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TRANSFER OF GENES FOR STRESS TOLERANCE INTO *TRITICUM AESTIVUM* L. FROM OTHER TRITICEAE RELATIVES

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Significant advances in wide hybridization in the Triticeae have been associated with the research momentum of the past two decades. The potential of alien germplasm utilization has since been receiving diversified attention with substantial success principally in the capabilities of overcoming crossability barriers and development of cytogenetic manipulation procedures for alien genetic introgression into wheat.

These reports have all predominantly considered the futuristic complex "intergeneric" hybridization research area where constraints are prevalent from hybridization to stabilization of alien genetic transfer/s capable of practical agricultural exploitation. More recently, there has been renewed emphasis on utilizing close wheat relatives for the crops improvement forming a comparatively simplistic "short-term" productivity research area. These close relatives involve sources that have complete or substantial homoeology with AA, BB or DD genome of hexaploid wheat (*Triticum aestivum* L. 2n=6x=42, AABBDD). Alien transfers are thus mediated by high recombination events as a consequence of interspecific hybridization and genomic homoeology.

The above "intergeneric" and "interspecific" research approaches are elucidated as they impinge upon transfer of stress tolerances from the Triticeae relatives to wheat.

MATERIALS AND METHODS

Aluminum tolerance. Polle et al. [1] followed by Takagi [2] developed a selection system involving a direct observation of wheat seedling roots under Aluminium (Al^{+++}) stress. The method is based on the fact that Al^{+++} tolerance in wheat is largely a function of Al^{+++} exclusion from the roots. This methodology has since been employed and described [3]. Scoring (1–3 scale) of root tip growth after immersion of the roots in a nutrient solution containing 46 ppm of aluminum and subsequent staining of the roots with an aqueous solution of 0.2% hematoxylin (in order to observe any continued root growth) correspond very well with Al^{+++} tolerance of the Triticeae germplasm. Higher concentrations (up to 95 ppm) have been used here for some alien sources. The list of the germplasm is included in Table 1 categorized under: (a) Conventional wheat cultivars, (b) *Secale cereale* cultivars, (c) Alien species and derivatives. For each entry, 20 seedlings were tested over various aluminum (Al^{+++}) concentrations and tolerant seedlings transplanted for seed increase for subsequent breeding utilization.

Table 1. Triticeae germplasm screened for aluminium tolerance under controlled laboratory conditions with tolerance level set at 46 ppm

Germplasm	Cultivars and accession numbers
Conventional cultivars	
Expt. 1. *Triticum aestivum* L.	CNT-1, Maringa, CS, Pavon, Glennson (Al^{+++} level 0 and 46 ppm)
***Secale cereale* cultivars**	
Expt. 2. *S. cereale* L.	Short, No. T-4776, Sardev, No. T-4777, Doukala, No. 4778 Turkey, No. 4779, Prolific, No. T-4781, White, No. T-4783 (Al^{+++} level 0, 46, 70 and 95 ppm)
Alien germplasm:	
Expt. 3. Alien species and controls	*T. aestivum* cv. Chinese Spring, *Ae. variabilis*, CS/*Ae. variabilis* *T. turgidum* cv. Laru, Laru/*Ae. variabilis* (Al^{+++} level 0 and 46 ppm)

Salt tolerance. In a CIMMYT collaborative program with AFRC–APSR, UK and Bangor, Wales (Drs. C. Law and G. Wyn Jones, respectively) several salt tolerant alien genera and conventional sources have been identified, further substantiated by other literature reports (Table 2). This cumulative information has added to the variability available for effecting alien transfers into *Triticum aestivum*. From this list, our priority making in CIMMYT has been with *Th. bessarabicum*, *Ps. juncea*, *L. elongatum*, *Th. disticha*, *E. giganteus* and *T. tauschii* (*Ae. squarrosa*). For intergration of variability into wheat, the initial step is the production of hybrids. The procedures for hybrid production and advance for meeting breeding goals have been documented earlier [4, 5, 6], leading to final products for the intergeneric combinations as disomic chromosome additions (22 bivalents, 44 chromosomes) or for interspecific combinations as synthetic hexaploids from the *T. turgidum* x *T. tauschii* cross. The material is then to be utilized for salt tolerance screening in hydroponics using NaCl as the salt between 0 to 250 mM concentrations. The methodology used is essentially similar to that cited earlier [7, 8] and also utilized for hydroculture [9, 10].

Copper efficiency. Copper deficiency reportedly effects the reproductive phase more than the vegetative [11] and testing has been conducted [12] over copper levels of 0 to 4.0 mg/pot with grain yield being measured. This identified *Secale cereale* cv. Imperial as a copper efficient source. Subsequently, the 5R addition was responsive and the 5A/5R translocation made available to us (AFRC-IPSR, T. E. Miller). We have attempted to transfer this 5A/5R translocation into some CIMMYT bread wheats by making F_1 hybrids, backcrossing (BC), selfing, using morphological and cytological markets to track the 5A/5R chromosome in the BC plus its selfed derivatives. The copper screening is not integrated in the 5A/5R transfer stages but will be conducted when the final product is realized based upon elite specific BC cultivar plant type and 5A/5R homozygosity.

