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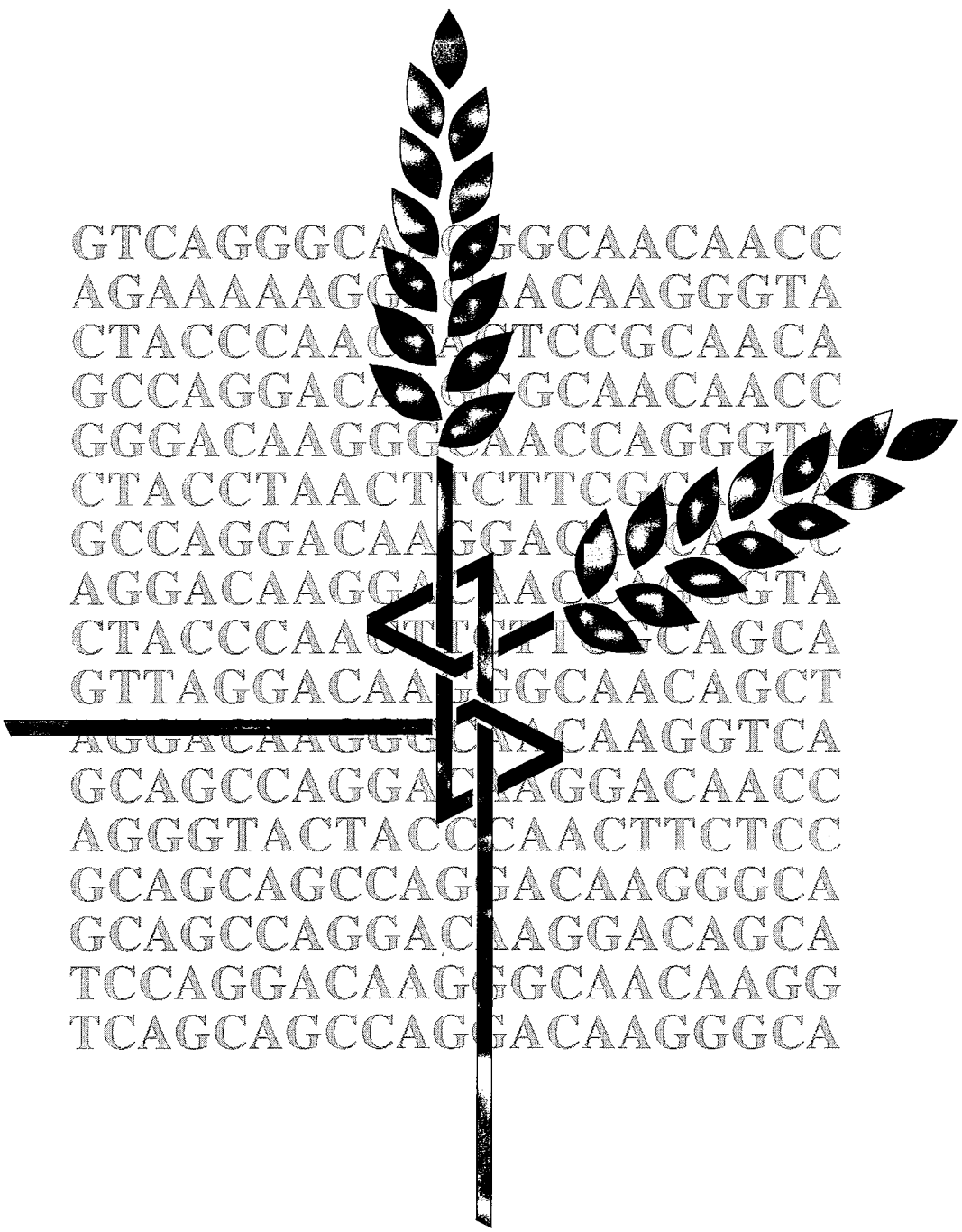

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Research article

Development and identification of short stalked wheat-*Thinopyrum ponticum* translocation lines

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Summary

From two dwarfing *Triticum aestivum-Thinopyrum ponticum* addition lines Shannong31504 and Shannong31505, two cytologically stable and short stalked translocation lines, named as Shannong31504-1 and Shannong31505-1, were developed. Their chromosome numbers in root tip cells were 2n=42, and 21 bivalents can be observed at PMC MI. Quadrivalent was observed in PMC MI of F_1 hybrid between Shannong31504-1 and different common wheat variety. Biochemical identification showed that the dwarfing translocation lines contained *Th. ponticum* chromatin, and three RAPD markers designated as $S36_{1800}$, $S37_{720}$ and $S97_{490}$ from 112 random primers, which can be used to detect the *Th. ponticum* chromatin in the translocation lines, were screened.

Key words: wheat, *Thinopyrum ponticum*, translocation line, dwarfing, identification

Introduction

During past several decades, the incorporation of dwarf genes into common wheat has resulted in dramatic yield increments. Now with the popularization of intensive farming system, more dwarfing or semi-dwarfing wheat cultivars with excellent agronomic characters will be urgently needed. Using dwarf genes to breed dwarfing or semi-dwarfing varieties is the most economical and effective approach to suit this needs. Until now, the fact that dwarfism is mainly controlled by major gene has been proved. Twenty-one major dwarf genes have been discovered and nine of them located on specific chromosome arms (Yang 1993). Among the twenty-one major dwarf genes, seventeen genes originated from common wheat and the others came from durum wheat. But only several dwarf genes, such as *Rht1, Rht2* (coming from Norin 10 source) and *Rht8, Rht9* (coming from Akakomugi source), have been successfully utilized in wheat breeding program, and the others are of little practical value as they severely reduce the plant height as well as grain yield (Gale 1985). It is, therefore, essential to search for additional source of dwarfism to improve wheat yields further and broaden the genetic bases of dwarfism.

Thinopyrum ponticum (= *Elytrigia elongata* = *Agropyron elongatum* = *Elymus elongatus* = *Lophopyrum elongatum*, 2n=10x=70) is one of the most important resources for wheat improvement (Li 1985). For it have many useful traits such as resistance to stem rust, leaf rust, wheat streak mosaic virus and barley yellow dwarf virus, high tolerance to salinity and aridity. Due to these useful traits and good crossing ability with common wheat, a number of useful genes have been transferred from this species to common wheat through wide cross, and several useful wheat germplasms and cultivars have also been produced. But there are still no reports about the transfer of dwarf gene from *Th. ponticum* to common wheat. In this paper, two wheat-*Th. ponticum* short

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stalked translocation lines, which came from the selfed progenies of two dwarfing *T. aestivum-Th. ponticum* addition lines Shannong31504 and Shannong31505, were bred. The biochemical methods and RAPD assay were used to detect the introgression of the *Th. ponticum* chromatin into the translocation lines.

Materials and methods

The plant materials used in this study are common wheat cultivars Lumai No.5, Chinese Spring (CS) and Marquis, *Th. ponticum*, wheat-*Th. ponticum* partial amphiploid Xiaoyan7631 (2n=8x=56), wheat-*Th. ponticum* disomic addition lines Shannong31504 and Shannong31505 (coming from the hybrids of Xiaoyan7631 and Lumai No.5) and the selected translocation lines Shannong31504-1 and Shannong31505-1. All the materials were preserved by our own laboratory.

Observation of mitosis and meiosis: Mitosis in root tip cells and meiosis in pollen mother cells were observed in accordance with the method of Kong (1999). Gliadin A-PAGE was carried out according to the methods of Yan (1989). The band patterns in the electrophoresis spectra were determined in the light of the nomenclature of Metakovsky (1991).

DNA extraction and PCR amplification: Genomic DNA was extracted from 2 week-old plants following the method of Sharp (1998). DNA concentration was measured by a spectrophotometer, and the DNA was diluted in 1xTE. Decamer oligonucleotide primer, $MgCl_2$, 10xbuffer, dNTP, Taq DNA polymerase were obtained from Sangon Technological Inc. PCR was performed in a 25 μl reaction volume consisting of 2.5μl 10xbuffer, 2 μl 2.5 mM dNTP, 2 μl 25 mM Mg^{2+}, 1.2 μl 8 pmol/μl primer, 1.25 U of Taq DNA polymerase, 50 ng template DNA, and an appropriate volume of water to make up the final volume to 25 μl. The reaction mixture was then overlaid with 25 μl of mineral oil (Sigma Chemical Co.) to avoid evaporation. The PCR reaction was run in a Gene Amp 9600 thermocycler for 46 cycles with the first cycle of 94°C for 3 min, 36°C for 1 min, 72°C for 2 min, followed by 45 cycles of 94°C for 15 seconds, 36°C for 30 seconds, 72°C for 1 min. After 46 cycles, there was a final extension step of 4 min at 72°C. The reaction mixture was cooled to 4°C and maintained at this temperature until gel electrophoresis. The PCR products were separated on a 1.2% agarose gel in 1xTAE(Tris-Acetic EDTA) buffer. Forty nanograms of ethidium bromide (EtBr) were added to the gel. A 2 kb DNA ladder was used as a size maker.

Table1. Main characters of wheat-*Th. ponticum* translocation lines and their relevant parents

Plant materials	Plant height(cm)	Plant type	Spike type	Spike length(cm)	Spikelet/ spike	Spikes/ plant	Awn	Yr†
Th. ponticum	150.9	loose	long and thin	54	31	51	no	1
Xiaoyan7631	126.5	loose	clubbed	18	21	8.5	no	2
Lumai No.5	70.3	compact	circular cone	7	18.4	4.4	short	4
Shannong31504-1	50.4	loose	clubbed	9.5	16.6	6.7	long	2
Shannong31505-1	50.9	loose	clubbed	9.7	16.5	6.2	long	2

†Reaction to yellow rust, 1: immune, 2: high resistance, 4: medium infection

Table2. Chromosome numbers and configurations of plant materials and F1 hybrids in RTC and PMC MI

Materials	Number of cells scored	Chromosome number	Univalent	Bivalent	Trivalent	Quadrivalent
Xiaoyan7631	34	56	0	28	0	0
Lumai No.5	41	42	0	21	0	0
Shannong31504-1	52	42	0	21	0	0
Shannong31505-1	50	42	0	21	0	0
Shannong31504-1/Lumai No.5	71		0	18.7	0.01	1.12
Shannong31505-1/Lumai No.5	69		1.9	20.0	0	0.04
Shannong31505-1/Shannong31504-1	89		1.9	20.1	0	0

Fig. 1. Shannong31504-1

Fig. 2. Shannong31505-1

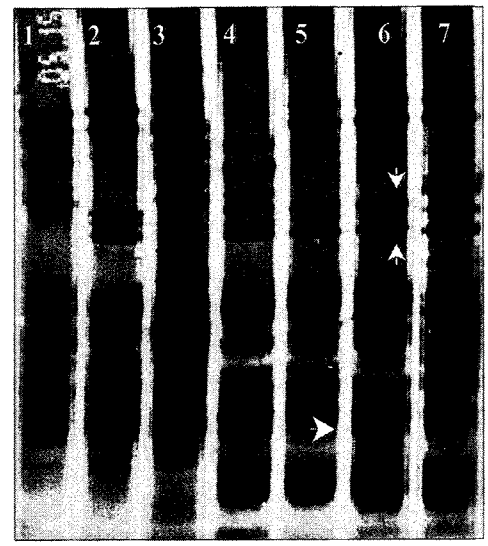

Fig. 3. Gliadin electrophoreogram of two translocation lines and their relevant parents. 1: Marquis, 2:CS, 3:*Th. ponticum*, 4:Xiaoyan7631, 5: Shannong31504-1, 6: Shannong31505-1, 7: Lumai No.5

