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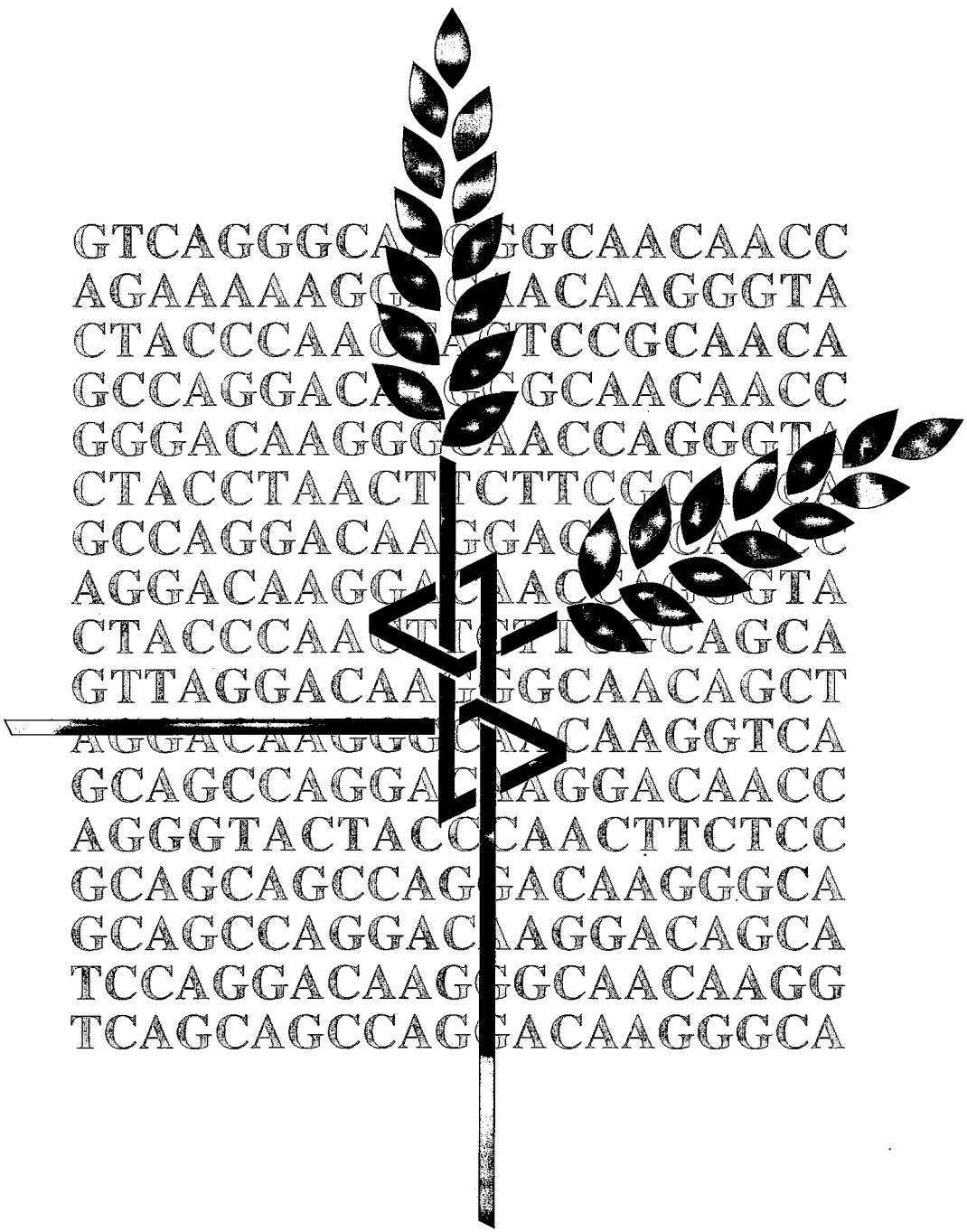

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Research article

Studies on the embryogenic processes in the *in vitro* culture of wheat somatic tissues by using a proliferative antigen of initial cells

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Summary

Embryogenic processes occurring in an *in vitro* culture of immature wheat (*Triticum aestivum* L.) embryos were studied using the proliferative antigen of initial cells, PAI – a cell-division-associated molecular marker for apical meristematic cells of cereal crops. With an immunochemical test-system based on polyclonal, monospecific anti-PAI antibodies, we found a correlation between PAI content and the embryogenic potential of calli of the wheat cultivar Saratovskaya 29 and its near-isogenic lines differing in the *Rht-B1c* alleles. We discuss the possible association of PAI with the *Rht* genetic system and the prospects for using this marker in breeding and bioengineering programs to screen newly raised wheat lines for their embryogenic capacity.