Table 2. Salt tolerant Triticeae relatives identified from sources in the literature and through collaborative efforts

Germplasm	Ploidy level or origin	Reference
Alien Sources:		
*Th. bessarabicum**	2n=2x=14	[20]
*E. elongata**	2n=2x=14	[17]
E. scirpea	2n=4x=28	"
E. pontica	2n=10x=70	"
E. junceiformis	2n=4x=28	"
*E. disticha**	2n=4x=28	"
*E. giganteus**	2n=4x=28	"
*Ae. squarrosa**	2n=2x=14	[7]
Ae. umbellulata	2n=2x=14	"
Ae. comosa	2n=2x=14	"
Ae. mutica	2n=2x=14	"
Ae. cylindrica	2n=4x=28	"
*Ps. juncea**	2n=2x=14	[21]
Conventional sources:		
T. aestivum cvs.	2n=6x=42	Personal communication
Kharchia	India	"
KRL 1-4	India	"
Lu26S	Pakistan	"
Shorawaki	Pakistan	"
Candeal	Mexico	"
Sakha-8	Egypt	"
Glennson 81	Mexico	"
Yecora	Mexico	"

*Priority for wide crossing currently in our locale.

RESULTS AND DISCUSSION

Aluminium tolerance. The low productivity of most crops in the acid soils of humid tropic and subtropic environments is mainly due to soil characteristics as : (i) low pH and high soluble aluminium, manganese, and iron concentrations, (ii) deficiency of available phosphorus and some microelements, and (iii) low calcium, magnesium, potassium and sulfur concentrations. Because of these problems, root growth of plant is reduced, resulting in a low intake of available water in the profile.

In general, aluminium toxicity is associated with low soil pH (below 5.5); it is severely increased when the pH falls below 5.0. Such low pH increase considerably the aluminium solubility in the soil where more than half of the cationic exchange sites can be taken by aluminium. The extent to which aluminium becomes soluble in the soil and the degree to which it becomes toxic to plants depends upon a number of factors in addition to soil pH such as the types of clay, salt and cation concentration, the buffering capacity of the soil and its organic matter content.

The soil acidity is associated with humus or organic matter, aluminosilicate clays, iron and aluminium oxides, interchangeable aluminium, soluble salts, and carbon bioxide. It is known that when interchangeable aluminium combines with basic cation losses, such as calcium, magnesium, and potassium, soils become acidic. Aluminum can also interfere with phosphorus metabolism by causing an accumulation of high quantities of inorganic phosphates within the roots, thereby reducing the root ability to absorb and transport this important element. There are management alternatives to correct acid soil problems. However, different cereal species and varieties within the same species also have wide variability in their tolerances to high aluminium levels in acid soils that could be exploited through breeding. For example, there is great genetic diversity in hexaploid wheat [3], but additional genes even for a static system [13] have their significance. *Secale cereale* is a potent source (Table 3) and each accession is highly tolerant even at 95 ppm. Mechanisms are in place to exploit this genomic variability and after the tremendous global agricultural success of 1B/1R translocation wheats [14, 15] the promise of using *S. cereale* (translocation or homoeologus exchange) is seemingly high.

Table 3. Aluminium tolerance at three concentrations in accessions of Secale cereale

Accession No.	Tolerance to different oncentrations of Al^{+++} (%)		
	46 ppm	70 ppm	95 ppm
T-4776	100	94	65
T-4777	100	100	92
T-4778	100	100	93
T-4779	100	100	86
T-4781	100	100	87
T-4783	100	95	95

The response of cv. Chinese Spring to Al^{+++} screening is slight tolerance, whereas Glennson 81 stands out as extremely sensitive (Table 4). It warrants further monosomic testing using the highly tolerant cultivars (CNT-1 or Maringa) and Glennson 81 monosomics (recently became available) to delineate the genetic effects using this more stricter analytical background of highly susceptible Glennson 81. Additional variability sources have identified *Aegilops variabilis* (2n=4x=28, UUSS) as an $A1^{+++}$ tolerant source which when hybridized to *T. aestivum* and *T. turgidum* has led to production of amphiploids of both combinations (2n=10X=70 AABBDDUUSS; 2n=8x=56 AABBUUSS). The tolerance of *Ae. variabilis* was expressed in the amphiploid (at 46 ppm). The potential to exploit this diverse variation is being actively pursued using the conventional addition line approach and utilizing the ph1b manipulation system [16].