Results and discussion

Breeding of short stalked alien translocation lines and their cytological identification: Among the named dwarf genes, none came from *Th. ponticum* (Yang 1993). Using common wheat crossing with *Th. ponticum*, a number of partial amphiploids and germplasms have been produced. But from these materials, no dwarf genes were reported. In the hybrids of Xiaoyan7631 and wheat cultivar Lumai No.5, two short stalked disomic addition lines named as Shannong31504 and Shannong31505 were selected. Their plant height were about 50 cm, resistant to yellow rust and not semilism (Li 1995). In the F_2 progenies between Shannong31504 and high wheat cultivars, plant height separated into three groups: the high plants, semi-high plants and short stalked plants, which their chromosome number were 2n=44, 43 and 42, respectively. This indicated that the dwarf gene was located on alien chromosomes, and has dosage effect (Li 1996). So we deemed that *Th. ponticum* contained a dwarf gene. Maybe, due to the compensating action, the gene effect was shielded in *Th. ponticum* and Xiaoyan7631. On the bases of Shannong31504 and Shannong31505, two short stalked individuals, which could be observed 21 bivalents in PMC MI, were selected from the selfed progenies of Shannong31504 and Shannong31505. Through two generations of selfed on the condition of covered of sacks, two strains Shannong31504-1(Fig. 1) and Shannong31505-1(Fig. 2), which their chromosome numbers were stably 2n=42 and 21 bivalents can be observed in PMC MI, were obtained and their main agronomic characters were shown in Table 1. In the PMC MI of F_1 hybrid between Shannong31504-1 and Lumai No.5, quadrivalent can be observed, but 21 bivalents were observed in Shannong31505-1/Lumai No.5. In the PMC MI of F_1 hybrid between Shannong31504-1 and Shannong31505-1, about two univalents and twenty bivalents can be observed. All of these data were shown in Table 2 and indicated that Shannong31504-1 and Shannong31505-1 may be two wheat-*Th. ponticum* translocation lines and they probably were permeated with different *Th. ponticum* chromatins.

Electrophoresis: the translocation lines Shannong31504-1 and Shannong31505-1, *Th. ponticum*, Xiaoyan7631 and wheat cultivar Lumai No.5, as well as CS and Marquis, were used in the gliadin electrophoresis analysis. The electrophoreogram was presented in Fig. 3. Gliadin bands patterns of Shannong31504-1 and Shannong31505-1 clearly discriminated to Lumai No.5, two *Th. ponticum* specific bands coded by *Gli-B1* (located on 1B), according to the nomenclature of Metakovsky (1991), presented in the two translocation lines and Xiaoyan7631, were absent in wheat cultivar Lumai No.5 (shown by the the arrow). So it is indicated that two translocation lines have *Th. ponticum* chromatins and chromosome structural recombination occurred on first chromosome group. In addition, one band shown by the arrowhead can be

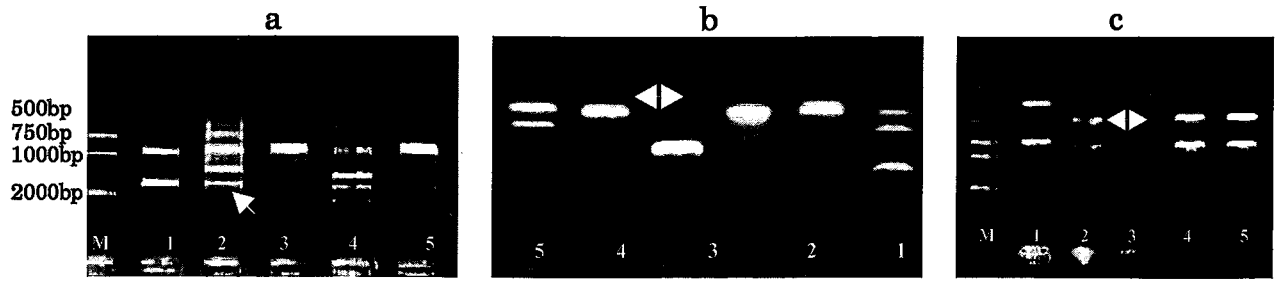

Fig. 4. Amplification profiles with the S36, S37 and S97 primers.
a) S36 primers, the arrow indicates *Th. ponticum* specific $S36_{1800}$ band; b) S37 primer, the arrow indicates *Th. ponticum* specific $S37_{720}$ band: c) S97 primer, the arrow indicates *Th. ponticum* specific $S97_{490}$ band. 1: *Th. ponticum*, 2: Xiaoyan7631, 3: Lumai No.5, 4: Shannong31504-1, 5: Shannong31505-1, M: size marker(2-kb DNA ladder).

used to differentiate two translocation lines.

RAPD analysis: a total of 112 decamer primers (S_{25}~S_{37}, S_{41}~S_{60}, S_{135}~S_{160}, S_{196} and S_{516}) were studied among the translocation lines Shannong31504-1, Shannong31505-1, maternal parent Lumai No.5, paternal parent Xiaoyan7631 and *Th. ponticum*. Three primers S36 (5'AGCCAGCGAA3'), S37 (5'GACCGCTTGT3') and S97 (5'ACGACCGACA3') could produce *Th. ponticum* specific amplification fragments in the translocation lines. With S36, Shannong31504-1 and Shannong31505-1 exhibited a fragment (about 1800bp, labeled as $S36_{1800}$) that was present in Xiaoyan7631 and *Th. ponticum* but absent in Lumai No.5 (Fig. 4-a); in the amplification products of primer S37, a *Th. ponticum* specific fragment (about 720bp, labeled as $S37_{720}$) was appeared (Fig. 4-b), the other primer S97 could also amplify a *Th. ponticum* specific product about 490bp (labeled as $S97_{490}$) (Fig. 4-c). So the presence of $S36_{1800}$, $S37_{720}$ and $S97_{490}$ in the translocation line amplification profiles and their absence in Lumai No.5 amplification profiles suggested that $S36_{1800}$, $S37_{720}$ and $S97_{490}$ were three makers to tag the *Th. ponticum* chromatin in the wheat-*Th. ponticum* translocation lines Shannong31504-1 and Shannong31505-1.

Acknowledgments

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Research article

Low temperature germinability and ABA sensitivity in wheat cultivars with pre-harvest sprouting tolerance

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Summary

To prevent pre-harvest sprouting (PHS) in wheat, selection of genotypes that maintain dormancy and imbibe even at low temperature during grain development is receiving considerable attention in breeding programs. However, little information is available for the genetic variation in low temperature germinability and its relation to the abscisic acid (ABA) sensitivity of an embryo. To evaluate low temperature germinability and ABA sensitivity, grains of 30 cultivars with various levels of PHS tolerance were incubated in water or an ABA solution at different temperatures. When grains at the dough-ripe stage were germinated at 12°C, grain germinability and ABA sensitivity varied even in PHS-tolerant cultivars. The dormancy of some PHS-tolerant cultivars broke with loss of ABA sensitivity at low temperature, suggesting that tolerance to PHS is in part genetically independent of low temperature germinability. A close relationship was found between low temperature germinability and the ABA sensitivity. For wheat production under cool moist conditions at maturity, it would be significant to develop cultivars that did not germinate at low temperature.

Key words: abscisic acid, low temperature germinability, pre-harvest sprouting, grain dormancy, *Triticum aestivum*

Introduction

Wheat (*Triticum aestivum* L.) cultivars are characterized by relatively weak embryo dormancy, and, under cool moist conditions at maturity, they show a developmental disorder and pre-harvest sprouting (PHS). PHS reduces grain yield and leads to deterioration in the milling performance and quality of the end product. The PHS problem often occurs in many major wheat growing areas of the world (Allan 2000). As reviewed by Li and Foley (1997), several morphological and physiological traits influence the expression of PHS, and grain dormancy is considered to be the most important factor in controlling PHS damage.

While high levels of dormancy are required to improve wheat cultivars, many environmental factors influence dormancy. Temperature and moisture during grain development and after ripening are especially important (George 1967; King 1993). While it has been reported that low temperatures during grain development enhance the level of grain dormancy (Kuwabara and Maeda 1979), mature wheat grains show dormancy when incubated at above 20°C but not below 12°C (Bewley and Black 1994). In turn, dormancy breaking at low temperature would lead to a problem in several wheat production areas where

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grain develops under relatively high humidity and cool temperatures of 12°C or less. This is the case in Japan, especially in Hokkaido. Therefore, selection of genotypes that maintain dormancy and imbibe even at low temperature during grain development has received considerable attention in breeding programs (Osanai and Amano 1996; Amano et al. 1999; Osanai and Amano 1999). By recurrent selection for low temperature germinability, Osanai and Amano (1993) developed a series of genotypes that are tolerant to PHS because they lack altogether or have weak low temperature germinability. On the other hand, Walker-Simmons (1987) indicated that the abscisic acid (ABA) sensitivity of the embryo was related to grain dormancy and PHS resistance. Noda and Kanzaki (1988) reported on the effectiveness of selection for ABA-sensitive genotypes by applying ABA to whole grains that almost lacked grain dormancy. This was done because embryo sensitivity to ABA of a PHS-tolerant cultivar is maintained even after its dormancy disappears. However, little information is available for genetic variation in low temperature germinability and its relation to the ABA sensitivity of the embryo.

In the present study, to evaluate the low temperature germinability and ABA sensitivity, germination tests were conducted at different incubation temperatures using 30 cultivars with various levels of PHS tolerance.

Materials and methods

Seventeen winter (9 red and 8 white) and 13 spring (8 red and 5 white) wheat cultivars and breeder's lines were used. As listed in Table 1, they have different PHS phenotypes when grown in Hokkaido (Kuwabara et al. 1996). For example, Lancer, RL4137, and Satanta are classified as PHS-tolerant, whereas Chihokukomugi, Verry S, and Haruyutaka are classified as PHS-susceptible. The materials were grown in the standard manner at the Memuro test field, Hokkaido National Agricultural Experiment Station. The winter wheats were sown in middle September, 1998, and the spring wheats, in late April, 1999.

Spikes of these cultivars were sampled at two grain filling stages: (1) the dough-ripe stage: 35-40% water content, with physiological maturity; and (2) full-ripe stage, when the water content is 25-30%, which occurred 7 days after the dough-ripe stage. To identify grain developmental stages, the classification system of Noda et al. (1994) and Kuwabara et al. (1996) was used, and the water content was measured from 30 days post anthesis (DPA) to 45 DPA. Collected spikes were dried in a forced-air oven at 35°C for 2 days and water content was reduced to less than 15%. Grains from primary and secondary florets of the central spikelets of spikes were then gently hand-threshed and stored in a freezer at -20°C, a storage temperature that is known to maintain the dormancy level (Mares 1983). Germination tests were carried out within 2 months of harvest.

Grains were surface-sterilized in sodium hypochlorite (5% available chlorine) for 20 min and immediately washed several times with distilled water. Duplicates of 50 grains were placed on sterilized filter papers wetted with 6 ml of distilled water or 50 μM ABA in Petri dishes. The dishes were incubated at two different temperatures, 12°C and 20°C, for 7 days in the dark and the number of germinated grains was scored. Following Mares (1984), germination was defined as occurring with the appearance of a 2 mm shoot and 3 distinct seminal roots. The incubation temperatures chosen in this experiment are representative to either keep grain dormancy or break it (Bewley and Black 1994), and the lowest incubation temperature is almost equal to the minimum summer temperature in the major wheat production areas of Japan.