Key words: *Triticum aestivum* L., embryogenesis, proliferative antigen of initials, *Rht* gene, immunochemical analysis

Introduction

The genetic determination of morphogenetic processes taking place in an *in vitro* culture of wheat somatic tissues is still an open question. For a long time, the important role of genotype has only been noted on the basis of cultivar and species differences in the frequency of embryogenic-callus formation (Maddock et al. 1983; Mathias 1990). Carman and Campbell (1990), analyzing monosomic and chromosome-substituted lines, revealed individual chromosomes and chromosomal regions involved in the genetic control of embryogenesis *in vitro*. Lange et al. (1995) found that the morphogenetic potential of callus tissues depends on the interaction among a large number of genes with major and minor effects. However, there are very few data on the influence exerted by individual genes on wheat somatic embryogenesis (Omelianchuk 1992).

These problems are conveniently handled by the use of genetic systems with a known functional linkage between gene expression and gene phenotypic manifestation. One of the best candidate systems is that of reduced-height (*Rht*) genes, particularly because the association of *Rht* genes with embryogenesis was noted earlier for a culture of immature wheat embryos (Mathias and Atkinson 1988; Djachouk et al. 2000; Lobachev 2000).

The embryogenic potential of callus tissues is dependent on many factors, including the functioning of "embryonal antigens" or "morphogenetic markers"

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– proteins associated with the embryogenic processes in an *in vitro* culture (Moiseeva 1991; Karabaev and Dzhardemaliyev 1994; Nato et al. 1997; Bai et al. 2000). In particular, the proliferative antigen of initial cells (PAI), characteristic of actively proliferating cereal-crop cells (Volodarsky 1985; Sumaroka et al. 2000) may be used as such a marker. The cellular PAI content in the rye-stem apex is correlated with growth processes in the whole plant (Volodarsky et al. 1986) and reflects the functional activity of wheat-apex meristematic cells during morphogenesis *in vitro* (Feshchenko et al. 1992).

In this work we investigate the hypothetical association of PAI with the embryogenic capacity of the wheat-callus tissue in an *in vitro* culture, with the example cultivar Saratovskaya 29 (S29) and its near-isogenic lines differing in the *Rht-B1c* alleles.

Materials and methods

The tall common-wheat (*Triticum aestivum* L.) cultivar S29 (tall wheat cultivar Saratovskaya 29) and its near-isogenic lines LA (dwarf near-isogenic Saratovskaya 29 lines carrying *Rht-B1c* alleles) and LB (tall sister near-isogenic Saratovskaya 29 lines carrying *Rht-B1a* alleles) were used. LA and LB were raised in a backcross by Lobachev (2000). The dwarf common-wheat line ANK11 (All-Russia Institute of Plant Industry's collection, St. Petersburg, Russia) was a donor and S29 a recipient of the *Rht-B1c* allele. The allele originates in the Tom Thumb form (McIntosh et al. 1998). The sister near-isogenic lines LA and LB are theoretically 99.2% identical in genotype to the recipient cultivar, S29. Phenotypically, the lines are identical except for the plant-height trait: LA is a dwarf line carrying the *Rht-B1c* allele, and LB is a tall line carrying the *Rht-B1a* allele.

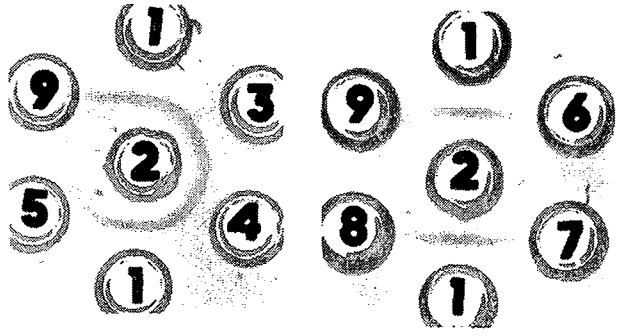

Fig. 1. Determination by agarose-gel double immunodiffusion of the PAI-concentration titer in S29 morphogenic callus on day 30 of culture. 1: the initial test-system with antibodies raised toward PAI (2), 3, 4, 5, 6, 7, 8 : successive twofold dilutions of PAI, 9: control (phosphate buffer).