Table 4. Tolerance of *Triticum aestivum* cultivars to aluminium in hydroculture

Cultivar	Tolerance to Al^{+++} at 46 ppm (%)
CNT-1	100
Maringa	100
Pavon 76	52
Chinese Spring	19
Glennson 81	0

Salt tolerance. With unequivocal acceptance that alien species express salt tolerance to high sodium chloride (NaCl) concentrations in a controlled hydroculture system the alien variability incorporation phase follows. Those fitting the intergeneric hybridization category are processed through production of alien disomic additions, substitutions and eventual subtle introgressions. Interspecific hybridization end goals are relatively simplistic, since these are based upon substantial A, B, D genomic homology. The choice of the alien source varies. In general, there exists an inverse relationship between genetic distance and ease of hybridization, a concept that has exception. Additionally, preference for alien species (distant or close) expressing complete or substantial homoeology with *T. aestivum* A, B and D genomes as means of variability introgression takes priority. Pragmatically, we accept that agricultural systems are quite complex and one exclusive aspect comprised of involved germplasm with a restricted genetic base will not resolve the ever-changing global situation of abiotic conditions that govern wheat production.

Unique genetic variability from a wide range of sources will be continuously needed for gene pyramiding by breeders to counter the incurred changes. The interspecific category does offer swift returns and the exact choice, e.g. *T. tauschii* for D genome manipulation and for more diverse sources (intergeneric) alien diploids stand at a priority. Consequently, for salt tolerance transfers *Thinopyrum bessarabicum, Lophopyrum elongatum* and *Psathyrostachys juncea* are preferred over the tetraploids *E. giganteus* (2s=4x=28) and *E. disticha* (2n=4x=28) or over the decaploid *El. pontica* (2n=10x=70) that inevitably is highly tolerant [17]. Even in diploids the traits may not be simply inherited as evidenced by the recent observations [18] where at least 3 of the 7 disomic additions (3E, 4E and 7E) of *L. elongatum* to wheat gave a positive effect for a salt response. These additions are in the Chinese Spring background. We have produced the reciprocal cross (*L. elongatum* x *T. aestivum* cv. Goshawk) allowing for inclusion of any cytoplasmic influence to be captured and the emphiploid has been obtained (2n=8x=56) from which disomic additions are being produced. Since the wheat background is an elite agronomic type the progenies are expected to be agriculturally better adapted. With the infusion of callus culture and polyhaploid methodology during the course of addition line development, multiple disomics will form a viable alternative to presumably integrate genes located on different chromosomes in one package [19].

The 5J disomic addition of *Th. bessarabicum* has been associated with bestowing salt tolerance or *T. aestivum*, 2J being susceptible and the amphiploid (2n=8x=56) being salt tolerant [9, 10]. Our initial results after testing these stocks are varied and the desired positive response based upon survival has not been obtained even at 150 mM NaCl for 5J effectivity. This implies that the alien addition line positive for salt tolerance still requires identification and the remaining *Th. bessarabicum* chromosome disomics need to be produced. So far, we have obtained morphologically and biochemically marked derivatives with 44 chromosomes, 22 bivalents and high fertility for homoeologous group, 1, 2, 3, 4 and 5 [19] in a Genaro background which shall enter the NaCl hydroculture testing. Hydroculture positive responses at 150 and 225mM have been further obtained for BC_1 derivatives of Inia/*Th. disticha*/ Inia (2n=8x=56, $AABBDDE_1E_2$; source Dr. Pienaar), *T. turgidum* x *Th.*

junceiforme amphiploid (2n=8x=56) with Kharchia 65 (control) the control performing positively. The wide spectrum of tolerance in the diverse germplasm is anticipated to have significance in providing novel genes for wheat improvement for the salt trait being assessed. In initial stages of the intergeneric program is a more distant genomic source being exploited (*Ps. juncea* 2n=2x=14, NN) [6] for which C-banding/isozyme markers are in place and advanced BC derivatives at disomic level have been obtained.

Interspecific hybrids (*T. turgidum* x *T. tauschii*) have led to production of several hexaploid synthetics (2n=6x=42, AABBDD) where screening may enable identification of DD genome salt tolerance. There could be genetic suppression in the synthetic hexaploids but the rapid utilization opportunities; if tolerance were to express; are remarkably high for wheat improvement for this complex character.

Copper efficiency. This does not have that magnitude of global importance (hectarage considered) as the previous two aspects but the constraints are prevalent in localized areas of Kenya and Australia. The transfer from 5AS/5RL for the efficiency trait is quite simplistic. The transfer of the translocation chromosome can be readily ascertained through the C-band positive sites of 5RL (rye chromosome arm) or the hairy (pubescent) neck marker on the segregating progenies. The backcrossing of this genetic stock has to proceed for another 5 generations before near isoline derivatives in 10 CIMMYT wheats are obtained which are homozygous for the 5A/5R translocation. The copper testing is curtailed at this stage of the research where focus is exclusively on germplasm development.

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