Results and discussion

Germination percentages of grains collected at the dough-ripe and full-ripe stages and imbibed at 20°C are given in Table 1. PHS tolerance was evaluated using a rain simulator for three seasons (Kuwabara et al. 1996). Germination test at 20°C of the grains of the dough-ripe stage indicated that PHS-tolerant cultivars showed lower level of germination than the susceptible cultivars. At the full-ripe stage the germination percentages increased in almost all the cultivars. However, most of the PHS-tolerant cultivars still showed a lower percentage of germination than the susceptible cultivars, although some of the tolerant cultivars, such as Norin 8, Norin 17, Retcital and CI-1, germinated as well as susceptible cultivars. The results agreed with the finding by Kuwabara et al. (1996), which indicated that the old cultivars, such as Norin 8, showed a high percentage of sprouting at the full-ripe stage, while the new cultivars, such as Taisetsukomugi, maintained high level of tolerance to sprouting at the full-ripe stage as well as the dough-ripe stage.

Germination percentages of the grains imbibed at 12°C are also shown in Table 1. The cultivar variation in germination percentage was smaller at

Table 1. Germination (%) of grains collected at the dough-ripe and full-ripe stages imbibed at 20°C and 12°C.

Winter wheat	20°C			12°C			Spring wheat	20°C			12°C		
	DRS	FRS	PHS	DRS	FRS	PHS		DRS	FRS	PHS	DRS	FRS	PHS
Red-grained													
Kitami 72	6	4	T	96	82	T	Park	0	0	T	54	82	T
Hokkai 251	8	42	T	48	94	T	Norin 61	0	8	T	96	90	T
Lancer	8	24	T	76	76	T	RL 4137	2	8	T	32	40	T
Satanta	12	36	T	90	96	T	Nishikazekomugi	2	4	T	76	68	T
Kitakei 1354	22	12	T	58	94	T	Zenkoujikomugi	8	8	T	46	52	T
Hokushin	32	90	MT	96	100	MT	Haruminori	8	18	MT	98	100	MT
Horoshiri	32	70	MT	94	96	MT	Harunoakebono	28	12	T	80	96	T
Chihokukomugi	34	100	S	94	100	S	Haruyutaka	40	52	S	68	80	S
Lewis	86	82	S	88	92	S							
White-grained													
Norin 8	2	88	T	46	90	T	AUS 1408	0	2	T	10	30	T
Clarks Cream	6	34	T	56	56	T	CI-1	8	68	T	42	76	T
Recital	12	64	T	60	70	T	Kanto 74	36	32	MT	80	84	MT
Norin 17	12	90	T	88	98	T	Gamenya	36	30	S	56	74	S
8096A58-1	16	34	MT	52	70	MT	Verry S	62	56	S	52	64	S
Hokkai 253	22	94	MT	100	98	MT							
Satsukei 67	34	92	S	88	94	S							
Hokkai 252	50	98	S	94	88	S							

DRS: Dough-ripe stage, FRS: Full-ripe stage, PHS: Tolerance to PHS evaluated in the Hokkaido area (T: tolerant, MT: moderately tolerant, S: susceptible)

12°C than in 20°C not only at the full-ripe stage but also at the dough-ripe stage. The germination percentages of many PHS-tolerant cultivars at 12°C were as high as those of susceptible cultivars. This tendency was strong at the full-ripe stage. Several PHS-tolerant cultivars, Clarks Cream, RL4137, Zenkoujikomugi, and AUS 1408, however, still maintained a relatively low germination percentage at 12°C compared with other cultivars at the full-ripe and dough-ripe stage.

These results agreed with the finding by George (1967), who reported that mature grains of several varieties in the U.S. showed dormancy at 30°C but not at around 10°C. The optimum temperature for germination increased after ripeness. This was confirmed in the germination percentages in grains imbibed at 20°C in which PHS-tolerant cultivars did not germinate until the full-ripe stage (Table 1). However, the degree of dormancy would be dependent on the temperatures during grain development, especially near maturity. Among the PHS-tolerant cultivars, genetic variation would appear as a result of the sensitivity to low temperature, as simulated in the 12°C incubation experiment (Table 1). If grains matured at low temperature and high humidity, the development of grain dormancy might reduce at both the dough-ripe and full-ripe stages.

Fig. 1 shows the effects of ABA treatment on the germination of the grains imbibed at 20°C at the dough-ripe and full-ripe stages. The germination was completely inhibited by ABA in most of the cultivars at the dough-ripe stage. The grains at the full-ripe stage were less sensitive to the inhibition effect of ABA, which was especially notable in the PHS-susceptible cultivars. For example, the PHS-susceptible Chihokukomugi germinated in ABA solution. By contrast, the germination of almost all the PHS-tolerant cultivars was still inhibited by ABA at the full-ripe stage.

Fig. 2 shows the effects of ABA treatment on the germination of the grains imbibed at 12°C at the dough-ripe and full-ripe stages. The inhibition effect of ABA on germination decreased with an increased in the germination percentage in water, and there were significant correlations between the germination in water and ABA solution ($r= 0.607$, $P<0.01$, in the winter wheat; $r=0.901$, $P<0.01$, in spring wheat at the dough-ripe stage, $r= 0.795$, $P<0.01$, in the winter wheat; $r=0.667$, $P<0.01$, in the spring wheat at the full-ripe stage; Figs. 1 and 2).

Most PHS-susceptible cultivars germinated in the ABA solution as well as in water, whereas the tolerant

cultivars, which germinated in water, were still inhibited by ABA. This result is in agreement with finding by Noda and Kanzaki (1988) who reported that the embryo sensitivity to the ABA of PHS-tolerant cultivars is maintained even after its dormancy disappears and can be estimated by applying ABA to whole grains that nearly lack dormancy. However, some of the PHS-tolerant cultivars germinated in ABA solution as well as in water, whereas most of the tolerant cultivars maintained low germinability in ABA solution. This was observed in the germination percentages of PHS-tolerant Lancer and RL4137 with only 20-30% germination, while Satanta and Norin 61 were up to 75-100% at both grain-filling stages (Fig. 2).

In the present study, grain germinability and the inhibition effect of ABA on germination were evaluated at different incubation temperatures to determine genetic differences for low temperature germinability. When grains harvested at the dough-ripe stage, corresponding to physiological maturity, were incubated at high and low temperatures, most of the PHS-tolerant cultivars retained lower germinability in water and maintained ABA sensitivity at both temperatures. However, several PHS-tolerant cultivars germinated in ABA solution as well as in water at a low temperature.

As mentioned earlier, Osanai and Amano (1993) demonstrated the effectiveness of recurrent selection for low temperature germinability. Their genetic lines, designated 'OS' and 'OW', expressed more potent tolerance to PHS than the most tolerant cultivars in this experiment (Osanai and Amano 1996, 1999). Those lines received attention as breeding materials to develop cultivars that are more tolerant to PHS than Lancer and Kitakei 1354 because, when

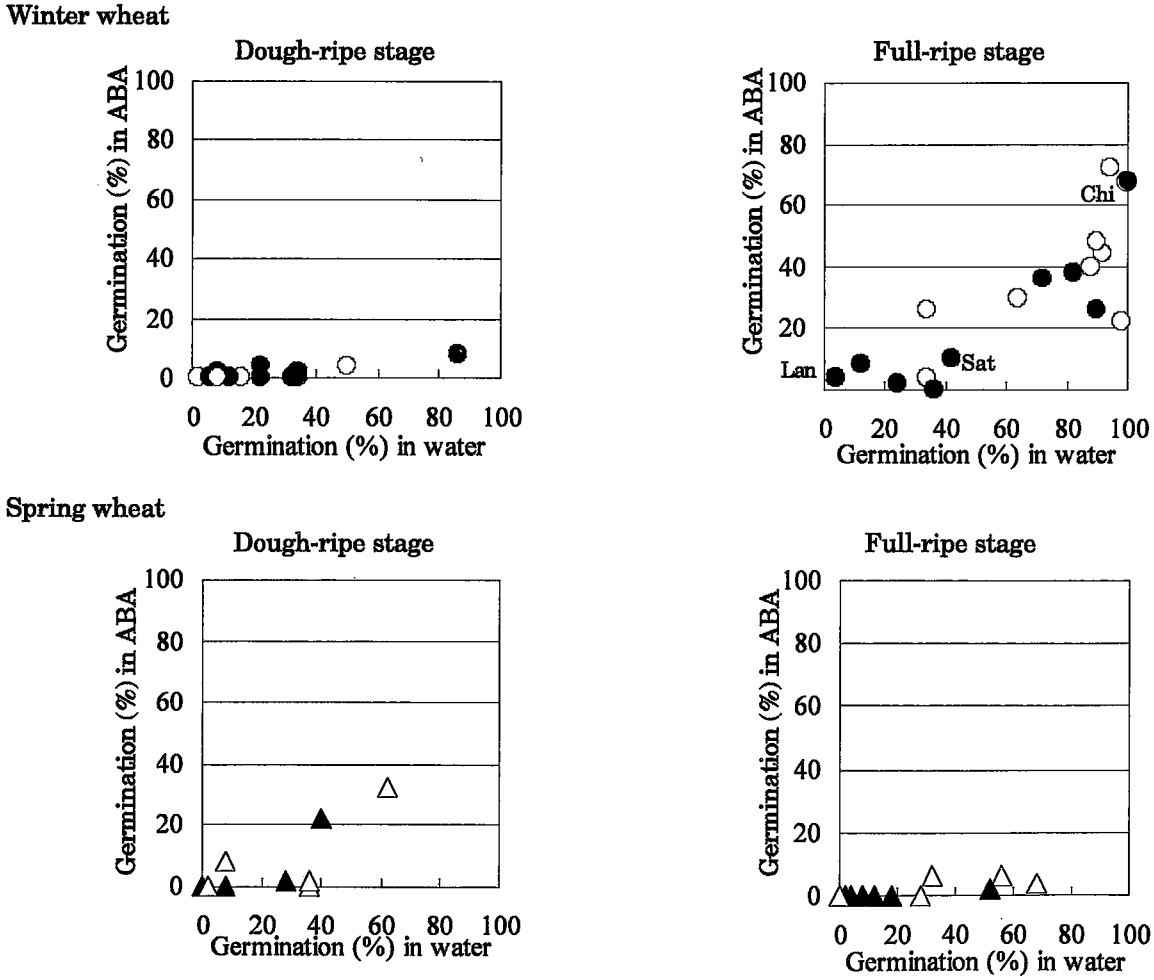

Fig. 1. Germination (%) of the dough-ripe and full-ripe stages of wheat grains incubated in water and ABA solution at 20°C

● : red, winter wheat ▲ : red, spring wheat ○ : white, winter wheat △ : whitge, spring wheat