For callus initiation, immature (14-day-old) wheat embryos were cultured on Linsmaier-Skoog medium (2,4 D: 2 mg ml^{-1}; kinetin: 0.5 mg l^{-1}). The embryogenic calli were regenerated on Blaydes medium (IAA: 0.5 mg ml^{-1}; kinetin: 0.5 mg l^{-1}).

Experiments to determine PAI (proliferative antigen of initials) content and to assess its time-course changes had six replicated samples. Each sample consisted of three calli chosen from each genotype at the start of the experiment (immature embryo); on days 8, 15, 22 and 30 (sampling from callus-initiation medium); and on days 32, 35, and 39 (sampling from regeneration medium). 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 (Volodarsky et al. 1979; Sumaroka et al. 2000).

The antigen concentration was estimated semiquantitatively as described in Clausen (1988). The concentration titer of PAI (maximum dilution that makes precipitation with antibodies visible to the unaided eye) was used as a measure of the antigen concentration. The concentration titer of 1:2 was taken as 100%. The total water-soluble proteins obtained from the stem apices of four-day-old wheat seedlings were used as the initial test-system. This was because PAI is localized not only in callus but also (and primarily) in wheat-stem apex (Sumaroka et al. 2000). By way of example, Fig.1 shows the concentration titer of PAI present in the embryogenic callus of LA on callus-initiation medium (day 30 of culture). Here the concentration titer was 1:4 (Fig. 1, well no. 5), because at a 1:8 dilution no precipitation band was observed (Fig. 1, well no. 6). To this titer corresponds the relative PAI-concentration $C = 200\%$. C was ultimately estimated by averaging over the data gathered from six replicated samples, which sometimes yielded C values non-divisible by 2.

The number of embryogenic calli of the genotypes under study was determined visually (Nabors 1982). Each experiment had three replicated samples, each sample consisting of 40 calli. The results were processed statistically by a one-factorial analysis of variance with determination of the mean square deviation (Maksimov 1984). The gene effect (%) was determined by comparing the mean values of the trait under study among LA, LB, and S29 only when there was a significant difference. In assessing the gene effect, we took as 100% the magnitude of yield of the LB embryogenic-callus on day 30 of culture (Lobachev

2000). In addition, the mean values of PAI content were compared among the calli of LA, LB, and S29 by the classification trees method described in Breiman et al. (1984).

Results and discussion

Immunochemical analysis of PAI content in embryogenic and non-embryogenic calli of cultivar S29, grown on callus-initiation and regeneration media, showed the absence of PAI in the non-embryogenic callus for both types of nutrient medium used (Fig. 2 a, b). However, the embryogenic callus contained PAI in either case. PAI content on regeneration medium was twofold greater than that on callus-initiation medium (Fig. 2 c, d).

Thus, PAI content possibly reflects the embryogenic capacity of the wheat-callus tissue, which depends on culture-medium composition as well as on genotype (Karabaev and Dzhardemaliyev 1994). We assume that the PAI-expressing cells of the embryogenic sites of callus-tissue origin behave similarly to the apical meristematic cells of an adult wheat plant.

Analysis of the time-course of PAI content in wheat embryos and callus tissue during callus formation and the subsequent regeneration is described in Fig. 3. PAI content was the same in the embryos of all three genotypes studied, possibly due to the presence of initial cells in developing embryonal

