white grain. From the five SH-based advanced derivatives, 25 doubled haploid derivatives per entry have been produced in anticipation that complete homozygosity may have a beneficial contribution in future evaluations of this germ plasm in Mexico and globally.

In order to combine drought tolerance with late heat tolerance, replicated trials of the candidates for HTWYT and WAWSN were sown under drought with the purpose of identifying potential parental material for the crossing

Table 11. Mean yields of the highest yielding entries sown with one preseeding irrigation in Obregon, Mexico, during the 1998–99 crop cycle. Yield data of lines is derived from different replicated trials.

Pedigree	Yield (t/ha)	% of Baviacora
PRL/VEE #6//Choix CMSS93Y01738S-54Y-010Y-010M-010Y-10M-0Y-0SY	4.256	114
Croc 1/*Ae. tauschii* (224)//Opata CMBW91Y00935S-80Y-11KBY-1KBY-010M-1Y-2M-0Y-0SY	5.197	111
TSI/VEE#5//Kauz ICW91.0295-3AP-0TS-0BR-1AP-0L-0AP-0SY	4.094	106
Croc 1/*Ae. tauschii* (224)//Opata CMBW91Y00935S-80Y-11KBY-1KBY-010M-1Y-3M-0Y-0SY	4.916	105
Chen/*Ae. tauschii*//2*Opata -41SSD-0Y	3.790	104
Altar 84/*Ae. tauschii*//2*Opata -76SSD-0Y	4.330	104
Croc 1/*Ae. tauschii* (224)//Opata CMBW91Y00935S-80Y-11KBY-1KBY-010M-1Y-1M-0Y/0SY	4.837	103
KAUZ/5/PAT10/ALD/PAT72300/3/PVN/4/BOW CMSS93B01334S-70Y-010M-010SY-010M-2SY-0M-0SY	4.067	100