Sat : Satanta, Lan : Lancer, Chi : Chihokukomugi, N61 : Norin 61, RL : RL4137

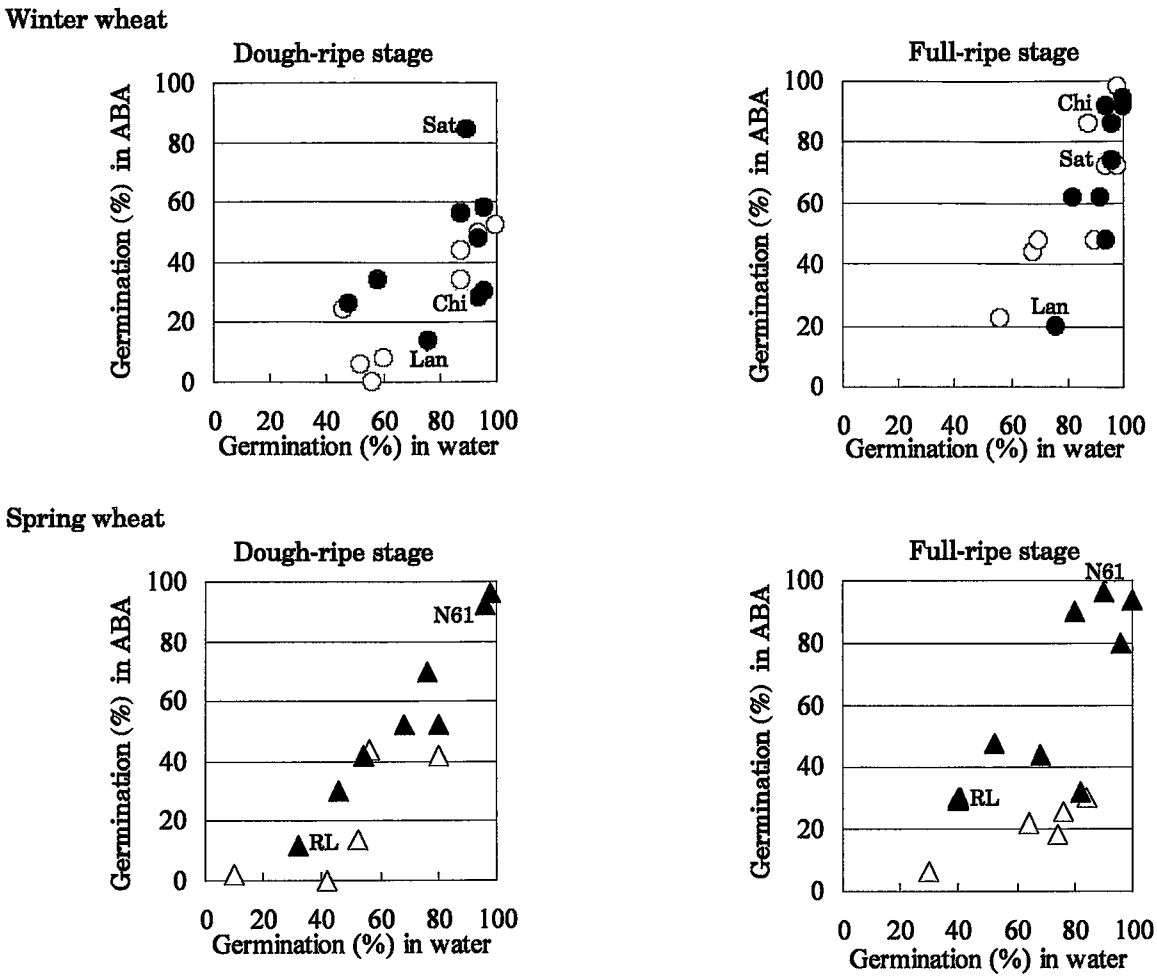

Fig. 2. Germination (%) of the dough-ripe and full-ripe stages of wheat grains incubated in water and ABA solution at 12°C

● : red, winter wheat ▲ : red, spring wheat ○ : white, winter wheat △ : whitge, spring wheat

Sat : Satanta, Lan : Lancer, Chi : Chihokukomugi, N61 : Norin 61, RL : RL4137

considering production under cool moist conditions at maturity in northern Japan, especially in Hokkaido, a high level of grain dormancy is desirable to prevent PHS damage.

For the enhancement of PHS-tolerance in wheat, it would be required to increase ABA sensitivity at lower temperatures during grain development. As revealed in Figs. 1 and 2, a strong correlation between low temperature germinability and ABA sensitivity was detected in this experiment. Hence we propose that it would be significant to improve sprouting tolerance with ABA sensitivity at low temperatures.

Acknowledgments

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Research article

Stress-induced changes in the content of the proliferative antigen of initial cells in the stem apices of two wheat cultivars differing in drought resistance

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Summary

The content of the proliferative antigen of initial cells (PAI) in the stem apex cells of two common wheat (*Triticum aestivum* L.) cultivars differing considerably in their drought resistance – Saratovskaya 29 and Opal – was measured at post-osmotic stress repair by using a special immunochemical test system. The PAI content of these cells was studied as normal and at post-stress repair. A marked response of droughted plants was obtained that was reflected by a change in the PAI content of stem apical cells. Saratovskaya 29 (resistant) did not differ from Opal (sensitive) in the post-stress resumption of leaf growth processes. However, differences in the content of PAI were observed during the post-stress regeneration of the meristematic tissues. These differences can serve as a basis for the study and prediction of plant resistance to unfavorable abiotic factors in the breeding of high-yielding wheat cultivars.

Introduction

Plant apical meristems are universal biological systems where the morphogenetic potential of stem cells (initial cells, or initials) is realized. The nature and function of these cells are poorly known, as are the molecular mechanisms of cooperative interactions among stem apex meristematic cells at stress. An in-depth analysis of such interactions requires that the functional activity of stressed cells be investigated on the molecular-cellular level.

The shift to research on the molecular-cellular level is feasible in principle if suitable molecular markers are available that are associated with cell division and reflect the functional activity of dividing cells. Such molecules have been studied both in vitro in cultures of synchronously dividing cells (Smith et al. 1988; Kodama et al. 1991; Kiyosue et al. 1991; Magyar et al. 1997; Meszaros et al. 2000) and in vivo in the cells of higher plant life (John et al. 1989; Hush et al. 1996; Schuppler et al. 1998; Mironov et al. 1999; Beemster et al. 2002). These investigations have been primarily to document the peculiarities of genetic system functions that control the proliferation and further differentiation of plant cells.

Previously, we and others (Volodarsky 1985; Moiseeva 1991; Sumaroka et al. 2000) showed that the apical meristem of the wheat stem contains a specific antigen (proliferative antigen of initials, PAI) that is characteristic of actively proliferating cells. The celluar PAI level in the rye stem apex is correlated with whole-plant growth (Volodarsky et al. 1986) and is associated with the embryogenic capacity of the wheat callus tissue in an in vitro culture (Evseeva et al. 2002). Those studies suggested that the PAI

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content of plant meristems will also permit one to assess stress-induced changes in meristem cell proliferative activity.

The aim of this work was to study osmotic-stress-induced changes in the content of PAI in the stem apices of two wheat cultivars differing in stress resistance.

Materials and methods

Two common wheat (*Triticum aestivum* L.) cultivars were used: Saratovskaya 29 and Opal. They have contrasting degrees of resistance to drought. Saratovskaya 29 originates in the steppe ecotype of the Volga region and is very tolerant of drought. The cultivar Opal originates from Germany and is drought sensitive. Yet, these cultivars are comparable in their vegetation period: they are not essentially different in their phenophase periods or in leaf growth rate (Kumakov 1985). The synchronism of their development is particularly important in studying the stress-induced disruption of cellular proliferation and the subsequent repair.

Wheat seedlings were grown in glass tubes tied up in pairs and placed in containers filled with water. The seedlings were placed in a KTLK 1250 (Nema, Germany) controlled environment cabinet (temperature: 22°C, light intensity: 30,000 lux, irradiance: 17.2 W/m^2, air humidity: 60%). After being grown for 3 days in the dark and then 1 day in continuous light, the seedlings were subjected to osmotic stress.

Osmotic stress was given by placing the plant root system in Knopp's nutrient solution supplemented with 24% (w/v) polyethylene glycol (PEG) (M_r = 6000; Serva Feinbiochemica GmbH, Heidelberg, Germany), a concentration that corresponds to an osmotic pressure of 3.7-MPa (Nechiporenko and Rybalova 1980). The stressed plants were then returned to their original growth conditions (see above) in order to study the repair processes. The growth response of the cultivars to osmotic stress was evaluated by the change in linear leaf size during post-stress repair.

To obtain the total meristem protein fraction containing PAI, we separated the seedling from the seed and excised a 2 to 3 mm piece of tissue with the stem apex and basal leaf meristems. The tissue was disrupted in liquid N_2 and was subjected to extraction for 1 h at 4°C in 50 mM Tris-HCl (pH 7.8) containing 0.02 M β-mercaptoethanol (Ferak Laborat GmbH, Germany) and 0.001 M phenylmethylsulfonyl fluoride (Fluka Chemie AG, Buchs, Switzerland). The homogenate was centrifuged for 30 min at 20,000x g. The supernatant liquid containing total water-soluble proteins was used to study post-stress repair.

The relative quantitative assay of PAI content was done with an immunochemical test-system using monospecific anti-PAI antibodies raised by us as described earlier (Evseeva et al. 2002). The PAI content in four-day-old seedlings of the control cultivar (Saratovskaya 29 grown under normal conditions, titer: 1:2) was taken as 100%.

The results of the PAI determination and growth evaluation were statistically processed. The arithmetic means (standard errors of the mean of four experiments, with three replicates per experiment) were calculated. Twenty-five seedlings were used in each replicate. The significance of differences in PAI content among the experiments was evaluated by a three-factorial analysis of variance (Maksimov 1980) at a significance level of 0.05.

Results and discussion

Studies of post-stress repair in plants showed that the most clear-cut distinctions in cultivars' resistance levels are manifested when the extreme factor is so severe that it decreases the productivity of a sensitive cultivar ca. twofold (Udovenko 1979). This principle of choosing stress conditions for the wheat cultivars

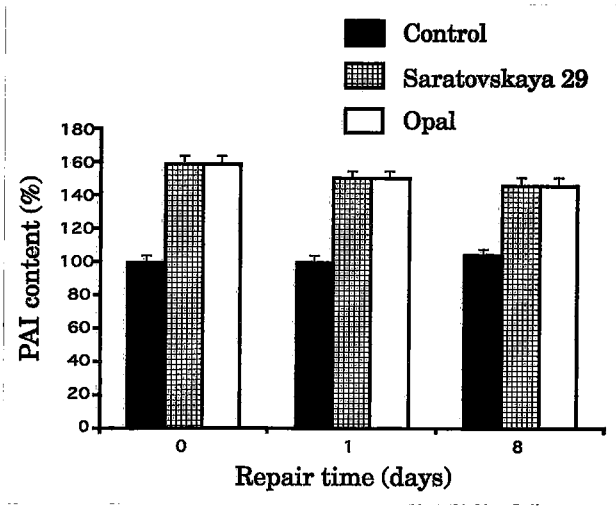

Fig. 1. Changes in the PAI content of the stem apical meristems of Saratovskaya 29 and Opal during post-stress repair. Stress conditions: 3.7-MPa, 2 days. The PAI content in four-day-old seedlings of the control cultivar (Saratovskaya 29 grown under normal conditions, titer: 1:2) was taken as 100%. Data are the arithmetic means of three replicated samples, each sample consisting of 25 plants. Standard error did not exceed 4%.

used in this work was applied by Demchenko (1994), who showed that the nearly twofold decrease in the leaf length of the susceptible cultivar occurs after a 3.7-MPa osmotic-stress treatment for 2 days.