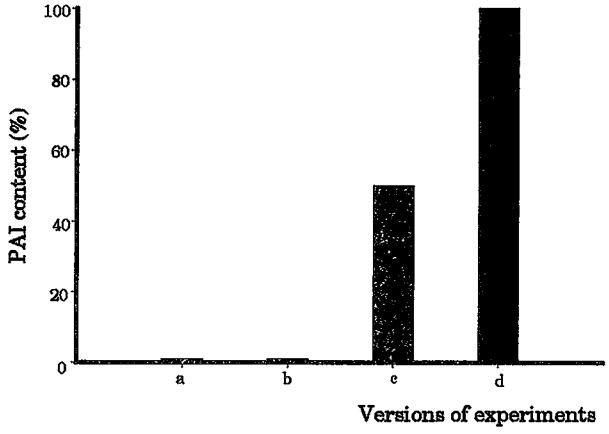

Fig. 2. PAI content in S29 calli with different embryogenic capacities on day 30 of culture (%). a: non-embryogenic callus on callus-initiation medium, b: non-embryogenic callus on regeneration medium, c: embryogenic callus on callus-initiation medium, d: embryogenic callus on regeneration medium.

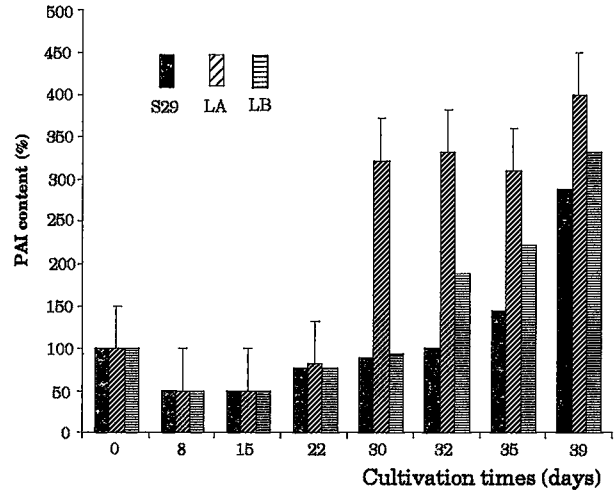

Fig. 3. PAI content of S29 (black), LA (inclined strokes) and LB (horizontal strokes) calli versus time of culture.

apices. There was a synchronous decrease in PAI content (to as low as 50%) on days 8 and 15 of callus formation. This decrease reflects de-differentiation and callus-tissue formation. From day 22 on, the callus PAI content was increasing, with significant PAI-content differences observed among the genotypes under study on day 30 of callus formation. The callus PAI content of LA was significantly greater than those of its sister line LB and cultivar S29 (Fig. 3). The number of embryogenic calli in LA was 51% greater than that in LB over the same period of culture (Fig.4).

The significant PAI-content differences among the genotypes under study were confirmed by additional statistical processing of the results by the classification trees method. Comparing the mean values of callus PAI content on day 30 of culture led us to group the genotypes as follows; (i) cultivar S29

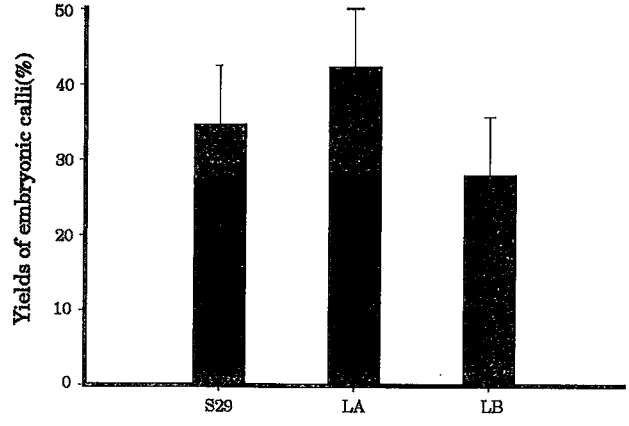

Fig. 4. Yields of embryogenic calli in S29, LA and LB on day 30 of culture.

and the tall line LB (mean value: 91.67) and (ii) the dwarf line LA (mean value: 283.3).

After the embryogenic calli had been transferred to regeneration medium, PAI content continued increasing in all the genotypes. This increase, however, was much more rapid and reached higher values in LA than in the other genotypes. On day 39 of regeneration (day 39 of culture), the genotypic differences smoothed out a little, possibly corresponding to a shift to whole-plant regeneration (Fig. 3).

On the basis of our estimates of the time-course of PAI content in wheat embryos during callus formation and the subsequent regeneration, we assume that this marker is an "embryonal antigen" (Moiseeva 1991), and its content is associated with the intensity of embryogenic processes occurring in wheat embryos. Because callus initiation and plant regeneration are different processes and are likely to be regulated by different genetic mechanisms (Karabaev and Dzhardemaliyev 1994), one can assume that the embryonal antigen used by us is associated, to a degree, with the *Rht* genetic system. This follows from the fact that the dwarf line, which has a greater embryogenic potential, had much more PAI than did the tall sister line and the parent cultivar.

