Indian J. Genet., **60**(1): 1-4 (2000)

CYTOGENETIC ANALYSIS AND DIFFERENTIAL RESPONSE OF RYE-INTROGRESSED BREAD WHEAT GENOTYPES FOR COLD TOLERANCE

RAKESH VAISHNAVI* AND G. S. SETHI

Department of Plant Breeding and Genetics,
Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176 062

(Received: August, 1999; accepted: January 2000)

ABSTRACT

Twenty three rye-introgressed bread wheat genotypes, two triticale parents, one rye and 2 wheat checks were examined for tolerance to cold stress and were subjected to cytogenetic analysis. On the basis of colorimetric estimation of the intensity of extracted formanzan, rye was found to be the most cold tolerant cereal, while five genotypes, viz., TW9321, RL75/83, RL6-3-4, TW9308 and TW9328 together with two triticale genotypes were found to be cold tolerant. Cold tolerance is suppressed to a great extent in triticale, making it difficult to transfer high levels of cold tolerance to wheat. The derivatives, TW9308 and TW9321 carried 7R(7D) substitution while the derivative TW9328 carried 6R (6D) substitution, indicating the association of cold tolerance with 6R and 7R chromosomes of rye.

Key Words : Rye, bread wheat (*Triticum aestivum* L.em Thell), cold tolerance, introgression, wheat-rye chromosome substitutions, cytogenetic analysis.

Wheat (*Triticum aestivum* L. em Thell) is grown under diverse agroclimatic conditions. During its life cycle the crop is often subjected to low temperature regimes. Cold stress is one of the factors limiting the yields both in winter wheats in temperate regions and spring wheats grown during the winter in subtropical regions. Low temperature affects the growth and development of wheat plant at different stages, leading to reduction in yield. The stress results not only in yield losses, but also prevents extension of the crop to new areas [1]. The cold affected plants generally experience water stress, often due to chilling of the intact root, which results in wilting [2, 3].

Since cold tolerance is a complex phenomenon, it is difficult to develop screening techniques to breed wheat for tolerance to this stress. Screening for cold tolerance can also be undertaken in conditions *in vitro* or *in vivo* [1]. Screening for cold

*Deptt. of Plant Breeding & Genetics, Regional Agricultural Research Station, SKUAST, R.S. Pura, Jammu

tolerance can also be undertaken in controlled conditions by subjecting the seedlings to artificial freezing for a specific time. The genotypes which exhibit recovery after stress are generally cold tolerant [1]. A wide range of pre-hardening and hardening conditions [4] can be used where freezing tests are employed to assist in identifying genotypes with greater winter survival ability.

Rye (*Secale cereale* L.) has been recognised as the most cold tolerant cereal. One of the objectives of synthesising triticale was to transfer cold tolerance of rye to wheat. However, triticale x *Triticosecale* Wittmack turned out to be only as cold hardy as wheat [5]. Sometimes, rye traits not expressing in the entire wheat genomic background could do so when a wheat chromosome carrying suppressors of the rye genes for cold gold tolerance are replaced by corresponding rye homeolegues [6].

The primary objective of the present study was to identify cold tolerant rye-introgressed wheat genotypes and characterise them cytogenetically.

MATERIALS AND METHODS

The material for the present study comprised 23 rye-introgressed wheat derivatives, 2 triticale genotypes, one rye check and 2 wheat checks (Table 1). All these genotypes were subjected to Triphenyl Tetrazolium Chloride (TTC) test. The seedlings were put in the refrigerator for hardening at 1 - 4°C for a period of 7 days. The hardened seedlings were subjected to freezing at – 9°C in the freezer for 24h followed by thawing at 1 - 4°C for 1 day and, thereafter, treated with 1% TTC solution. Colorimetric estimation of formazan was done as described elsewhere [7].

Alien chromosome substitution were detected through the study of meiosis in F_1 hybrid raised by crossing the derivatives with bread wheat, and with three confirmed substitution lines viz., RL4:1R(1D), RL22:6R(6D) and RL83:7R(7D) [8]. For meiosis, the young spikes were fixed in aceto-alcohol (1:3), transferred to 70% alcohol after 24h of fixation and preserved at 4 ± 1^0 C till study. For chromosome homology studies, anthers were squashed in aceto-carmine and were made permanent with Eupral. About 50 cells each at different meiotic stages of each line were studied.

RESULTS AND DISCUSSION

In the healthy cells, oxidized form of TTC, which is colourless, is reduced to a coloured form (formazan) and the intensity of colour was estimated colorimetrically (Table 1). The two triticale genotypes, viz., TL1210 and TL1217 were as cold hardy as some of the derivatives under study, while rye was categorized as highly cold tolerant (>0.200). Five genotypes, viz., TW9321, RL75/83, Rl6-3-4, TW9308 and TW9328

Table 1. Parentage, colorimetric estimation of formazan and relative ranking of the genotypes under test for cold tolerance