Those conditions, however, did not permit us to find any distinct differences between Saratovskaya 29 and Opal with respect to post-stress repair (Fig. 1). The normal content of PAI present in the stem meristem cells (non-stress conditions) was the same in both cultivars; as a result, only one cultivar, Saratovskaya 29, was used as a control (Fig. 1). Further work investigated the effects of various times of the 3.7-MPa treatment (4, 6, and 8 days). Increasing the time of osmotic stress stopped leaf growth; the upper part of the lamina and the wheat-root coleoptile began to wilt. On the eighth day of stress, the coleoptile and the leaves dried out; only the shoot apical meristem and the adjacent basal leaf meristems remained viable. At first the seedlings looked lifeless, but at post-stress repair the leaves renewed their growth, indicating the preserved viability of the apex. The longer was the stress time, the later commenced the re-growth of the youngest leaf under these conditions and the lower was the re-growth rate. The cultivars did not differ in the re-growth dynamics of the youngest leaf (Fig. 2).

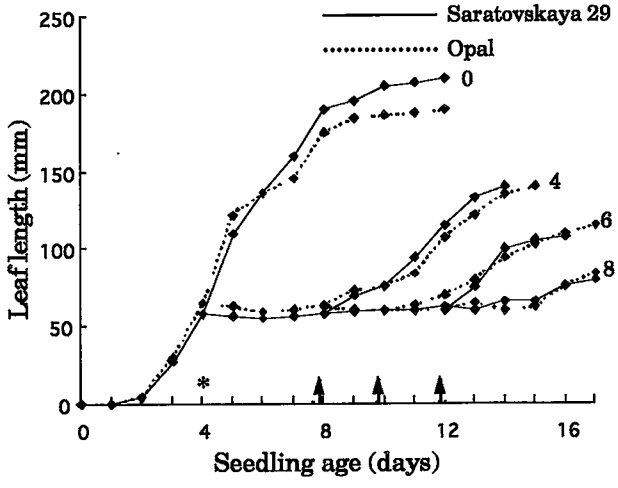

Fig 2. The length of the youngest leaf of seedlings of Saratovskaya 29 (solid lines) and Opal (dotted lines) during and after osmotic stress (3.7-MPa). The numbers (0, 4, 6, and 8) at the curves represent stress period in days. The asterisk shows the onset of stress, and the arrows mark its ending. Data are the arithmetic means of three replicated samples, each sample consisting of 25 plants. The experiment was repeated four times. Standard error did not exceed 4%.

The differences in the content of PAI during repair were greatest after a 3.7-MPa osmotic-stress treatment for 8 days (Fig. 3). In particular, we noted that the normal content of PAI in both cultivars increased monotonically during ontogenesis (Fig. 3). The content of PAI in the stem meristems at 2 h after osmotic stress increased ca. fourfold (Saratovskaya 29) and ca. eightfold (Opal), as compared with the control. During repair, PAI content decreased to the control value in Saratovskaya 29 and below the control value in Opal (Fig. 3).

Our results show that the stem apical cells have a higher degree of resistance than do the cells of differentiated wheat tissues. As Gudkov (1985) notes, the reliability resources of the apical meristem are determined not by resistance of single cells but by cooperative interaction of individual meristem cell populations at various cell cycle phases. As with all eukaryotes, the cell cycle in plants is regulated through the G_1/S and G_2/M transitions. The transitions serve as control points at which cell division is synchronized under the action of adverse factors (Doerner 1994; Dewitte and Murray 2003). In particular, the synchronizing action of osmotic stress on the cell population of the plant apical meristem is associated with a change in the phytohormone balance (Bray 1993), leading to a change in the activity of cyclin-dependent kinases (CDKs) (Schuppler et al.

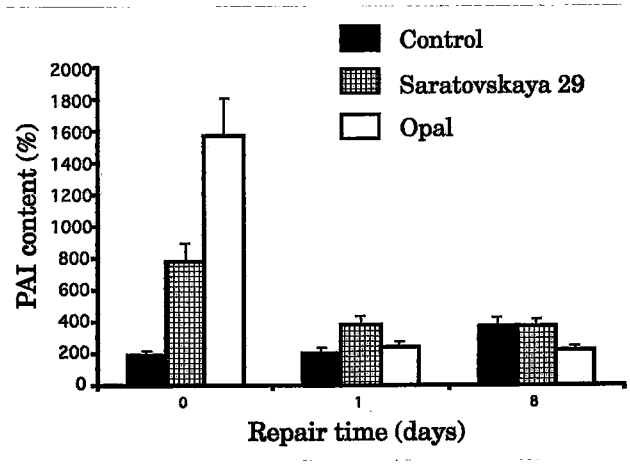

Fig. 3. Changes in the PAI content of the stem apical meristems of Saratovskaya 29 and Opal during post-stress repair. Stress conditions: 3.7-MPa, 8 days. The PAI content in four-day-old seedlings of the control cultivar (Saratovskaya 29 grown under normal conditions, titer: 1:2) was taken as 100%. Data are the arithmetic means of three replicated samples, each sample consisting of 25 plants. Standard error did not exceed 6%.

1998). One can assume that PAI's function is similar to that of cdc2-activating kinases, among which is the protein kinase $P34^{cdc2}$, regulating the entry of eukaryotic cells into mitosis (John et al. 1989; Dudits et al. 1998). A sharp increase in the PAI content immediately after stress seems to reflect a synchronous entry of meristematic cells into mitosis (Figs. 1 and 3). We note that the content of PAI depends on the severity and duration of stress, possibly reflecting the degree of cell division synchronization and the genotypic differences between resistant and sensitive cultivars.

At repair, Saratovskaya 29, having a higher degree of reliability of the cell homeostasis in the stem apex, showed a near-normal PAI content, whereas in Opal the PAI content decreased twofold as compared with the control. This possibly reflects a disturbance in the mechanisms regulating the entry of individual meristem cell subpopulations into proliferation (Fig. 3). Thus, determination of the content of PAI in the stem apex enabled us not only to assess the functional state of cells of the stem apical meristem, but also to reveal the degree of their reliability in the post-stress regeneration of plant tissues and organs.

Our data suggest that the resilience of wheat cultivars to extreme factors can be predicted and assessed at the cellular level with our immunochemical test system for PAI. More work to explain the nature of PAI and the molecular mechanisms of its expression will aid in obtaining a more penetrating insight into the regulatory mechanisms underlying cell division and further cellular differentiation in plant apical meristems under the influence of various environmental factors.

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Research article

Genetic studies of grain filling period in durum wheat under normal and late sown environments

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Summary

Parental, F_1, F_2, BC_1, BC_2, BC_{11}, BC_{12}, BC_{21}, BC_{22}, BC_1 self and BC_2 self generations of three crosses involving six cultivars of durum wheat (*Triticum durum* Desf.) were studied for grain filling period under normal and late sown environments to analyze the nature of gene effects. Six-parameter model in the cross Raj 911 x DWL 5002 in normal sown and 10-parameter model in other crosses under both normal and late sown plantings were found adequate to explain the inheritance of this trait. Additive (d) and dominance (h) gene effects were frequently observed significant. Of the epistatic interactions, one or the other digenic and / or trigenic interactions played significantly greater role in controlling the inheritance of this trait. Non-fixable gene effects were much higher than the fixable gene effects in all the crosses in both the sowing environments. Significant positive heterosis (over better parent) was observed in the crosses Cocorit 71 x A-9-30-1 under both the planting dates and in the cross HI 8062 x JNK-4W-128 in normal planting. The major combined effects of digenic as well as trigenic epistatic interactions attributed the significant heterosis. Significant inbreeding depression was not frequently observed. Biparental mating and/or diallel selective mating have been suggested for the improvement of this trait in durum.

Key words: durum wheat, non-allelic interactions, gene effects, epistasis, heterosis

Introduction

The grain yield in cereals can be defined in terms of the rate of dry matter accumulation in grain per unit time and the length of grain formation period. Hence, breeding of early maturing varieties is an important objective in durum wheat research in view of the increasing importance of intensive crop sequences. Short duration genotypes can be adjusted easily in double cropping and are also suitable for conditions of short winter. Existing wheat varieties are differentiated in length of period from flowering to maturity, the differences are considerable, and ranged from 30 to 70 days. In general, varieties having a long period of seed development, flower earlier and this conditioned trait are undoubtedly genetic and even have ecological significance. This trait plays a different role in various regions. Hence, when this trait will be taken into account, the length of period from flowering to maturity can be used as a marker for selection. Results of various experiments (Sterzycki 1978; Sinha et al. 1979) clearly indicated that among varieties of wheat considerable variation according to earliness and length of grain formation period is observed and the length of the period from flowering to maturity determines the quantum of yield. Nasyrov (1978) led emphasis on physiological components for increasing the yield potential of wheat. Further breakthrough in yield may be obtained by

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exploiting the genetic information for important physiological trait related to yield (Austin et al. 1980). Thus, there is a need for exploitation of such important character for further amelioration of wheat production under diverse environments.

Early grain filling period is an index of the general efficiency of a genotype in terms of giving maximum returns per unit time. The number and the weight of the grains determine the final yield of grain per spike of wheat, it contains. The weight attained by any grain depends on the duration of the grain filling period; rate of supply of assimilates to grain and the rate of incorporation of these into its structure from anthesis onwards (Singh et al. 1977; Prabhu and Sharma 1984; Dasgupta and Mondal 1989; Sood and Dawa 1999). The yield of late sown durum wheat is reduced mainly due to reduced grain filling period and not due to reduced rate of supply of assimilates. Experimentally it is evident that seed number and seed weight are two most important yield components in durum wheat. Weight of any grain depends on duration of the grain filling period. Grain filling is a very critical physiological phase in source-sink relationship. It is the period when photosynthetic assimilates are supplied and incorporated into the seed. There are scanty references for genetic studies of grain filling period in durum wheat. Information on genetics of parents and mode of gene action governing duration of grain filling would be useful in devising suitable breeding procedures for developing early genotypes suitable for varied environmental conditions.

Very limited studies are reported on the inheritance of grain filling period particularly in durum wheat. The present research work was, therefore, undertaken through generation mean analysis in twelve generations of the three crosses of durum wheat to provide information on: (a) the relative significance of different types of gene action viz., main effects, digenic and trigenic epistatic interactions and their components for grain filling period in normal and late sown environments. (b) Role of heterosis and inbreeding depression in the grain filling period in normal and late sown environments and the gene actions involved in manifestation of these phenomena. Based upon the results achieved, an attempt was made to suggest breeding methodology to be followed in durum wheats for further improvement of grain filling period for further amelioration of grain yield.

Materials and methods

Materials, field design and standard statistical procedures used in this study are the same as in the previous one (Sharma and Sain 2003). The number of days taken from the date of heading to the date of physiological maturity (turning yellow) of the selected plant was recorded as a grain filling period.