Thus, the immunochemical determination of PAI is fully suited for breeding and bioengineering programs to estimate the embryogenic capacity of the newly raised wheat lines in the *in vitro* culture.

Acknowledgments

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Research article

In vitro screening and production of karnal bunt resistant doubled haploids in wheat

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Summary

Two Karnal bunt susceptible wheat varieties viz. UP2338 and WH533 were crossed with two Karnal bunt tolerant varieties viz. HD2285 and PBW343. Resultant F_1's were crossed with different maize lines (pollinator) and embryos were rescued between 13 to 16 days post pollination. To enhance embryo survival, 2,4-D + GA_3 solution was injected in the upper node and also applied in the florets. Rescued embryos were cultured on MS medium containing 5mg/l 2,4-D. The callus as well as the callus regenerated haploid plantlets were screened *in vitro* at threshold concentration of culture filtrate. The resistant haploid calli and plantlets were selected and plated on colchicine mediated MS medium for doubling their chromosomes and production of Karnal bunt resistant doubled haploids.

Key words: wheat, karnal bunt, wide hybridization, wheat x maize cross, doubled haploid

Introduction

The Karnal bunt (KB) of wheat caused by *Neovossia indica* (Mitra) Mundkur has been of great concern worldwide. Beside India and Pakistan, KB is reported from Syria (Williams 1983), Afganistan, Mexico (Joshi et al. 1983), Nepal (Singh et al. 1989), USA (Yakema et al. 1996) and Iran (Torarbi et al. 1996) etc.

Karnal bunt causes heavy losses during epidemic years and adversely influence wheat grain end use quality and thus consumer acceptability besides quarantine related procedural wrangles create trade barriers. KB is difficult to control by seed treatment by fungicides. Moreover, genetic variability for KB resistance is limited, therefore, wheat improvement program for KB resistance must proceed with creation of novel genetic variability through *in vitro* methods as the conventional breeding has not paid much dividend. Studies have shown that genes for KB resistance are scattered over a number of wheat genotypes. Concentration of favorable alleles governing Karnal bunt resistance and high yield in few genetic backgrounds is one of the most important task, wheat breeders would like to accomplish. Any method, which offers infinite F_2 population in homozygous state, would cut short breeding time and help increase selection efficiency for KB resistance. In this context, doubled haploid (DH) technique would facilitate creation of new genetic variability in the form of multitude of homozygous true breeding lines. Wheat x maize crosses, being free of crossability alleles, have proved efficient mean of haploids and hence doubled haploids production.

Materials and methods

Crossing program and embryo rescue: Karnal bunt susceptible wheat varieties UP 2338 and WH 533 were crossed with Karnal bunt tolerant varieties HD2285

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and PBW343. The resultant four F_1's were crossed with different maize lines (Prabhat, Vijay, Accessions; 803, 1344, 645 and 1040 used as pollinator). As soon as the primary spikes of healthy, vigorous plant emerged from the boot, the outer two florets were emasculated and the inner one was removed. The pollination was done after 3 to 5 days after emasculation. Maize pollens were collected by tapping the tassels into a petriplate. The wheat pistils were pollinated using a fine brush and then bagged.

Immediately after pollination the solution containing 50 mg/l, 2,4-D + 100 mg/l GA_3 was injected about a centimeter above the upper most node through leaf sheath as suggested by Pienaar et al. (1997). Spikelets of these pollinated spikes were again flooded with the same solution after 24 hours using injection syringe and covered with another brown paper bag. The treated spikes were harvested between 13 to 16 days after pollination (Dhaliwal et al. 1995; Riera-Lizarazu and Mujeeb-Kazi 1990; Suenaga et al. 1997) and were taken to the laboratory in the above-mentioned solution. The green parthenocarpic caryopses (GPCs) were removed from florets by bending them backward with a forceps. GPCs were surface sterilized and the haploid embryos from the disinfected GPCs were excised under laminar flow hood with scalpel and plated on callus induction medium i.e. MS_1 (MS medium + 5mg/l 2,4-D).