Genotype	Parentage	Absorbance	Rank
TW9308	TL1217/CPAN 1922	0.156	07
TW9309	"	0.144	10
TW9321	"	0.181	02
TW9322	"	0.081	20
TW9325	"	0.065	22
TW9326	"	0.112	16
TW9327	"	0.132	13
TW9328	"	0.152	08
TW9333	CPAN 1922/RL6	0.150	09
TW9334	CPAN 1922/RL 24	0.062	23
TW9335	RL68/VL 616	0.121	14
TW9336	CPAn 1922/RL 24	0.104	18
RL 75/83	UPT 74303/Sonalika//TL 161/Sonalika	0.176	03
$RL24/4P_2$	TL161/Sonalika//TL 68/Sonalika	0.104	18
$RL16/83P_2$	TL68/Girija//TL 161/Sonalika	0.085	19
$RL78/4P_2$	TL68/HS 74//TL 68/Sonalika	0.134	12
Rl88/22	UPT72142/HS 74//TL 68/Shailaja	0.085	19
RL23/83	TL 161/Sonalika//TL 161/Sonalika	0.055	24
$RL20/83P_1$	UPT 74303/Sonalika//TL 161/Sonalika	0.076	21
$RL20/83P_2$		0.115	15
RL22-2-1	CPAN 1922/RL22	0.111	16
Rl22-37	RL 22/RL 37	0.112	17
RL6-3-4	HD 2323/RL 6	0.160	06
HPW42	VEE"S"/4/PVN"S"/CBB//CNO"S"/3/JAR/ORZ"S"	0.132	13
HS240	AU/KAL/BB/WOP"S"/PVN"S"	0.143	11
TL1210		0.162	05
TL 1217		0.174	04
Rye		0.218	01

together with 2 triticale genotypes were found to be cold tolerant (0.151-0.199). Eleven derivatives, viz., TW9309, TW9326, TW9327, TW9333, TW9335, TW9336, $RL24/4P_2$, $RL78/4P_2$, RL22-37, $RL20/83P_2$ and RL22-2-1 alongwith the checks HPW42 and HS240, were categorised as moderately tolerant (0.100-0.150). The remaining 7 derivatives, viz., TW9321, TW9322, TW9325, TW9334, $RL16/83P_2$, RL88/22, and $RL20/83P_1$, were susceptible to cold stress (< 0.099).

Of the line cold tolerant derivatives, four (TW 9308, TW9321, TW9328 and RL-6-3-4) were examined for rye chromosome substitutions; the fifth genotype (RL 75/83) could not be examined. Two univalents at metaphase 1 were observed in the F_1 hybrids of TW9308 with HD2380 in most of the meiocytes scanned, indicating the substitution of a pair of wheat chromosomes by a pair of rye chromosomes in this derivative. When this derivative was crossed with RL4 and RL22, 4 univalents and laggards were observed at metaphase 1 and telophase 1, respectively, in most of the cells. Out of these, 2 were clearly longer (rye) and the remaining 2 were smaller (wheat). These results exclude the chances of 1R or 6R substitutions in this derivative. When crossed to RL83, the derivative (TW 9308) exhibited normal meiotic stage in most of the meiocytes, suggesting the substitution of 7R in this derivative. The derivative TW9321 gave similar results suggesting the substitution of 7R in this derivative also. Using the same criterion, TW9328 was found to carry 6R 6D substitution. Thus, of the five derivatives, identified as cold tolerant on the basis of TTC test, TW9308 and TW9321 carried 7R(7D) substitution, while the derivative, TW9328 carried 6R(6D) substitution. This indicates the association of high levels of cold tolerance with 7R and 6R chromosomes of rye, which could be further exploited systematically for the transfer of cold tolerance genes into wheat.

REFERENCES

1. G. S. Sethi, P. Plaha and K. S. Thakur. 1993. Breeding bread wheat for cold stress. Ann. Biol., **9**: 165-173.

2. M. N. Christiansen and J. B. St. John. 1981. *In*: Analysis and Improvement of Plant Cold Hardiness. Olien, C.R. and Smith, M.N. (eds.), C.R.C. Press, Boca Raton, Florida.

3. J. R. Mc William. 1983. *In*: Crop Reactions to Water and Temperature Stress in Humid Temperate Climates. Raper, D. Jr. and Kramer, P.J. (eds). Westview Press, Boulder, Colo. pp. 113.

4. G. M. Bridgar, D. E. Falk, B. D. Mc Kersie and D. L. Smith. 1996. Crown freezing tolerance and field winter survival of winter cereals in Eastern Canada. Crop Sci., **36**: 150-157.

5. J. Dvorak and B. B. Fowler. 1978. Cold hardiness potential of triticale and tetraploid rye. Crop Sci., **18**: 477-478.

6. S. Singh and G. S. Sethi. 1992. Expression of 17 rye (*Secale cereale* L.) traits in a range of durum wheat (*Triticum durum* Desf.) and bread wheat (*T. aestivum* L.) genetic backgrounds. Euphytica., **60**: 37-44.

7. D. L. Kittock and A. G. Law. 1968. Relationship of seedling vigour to respiration and Tetrazolium chloride reduction by germinating wheet seeds. Agron. J., **60**: 286-288.

8. G. S. Sethi and P. Palha. 1988. The nature of rye (*Secale cereale* L) chromatin introgression into wheat (*Triticum aestivum* L. em. Thell.) via triticale (× *Triticosecale* Wittmack). Proc. 7th Int. Wheat genet. Symp., Cambridge, U.K. pp 433-438.