Results and discussion

The results of generation mean analysis and standard errors for grain filling period in the three inter-varietal crosses of durum wheat under two dates of plantings revealed significant differences among all the twelve generation means indicated presence of genetic variability for this trait in the materials studied. The joint scaling test exhibited that subsequent fitting of different models viz., non-epistatic model (3-parameter model), epistatic models assuming presence of digenic interactions (6-parametr model) and trigenic interactions (10-parameter model), 10-parameter model showed non-significant values of chi-square with maximum significant estimates of parameters in all the crosses under both the sowing dates except in the crosses HI 8062 x JNK-4W-128 and Raj 911 x DWL 5002 in normal sown environment (Table 1). This indicated adequacy of this model to account the genetic variance among the generation means existing in the crosses. In the cross HI 8062 x JNK-4W-128, 3-parameter and 6-parameter models gave very high chi-square values in normal sowing. Subsequent fitting of 10-parameter model did not give probability higher than 0.001, revealed that even 10-parameter model did not fit to the data indicated involvement of more complex interactions or linkage in the inheritance of this trait. The various gene effects in this cross were, however, estimated following the trigenic interaction model, in view of the fact that the chi-square value for this model was the lowest. In the cross Raj 911 x DWL 5002 (normal sown) the chi-square value for 3-parameter was significant. Subsequent fitting of 6-parameter and 10-parameter models showed non-significant chi-square values, however, the probability of fitness of the 6-parameter model was the highest along with maximum significant parameters. Therefore the 6-parameter model was considered more appropriate to explain the genetic variation among the generation means existing in this cross in normal sown condition (Table 1). These results clearly indicated that epistatic interactions had a greater role in controlling the inheritance of grain filling period in all the crosses under optimum and sub-optimum sowing environments. The results of the cross Raj 911 x DWL

5002 in late sown environment further exhibited that environment played a greater role to the expression of different non-allelic interactions in such a way that a significant contribution for trait changed drastically in changing the environments. As a matter of fact that when sowing is delayed, the genotypic expression is affected hence, the possibility is that the true phenotypic difference are not resolved leading to observation of non-significant differences between the different genetic parameters estimated in normal and delayed sowing. In such situations the estimates made under late sowing are found to be less or non-significant in comparison to the normal environments unless the parents involved are specially selected for the targeted environment. It was also noted in the cross HI 8062 x JNK-4W-128 that changes in planting dates (normal to late) somehow reduce/break the complex genetic barrier of different gene effects in late sown condition.

The analysis of gene effects revealed that both additive (d) and dominance (h) gene effects were significant in the crosses HI 8062 x JNK-4W-128 and Raj 911 x DWL 5002 under both the sowing dates. However, in the cross Cocorit 71 x A-9-30-1 only additive (d) gene effect was significant in late planting. In the present study relative magnitude and direction (signs) of the main effects [(d) and (h)] changed in change in cross as well as sowing environment. Results further revealed that dominance (h) had a higher magnitude in most of the cases than additive (d) gene effect indicated preponderance of dominance (h) gene effect in the expression of this trait. A perusal of the results of digenic epistatic interaction [(i), (j) and (l)] revealed that additive x additive (i) and dominance x dominance (l) were equally and significantly contributed towards the inheritance of the trait in most of the crosses under both the planting dates, however, magnitude of the dominance x dominance (l) was observed higher. Additive x dominance (j) digenic interaction was not significantly contributed so frequently towards the genetic control of this trait (Table 1). The results of trigenic epistatic

Table 1. Results of joint scaling test and gene effects for grain filling period in durum wheat under normal and late sown conditions

Effects	Cocorit 71 x A-9-30-1		HI 8062 x JNK-4W-128		Raj 911 x DWL 5002	
	Normal	Late	Normal	Late	Normal	Late
M	40.93**	35.96**	36.46**	41.76**	39.27**	37.73**
	±0.45	±0.80	±0.63	±0.62	±0.45	±0.90
(d)	0.50	–1.34*	4.59**	2.17**	2.17**	2.12**
	±0.41	±0.57	±0.51	±0.55	±0.33	±0.67
(h)	–0.26	1.45	–4.12**	–4.43**	–5.01**	3.20*
	±0.81	±1.15	±1.03	±1.11	±0.71	±1.36
(i)	–16.59**	4.12	10.74**	–5.34**	–6.11**	1.29
	±1.09	±2.44	±1.6	±1.51	±0.78	±2.51
(j)	–3.51	6.86*	–8.45**	0.39	36.89**	–2.18
	±2.19	±0.67	±0.67	±2.86	±1.05	±2.95
(l)	–48.27**	–3.15	37.42**	–11.39*	7.60**	2.25
	±3.33	±8.06	±5.93	±5.09	±1.77	±8.98
(w)	–0.58	10.76**	15.39**	–3.53*	-	–0.40
	±1.01	±1.53	±1.90	±1.69		±2.65
(x)	–68.77**	8.03	45.38**	–17.59**	-	4.89
	±4.59	±10.45	±7.39	±6.54		±11.19
(y)	–12.22**	–20.20*	37.24**	10.21	-	–7.04*
	±4.26	±8.37	±4.94	±5.80		3.08
(z)	79.45**	5.01	–23.14*	34.90**	-	–18.68**
	±7.48	±15.24	±10.14	±10.70		±3.95
χ^2 value for adequate model†	0.42 (2)	1.96 (2)	24.96 (2)	2.58 (2)	9.63 (6)	2.36 (2)

*, ** Significant at 0.05 and 0.01 levels, respectively.

† Figures in parentheses indicate degrees of freedom

interactions [(w), (x), y) and (z)] showed that all the interactions played a significant role in controlling the inheritance of this trait in the cross HI 8062 x JNK-4W-128 in both sowing conditions except additive x dominance x dominance (y) in late sowing. In the cross Cocorit 71 x A-9-30-1 additive x additive x dominance (x) and additive x dominance x dominance (y) in normal planting, whereas in late planting additive x additive x additive (w) and additive x dominance x dominance (y) were responsible in governing the inheritance of this trait. In the cross Raj 911 x DWL 5002 additive x dominance x dominance (y) and dominance x dominance x dominance (z) were significantly contributed in the inheritance under late sowing. These results further confirmed that non-allelic interactions played a significantly greater role than the additive (d) and dominance (h) gene effects in the genetic control of inheritance of this trait in durum wheat. Earlier studies (Singh et al. 1977; Edward et al. 1976; Dasgupta and Mondal 1989; Nayeem 1994; Sood and Dawa 1999) also confirmed the role of epistatic interactions in the inheritance of grain filling period in wheat.

Relative magnitude of gene effects (percent) revealed that in the cross Cocorit 71 x A-9-30-1, maximum contribution was made by dominance x dominance x dominance (z) followed by additive x additive x dominance (x), dominance x dominance (l), additive x additive (i) and additive x dominance x dominance (y) in normal sowing. However, in late sowing additive x dominance x dominance (y) followed by additive x additive x additive (w), additive x dominance (j) and additive (d) contributed maximum towards genetic control of the inheritance of this trait. In the cross HI 8062 x JNK-4W-128 almost all the gene effects were significantly contributed in governing the inheritance of this trait. However, additive x additive x dominance (x) followed by additive x dominance x dominance (y) in normal planting; dominance x dominance x dominance (z) followed by additive x additive x dominance (x) in late planting were contributed maximum in the inheritance of this trait. In the cross Raj 911 x DWL 5002 the main effects and all digenic interactions contributed significantly, however, additive x dominance (j) followed by dominance x dominance (l) and additive x additive (i) contributed maximum in governing the inheritance in normal sowing. Under late sowing dominance x dominance x dominance (z) followed by additive x dominance x dominance (y), dominance (h) and additive (d) were contributed maximum to genetic control of the inheritance of the grain filling period (Table 1).

The results of generation mean analysis further exhibited that absolute totals (ignoring signs) of second order interactions [(w) + (x) + (y) + (z)] were much higher than the main effects and first order interactions [(i) + (j) + (l)] in all three crosses under both the sowing dates except in the cross Raj 911 x DWL 5002 in normal planting (Table 2). In this cross first order interactions contributed maximum than the main effects. These results of this study confirmed the greater role of non-allelic interactions in the genetic control of the inheritance of grain filling period in durum wheat. Experimental results showed that parameters (h), (l) and (z) were significant in the cross HI 8062 x JNK-4W-128 in both the sowing dates. The relative signs of (h) and (z) were in same direction but opposite to (l) in normal sowing whereas in late sown the relative signs (h) and (l) were in same direction but opposite to (z), which indicated that all the three genes were showing duplicate type of epistasis. The parameter (h) was observed significant

Table 2. Absolute totals of epistatic effects, fixable and non-fixable gene effects for grain filling period in durum wheat under normal and late sown conditions

Cross	Environment	Main effects		Epistatic effects†		Total gene effects‡	
		(d)	(h)	I order	II order	Fixable	Non-Fixable
Cocorit 71 x A-9-30-1	Normal	0.50	-0.26	68.37	161.02	17.67	212.48
	Late	-1.34	1.45	14.53	44.00	16.22	73.11
HI 8062 x JNK-4W-128	Normal	4.59	-4.12	56.61	121.15	30.72	155.75
	Late	2.17	-4.43	17.12	66.23	11.04	78.91
Raj 911 x DWL 5002	Normal	2.17	-5.01	50.6	-	8.28	49.50
	Late	2.12	3.20	5.72	31.01	3.81	38.24

†First order interactions: [(i), (j), (l)]; Second order interactions: [(w), (x), (y), (z)]

‡Fixable components: [(d), (i), (w)]; Non-fixable components: [(h), (j), (l), (x), (y), (z)]

negative and (l) was significant positive in the cross Raj 911 x DWL 5002 in normal planting indicated duplicate type of epistasis at digenic level (Table 1). Conclusion regarding any type of epistasis could not be drawn in other cases because among (h), (l) and (z) one or the other parameter was found non-significant.

A perusal of absolute totals of fixable [(d) + (i) + (w)] and non-fixable [(h) + (j) + (l) + (x) + (y) + (z)] gene effects revealed that in all the crosses non-fixable (non-additive) gene effects were many times higher than the fixable (additive) gene effects indicated greater role of non-additive gene effects and hence, the requirement of complicated procedure of breeding for their further exploitation is needed (Table 2). Other studies (Prabhu and Sharma 1984; Singh and Rana 1987; Raghuvanshi et al. 1988; Srivastava et al. 1992 and Singh 2002) also led to similar conclusion.

Analysis of heterosis (over better parent) exhibited that significant positive heterosis was observed in the cross Cocorit 71 x A-9-30-1 in both the normal and late planting dates and in the cross HI 8062 x JNK-4W-128 in late planting (Table 3). The present results were in conformity with the findings of many wheat workers (Randhawa and Minhas 1977; Gautam and Jain 1985; Prasad et al. 1998). Significant positive heterosis is not desirable in durum wheat because F_1 hybrid showed longer grain filling period than its parents. Component analysis of heterosis revealed that in the cross Cocorit 71 x A-9-30-1, dominance x dominance x dominance (z) followed by additive x additive x dominance (x), dominance x dominance (l), additive x additive (i) and additive x dominance x dominance (y) in normal sowing, whereas, in late sowing, additive x dominance x dominance (y) followed by additive x additive x additive (w), additive x dominance (j) and additive (d) contributed towards significant positive heterosis. In the cross HI 8062 x JNK-4W-128 most of the gene effects contributed towards significant positive heterosis, however, maximum contribution was made by dominance x dominance x dominance (z) followed by additive x additive x dominance (x) in the late sown environment. Absence of significant heterosis in remaining cases could be explained due to the internal cancellation of heterosis components. Significant negative inbreeding depression was recorded in the cross Cocorit 71 x A-9-30-1 in normal sowing, which is not desirable because F_2 had longer grain filling period than the F_1. Significant positive inbreeding depression was observed in the cross HI 8062 x JNK-4W-128 in normal sowing because better homeostatic power, due to segregational variation in F_2 or favourable gene dispersion in this generation can make the F_2 cross superior to F_1 for grain filling period (Table 3).

Thus, the results of the present investigation exhibited that epistasis was an integral component of the genetic architecture of grain filling period in

Table 3. Percent heterosis (over better parent) and inbreeding depression and components of heterosis for grain filling period in the three crosses of durum wheat in normal and late sown conditions

Components	Cocorit 71 x A-9-30-1		HI 8062 x JNK-4W-128		Raj 911 x DWL 5002	
	Normal	Late	Normal	Late	Normal	Late
(h)	–0.26	1.45	–4.12	–4.43	–5.01	3.20
–(i)	16.59	–4.12	–10.75	5.34	6.11	1.29
1/2(x)	–34.38	4.01	22.69	–8.775	-	2.44
1/4 (z)	19.86	1.25	–5.78	8.72		4.67
–(d)	–0.50	1.34	–4.59	–2.17	–2.17	–2.12
1/2(j)	–1.75	3.43	–4.22	0.19	18.44	–1.09
–(w)	0.58	–10.76	–15.39	3.53	-	0.40
–1/4 (y)	3.05	5.05	–9.31	–2.55	-	1.76
F_1-BP	3.19	3.54	2.49	1.82	0.94	0.62
S.E.	±0.63	±0.22	±1.34	±0.89	±0.58	±0.91
Heterosis(%)	9.02**	10.50**	6.74	4.92*	2.69	1.61
F_1-F_2	–2.28	0.57	5.23	–0.76	–0.92	–0.38
S.E.	±0.48	±0.58	±0.79	±0.71	±1.02	±1.07
Inbreeding depression (%)	–5.91**	1.53	13.28**	–1.95	–2.56	–0.97

*, ** Significant at 0.05 and 0.01 levels, respectively.

durum wheat and hence detection, estimation and consideration of this component are important for the formulation of breeding program. As a consequence of higher magnitude of interactions particularly of trigenic type, the non-fixable gene effects were higher than the fixable indicated the major role of non-additive gene effects. Naturally, the successful breeding methods will be the ones, which can mop-up the genes to form superior gene constellations interacting in a favorable manner. Some forms of recurrent selection namely, diallel selective mating (Jensen 1970) or biparental mating in early segregating generations (Joshi and Dhawan 1966) might prove to be effective alternative approach. The restricted recurrent selection by the way of intermating the most desirable segregants followed by selection (Joshi 1979) might also be a useful breeding strategy for the exploitation of both additive as well as non-additive type of gene actions. These breeding approaches could be helpful in developing durum wheat populations, which upon selection will result in shorter grain filling period as well as higher economic yield in durum wheat varieties under different sowing environments through development of new plant type. Furthermore, as the duplicate type of epistasis was observed in the crosses HI 8062 x JNK-4W-128 under both the normal and late sown conditions and Raj 911 x DWL 5002 under normal sown environments, so the selection intensity should be mild in the earlier and intense in the later generations to achieve the desirable improvement in this trait in durum wheat. The study also showed that inheritance is highly affected by environment, hence an appropriate choice of the environment should be made in such a way that grain filling period show relatively simple inheritance for further tangible advancement of this trait in durum wheat.

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Quality and agronomic comparisons between nearly isogenic wheat lines differing for spike density

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Summary

Quality and agronomic comparisons were made between soft white winter wheat selections of Early Blackhull/7*Paha and Suweon 185/7*Paha that were nearly isogenic for club (*CC*) and lax (*cc*) spike density. The objective was to determine whether differences between the two sets of nearly isogenic lines (NILs) were directly associated with the *C* or *c* allele. Differences were detected for 7 of 13 quality traits measured between the club and lax NILs during one or both seasons and for one or both populations. Flour yield, break flour yield, and milling score differed between the NILs of both populations when averaged across seasons. Values of these three traits were lower for club NILs versus their lax sibs. Less consistent differences occurred between the club and lax NILs for wheat protein, mixing type, cake volume, and cake score for one but not both populations. When quality trait differences occurred between club and lax NILs, the lax sibs consistently had more optimal values than their club sibs.No differences occurred between the club and lax NILs for grain yield, test weight, and spike number in either population. Club NILs of both populations had 19% more kernels per spike than lax sibs, but their kernels weighed 7 to 14% less. Club NILs sustained less lodging than lax NILs. In this study the club (*CC*) genotype did not enhance soft wheat quality and actually adversely affected some quality traits.

Key words: *Triticum aestivum* L., market class, soft wheat

Introduction

Major market classes of soft wheat grown in the Northwestern USA are soft white club and soft white common. Club cultivars have the *C* allele for dense spike while common cultivars have the *c* allele for lax spike. As a group club cultivars generally have superior milling and baking quality than common cultivars. In contrast common cultivars usually have higher test weight and grain yield potentials than club cultivars. The objective of this study was to determine if quality and agronomic trait differences between club and lax spike nearly isogenic lines (NILs) are directly associated with the *C* locus.

Material and methods

Four pairs each of NILs having either the *C* allele for dense spike or the *c* allele for lax spike were developed in two populations. The populations were Early Blackhull/7*Paha and Suweon 185/7*Paha. Paha is a soft white winter club cultivar and was the source of the *C* allele in both populations. Sources of the *c* allele were Early Blackhull, a hard red winter cultivar and Suweon 185 a soft red winter breeding line from South Korea. The NILs with either the *C* or *c* allele were selected in the F_4 generation after six backcrosses to Paha.

Agronomic data was obtained on eight traits in 6 to 7 trials for the Early Blackhull/7*Paha NILs during

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1995 and 1996 and from 6 to 12 trials for the Suweon 185/7*Paha NILs during 1994 to 1996. Trials comprised three eastern Washington environments differing in growing degree days, cropping systems and moisture availability. Each trial consisted of a single replication of the NILs and Paha. Analyses of variance were performed across trials for each trait. The four NILs of each spike density type did not differ significantly among each other for any of the eight traits. Hence data was pooled across the club NILs and the lax NILs.

Quality data was obtained from three trials each in 1995 and 1996. A single 250 g sample of the NILs and Paha from each trial was analyzed for 13 quality traits. They were flour yield, break flour yield, kernel hardness, wheat protein, flour protein, flour ash, milling score, mixograph absorption, mixing type, cookie diameter, cookie top grain score, sponge cake volume, and score. Methods for evaluating these traits are described by the American Association of Cereal Chemists (1995). The data was analyzed for each year and across both years to determine whether differences occurred between the club and lax NILs for each trait.

Results and discussion

Among the 13 quality parameters measured, no differences (P>0.05) occurred between club and lax NILs for kernel hardness, ash content, flour protein, water absorption, cookie diameter or cookie score.

Table 1. Means of club and lax near-isolines that differed for seven quality traits each year or averaged across years

Trait	Population†	1995		1996		Avg.	
		Club	Lax	Club	Lax	Club	Lax
Flour yield (%)	SU/Paha	74.9	75.3	71.8	73.8*	73.4	74.6*
	EB/Paha	75.0	75.5	71.0	72.3*	73.0	73.9*
Break flour yield (%)	SU/Paha	50.7	51.6*	47.2	48.4*	49.0	50.0*
	EB/Paha	51.4	52.6	46.5	48.3*	49.0	50.1*
Milling score	SU/Paha	92.9	93.8	88.3	90.5*	90.6	92.2*
	EB/Paha	92.9	93.5	87.0	89.5*	90.0	91.5*
Wheat protein (%)	EB/Paha	11.0*	10.5	10.3	10.0	10.6	10.3
Mixing type	EB/Paha	1.17	1.40	1.67*	1.00	1.42*	1.20
Cake volume (cc)	EB/Paha	1288	1283	1263	1290*	1274	1287
Cake score	SU/Paha	70	74*	70	71	70	73*

*Means of club and lax NILs differ at P<0.05.
†SU/Paha and EB/Paha designate Suweon 185/7*Paha and Early Blackhull/7*Paha populations, respectively.

Table 2. Means of eight agronomic traits for club and lax near-isogenic lines of two winter wheat populations

Trait	Tests	Early Blackhull/7*Paha		Tests	Suweon 185/7*Paha	
		Club	Lax		Club	Lax
Plant height (cm)	7	100	101	12	94	101*
Lodging (%)	7	31	41*	12	29	38*
Days to heading (d)	7	158*	157	9	155	154
Grain yield (g/m^2)	6	442	428	12	465	474
Test weight (g/l)	7	801	793	12	804	808
Kernel weight. (mg)	6	30	35*	6	27	29*
Spikes ($0.3m^2$)	6	177	195	6	209	222
Kernels per spike	6	80*	67	6	70*	59

*Club and lax near-isoline means differ at P<0.05.

There were significant (P<0.05) differences between the club and lax NILs for flour yield, break flour yield, milling score, wheat protein, mixing type, cake volume and cake score for one or both years or for one or both populations (Table 1). In 1995 the club and lax NILs differed for break flour yield, wheat protein and cake score but only for one of the two populations (Table 1). In 1996 the club and lax NILs differed for mixing type and cake volume for the Early Blackhull/7*Paha population; they differed for flour yield, break flour yield and milling score for both populations (Table 1). When averaged across both years, the club and lax NILs differed for mixing type in Early Blackhull/7*Paha and for cake score in Suweon 185/7*Paha. Club and lax NILs differed only for three traits of both populations when averaged across years. These were flour yield, break flour yield and milling score (Table 1). In all instances where differences occurred between quality traits of club and lax NILs, the lax sibs consistently had a more optimal value than did the club sibs. Generally the means of the four club and four lax NILs of both populations had similar quality trait values to Paha. The only exception was the mean of common NILs of Suweon 185/7*Paha exceeded Paha for milling score.

These results were somewhat unexpected. In the U.S. Pacific Northwest soft white winter club cultivars generally have better soft wheat quality than common soft white winter cultivars. This is especially true for milling quality. In this study the *C* allele for dense spike of Paha failed to enhance flour yield, break flour yield and milling score. Conversely the lax spike *c* allele may enhance these three quality traits. Differences also occurred between club and lax sibs for mixing type and cake score but not in both populations. Hence it is unlikely that there is a direct effect between the *C* locus and these two traits.

Among the eight agronomic traits measured the club and lax NILs differed (P<0.05) for kernel weight, kernels per spike, and percent lodging in both populations (Table 2). Club NILs averaged 19% more kernels per spike, with kernels that were 7 to 14% lighter than their lax sibs. They also sustained about 25% less lodging than their lax sibs. Lax NILs of the Suweon 185/7*Paha population were significantly taller (P<0.05) than their club sibs. Club NILs of Early Blackhull/7*Paha headed 1d later (P<0.05) than their lax types. They also headed 1d later in the Suweon 185/7*Paha population but this difference was not significant (P>0.05).

These results agreed in part with those of Gul and Allan (1972a, b). They found that club NILs had increased kernels per spike, decreased kernel weight and reduced plant height compared to their lax sibs in a Suweon 92/8*Omar population. They reported that club NILs had increased grain yields and test weights compared to their lax sibs whereas no differences occurred for these two traits in this study.

The *C* allele in the earlier and in this study was derived from Omar. Paha is a backcross-derived club cultivar, selected from a Suweon 92/4*Omar population. Paha and Omar differ mainly in plant height. Paha is a semidwarf cultivar while Omar is a tall, non-semidwarf cultivar. In this study the club and lax NILs were semidwarfs while in the Gul and Allan (1972a) study they were tall, non-semidwarfs. Perhaps semidwarf versus non-semidwarf plant height determines whether the *C* allele affects certain traits such as grain yield and test weight.

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Research information

Raj Molya Rodhak 1, a first commercial unique wheat variety resistant to cereal cyst nematode in India

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Of the several constrains towards realizing potential yield in wheat, losses incurred due to molya disease is enormous. This disease is used to cause about 40-50 percent yield loses, which may attain up to 60-65 percent (Mathur 1969; Mathur et al. 1980). Looking towards the state wheat production (5.99 m tones), the cereal cyst nematode (CCN) infested area (0.15 m ha) instead of producing 0.43 m tones, only yielding 0.22 m tones (2001-02) and grain loss in terms of money amounts about Rupees 126 million. It is observed that high yielding and well adopted modern varieties when grown extensively under CCN infested areas, the high incidence of this disease results in epidemic proportions and causes grain as well as straw yield losses. Earlier certain efforts using cultural practices and chemicals were also exercised to overcome the threat of CCN in naturally infested soils of Rajasthan, as the basis of genetically defined CCN resistance sources during this period was not known. It was felt that deployment of CCN resistance resources for this disease resistance could assist in achieving yield stability without resorting potentially harmful chemicals, at the same time preventing environmental degradation and benefiting the resource-poor farmers who can ill afford the use of costly chemicals to sustain the yield in infested soils (Mathur et al. 1998). Hence, breeding for CCN resistance based on genetic principles was initiated in 1991, soon after the identification of AUS 15854, a Turkish wheat line received from Australia, demonstrated for the first time that resistance to CCN in wheat was controlled by a single dominant gene under warmer condition of Rajasthan, India. The progress achieved to check the severe losses of yield due to the cereal cyst nematode (*Heterodera avenae*) by developing a unique CCN resistant wheat variety CCNRV 1 (Raj Molya Rodhak 1), dealt with in this paper.

The breeding program was initiated in 1991 for development of CCN resistant wheat variety for sustained and maximization of wheat production in molya infested areas of the country. Seven popular high yielding bread wheat (*Triticum aestivum* L.) varieties namely J-24, Raj 2184, Raj 3077, HD 2329, Raj 2535, HD 2009 and Kalyansona were selected for hybridization program. The AUS 15854 genotype was used as a male parent. Pedigree method was followed in handling the segregating generations under artificially CCN infested field in each generation. In the first instance, selection was based on visual assessment of single plant in the sick field, backed up by rapid CCN resistant and grain quality tests. Yield traits and CCN resistance were fixed in the fifth generation and a new variety is typically multiplied up from a single plant in sixth generation. From each cross combination one of the best plant progeny was selected and bulked on the basis of CCN resistance and other desirable traits for further evaluation in yield trials at various locations. The selected seven plant progenies were designated as CCNRV 1 (J-24 x AUS 15854), CCNRV 2 (Raj 2184 x AUS 15854), CCNRV 3 (Raj 3077 x AUS 15854), CCNRV 4 (HD 2329 x AUS 15854), CCNRV 5 (Raj 2535 x AUS 15854), CCNRV 6 (HD 2009 x AUS 15854) and CCNRV 7 (Kalyansona x AUS 15854) for convenience in testing in the trials.

Initially all the selected seven CCN resistant varieties were tested along with check variety Raj 3077 in randomized block design in three replications at twelve locations during 15th–25th November, 1996-

97. Three varieties CCNRV 1, CCNRV 3 and CCNRV 7 were observed promising for yield attributes along with CCN resistance in trials conducted during 1996-97. To raise the crop, 100 N : 40 P_2O_5 : 60 K_2O kg/ha fertilizers were used. Other recommended agronomic practices were also used to raise the crop under naturally infested as well as normal (nematode free) soils. Grain and straw yields were recorded per plot in kilogram, however, this unit was converted in to q/ha for interpretation of the results obtained in the present study. Standard scale as suggested by Swaroop (1986) was used to record the nematode reaction in infested soils viz., resistant (0.0-4.0 cysts/plant), moderately resistant (4.1-9.0 cysts/plant), susceptible (9.1-20.0 cysts/plant) and highly susceptible (20.1 and above cysts/plant). The data of trials were analyzed as per standard statistical methods suggested by Panse and Sukhatme (1967).

The pedigree of wheat variety CCNRV 1 (Raj Molya Rodhak 1) is J 24/AUS 15854. This variety is the first cereal cyst nematode resistant wheat variety in the country developed by transferring resistant dominant gene from exotic germplasm- AUS 15854 (Sharma and Sharma 2000). The main characteristics of CCNRV 1 are intermediate growth habit, light green foliage color at boot stage, flag leaf waxy (dorsal side), spike shape tapering, intermediate to compact, turn dusty white at maturity, awns length medium and awns shed before maturity. It matures in 115-120 days and possesses medium bold, amber and lustrous grains. The results obtained in different experiments are explained below:

The results of twelve locations of CCNRV 1 (1996-97) revealed that all the seven CCN resistant varieties significantly out yielded over the Raj 3077 check variety under infested soils (Table 1). Similarly these varieties also gave significantly higher straw yield over the check. The maximum grain yield was recorded for CCNRV 1 followed by CCNRV 3 and CCNRV 7 and almost similar trend was observed for straw yield in this experiment. All the seven varieties showed resistance against nematode and number of cysts per plant ranged 1.7-3.5. All the CCN resistant varieties showed superiority for plant height and tillers per plant as compared the Raj 3077, however, only three varieties viz., CCNRV 1, CCNRV 3 and CCNRV 7 were found significantly superior for spike length. These results indicated that among the seven newly developed CCN resistant wheat varieties only CCNRV 1 had excellent potential for grain and straw yield along with other desirable agronomical traits. The results of overall mean performance of CCNRV 1 variety under multi-location trials under infested soils (1997-99) revealed that this variety gave 78.7 percent higher grain yield over Raj 3077 (Table 2). Similarly it also gave 60.1 percent higher straw yield over the widely cultivated popular wheat variety Raj 3077 in CCN infested soils at 23 locations. Results further exhibited that CCNRV 1 has excellent resistance against nematode and average number of cysts per plant over the three years was recorded 1.5 cysts per plant whereas Raj 3077 had 14.0 cysts per plant. Overall results of three years experiments at 23 locations showed that newly developed wheat variety has good yield potential and superiority of resistance against molya disease in sandy soils of Rajasthan. Thereby, it is envisaged that this variety will open up new vistas of boosting wheat production and alleviate the socio-economic status of the subsistent Indian farmers of the CCN infested areas. Thereby, State

Table 1. Performance of CCN resistant wheat varieties in multi-location trials under infested soils during 1996-97.

Variety	Grain yield (q/ha)	Straw yield (q/ha)	No. of cysts /plant	Nematode reaction	Plant height	Spike length	Tillers /plant
CCNRV 1 (J-24 x AUS 15854)	39.2*	70.2*	1.7*	R†	82.3*	12.0*	5.6*
CCNRV 2 (Raj 2184 x AUS 15854)	32.0*	61.2*	3.5*	R	106.1*	8.1	5.1*
CCNRV 3 (Raj 3077 x AUS 15854)	38.6*	70.7*	2.5*	R	78.2*	13.0*	7.8*
CCNRV 4 (HD 2329 x AUS 15854)	32.0*	61.4*	3.1*	R	91.3*	8.1	5.6*
CCNRV 5 (Raj 2535 x AUS 15854)	30.3*	58.7*	3.0*	R	87.2*	8.3	5.0*
CCNRV 6 (HD 2009 x AUS 15854)	29.7*	58.9*	2.2*	R	92.8*	8.5	5.5*
CCNRV 7 (Kalyansona x AUS 15854)	38.5*	66.1*	3.4*	R	82.2*	11.5*	6.0*
Raj 3077 (C)	21.6	44.5	13.3	S‡	54.0	7.6	2.8
C.D. at 5%	1.8	2.6	0.7		2.4	1.4	1.5

*Significant at 5% level, †Resistant (0.0-4.0 cysts/plant), ‡ Susceptible (9.1-20.0 cysts/plant)

Table 2. Mean performance of CCNRV 1 and Raj 3077 (C) varieties in multi-location trials under infested soils (1997-1999)

Year	No. of location	Grain yield (q/ha)		C.D.	Increase over check(%)	Straw yield (q/ha)		C.D.	Increase over check(%)	No. of cysts/plant		C.D.	Nematode reaction	
		CCNRV1	Raj 3077			CCNRV1	Raj 3077			CCNRV1	Raj 3077		CCNRV1	Raj 3077
1996-97	12	39.2*	21.6	1.8	80.8	70.2*	44.5	2.6	57.8	1.7*	13.3	0.7	R	S
1997-98	6	40.7*	24.1	3.0	68.3	72.5*	44.0	1.2	64.7	1.7*	12.8	1.9	R	S
1998-99	5	43.9*	23.4	0.9	87.6	71.3*	45.2	1.4	57.7	1.2*	15.9	2.3	R	S
Mean		41.2	23.0		78.7	71.3	44.5		60.1	1.5	14.0		R	S

Table 3. Mean performance of agronomic and grain quality parameters of CCNRV 1 and Raj 3077 (C) varieties under infested soils

Variety	Agronomic traits					Grain quality parameters				
	Days to maturity	Plant height	Spike length	Tillers /plant	1000-grain weight	Hectoliter weight	Grain appearance	Protein (%)	Sedimentation value
CCNRV 1	117.5	83.4	12.0	5.8	41.2	76.0	7.0	12.7	55.0
Raj 3077(C)	121.0	54.8	7.5	2.6	29.0	54.5	5.5	11.8	58.0

Seed Sub-Committee on Crop Standards (Notification and Release of Varieties meeting held on 28th September, 2002) released CCNRV 1 for cultivation in Rajasthan state, which has resistance genes against nematode and good yield potential along with various other desirable parameters (Table 3). It is hoped that this wonderful wheat variety will help in achieving higher yield levels throughout the country, where molya disease is a threat for wheat cultivation.

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Editorial remarks

Due to managing trouble, we regret not to publish WIS No. 98 on time.

The catalogue supplement of gene symbols for wheat has been printed in WIS for last two decades. However, since the modern system through internet is more convenient and efficient than hard-copy, WIS decided to notify only the announcement of each supplement. Supplement for 2004 appeared on following web site: http://shigen.lab.nig.ac.jp/wheat/komugi/genes/macgene/supplement2004.html

The most recent edition of the catalogue, produced and presented at the 10th International Wheat Genetics Symposium is available on CD. 'MacGene' was produced by Dr. Y. Yamazaki in collaboration with Dr. R.A. McIntosh. The catalogue is also displayed on the GrainGenes Website (http://wheat.pw.usda.gov) and KOMUGI database (http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp).

WIS No. 98 contains six qualified research articles and other information. Since we had announced in the last issue or at 10th International Wheat Genetics Symposium (Paestum, Italy), WIS is going to be closed in 2005. We have a plan to publish the special issue for the centennial and final number in the end of 2005. The editorial office has already enough manuscripts for publication of No. 99, so that please stop sending manuscript anymore. We appreciate your kind understandings, and be proud of comments which have sent to the office from many subscribers and wheat researchers.

If you want to keep back-numbers of WIS, these are available on request (early one should be copied ones).

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