In vitro screening at callus level: Well developed calli of different genotypes of wheat (HD2285 as tolerant and WH157, PBW373, KH65 and WH147 as susceptible to Karnal bunt) were transferred to fungal filtrate mediated callus induction media (MS_1) with 2 to 50 ml of fungal toxin and without it (control). The callus cultures were incubated at 25±1°C for 20 days. The final weight of the plated calli was recorded on 21st day before their transfer to culture filtrate free medium. On the basis of decrease in callus weight in comparison to control the threshold level (T) was determined.

For *in vitro* screening, haploid calli obtained from embryo rescue, were transferred to MS_1 medium having threshold level of culture filtrate and the callus growth was recorded. The well-developed calli were cultured and subcultured on regeneration media i.e. MS_3 (MS medium supplemented with 0.5 mg/l of IAA and 1.0 mg/l of BAP) to get embryoids that further developed into plantlets.

Converting haploids into doubled haploids: The haploid calli differentiated embryoids were transferred to MS_1 medium having 400 mg/l colchicine for three days and again the embryogenic calli were transferred to regeneration (MS_3) media to get doubled haploid plantlets. As an alternative approach for converting haploid plantlets obtained from haploid calli into doubled haploids the plantlets having two to three sprouts were taken. The roots of these plants were trimmed and washed. Colchicine solution (0.05%) was taken in a tube and a piece of filter paper was floated. This solution was sterilized in autoclave, on which subsequently the plant was placed for 24 hrs at 24°C. The plants were rinsed in tap water for half an hour and then placed in a similar test tube having distilled water and a filter paper floating on it. Then, the plants were placed on filter paper and kept for three days at 4°C. The shoots of the treated plants were cut back to 15 cm before transferring to the pots. Root tip analysis was conducted following acetocarmine stain procedure to determine the ploidy level.

Results and discussion

In vitro screening and organogenesis of haploid calli: For *in vitro* screening of haploid calli, the calli were transferred on threshold level (T medium i.e. MS_1 supplemented with 10 mg/l of culture filtrate) and growth was recorded (Table 1). The haploid calli of the cross (UP2338 x HD2285) F_1 x maize were transferred to MS_1 and T media and it was observed that callus growth was 16.1 per cent in the former and 1.4 per cent in later medium. However, for (WH533 x PBW343) F_1 x maize cross the callus growth was 8.8 per cent in MS_1 and 1.0 per cent in T medium. The per cent callus growth was decreased in both the crosses in culture filtrate treatment. It was observed that there are chances of obtaining resistant DH plants from these screened calli. Whereas, other two crosses namely (UP2338 x PBW343) F_1 x maize and (WH533 x HD2285) F_1 x maize failed to show any callus development and hence plantlet regeneration. The possible reason can be the timing of embryo rescue, for these two crosses may be somewhat different or some modifiers may be affecting embryo recovery.

Haploid callus survival at threshold level was observed 56.5 per cent for (UP2338 x HD2285) F_1 x maize and 57.1 per cent for (WH533 x PBW343) F_1 x maize cross (Table 1). In both the crosses after callus screening the frequency of embryoids per callus was less than one. Whereas, per cent plant obtained after converting to DH using colchicine was 30.8 and 16.7 for the crosses (UP2338 x HD2285) F_1 x maize and (WH533 x PBW343) F_1 x maize, respectively. However, number of regenerated DH shoots from plated calli were 4 and 2 for the respective crosses.

Table 1. Effect of threshold concentration of *Neovossia indica* culture filtrate on callus growth, callus viability and plant organogenesis of wheat F1's x maize derived haploid calli and doubled haploid plantlets

Character	Cross		Medium
	UP2338 x HD2285	WH533 x PBW343	
Callus growth			
Mean callus weight (mg)			
Initial weight	276.7	320.0	MS_1†
Final weight	321.1	348.0	MS_1
Increase in callus wt.(mg)	44.4	28.0	MS_1
Callus growth (%)	16.1	8.8	MS_1
Mean callus weight (mg)			
Initial weight	235.0	224.4	T‡
Final weight	238.3	226.7	T
Increase in callus weight (mg)	3.3	2.2	T
Callus growth (%)	1.4	1.0	T
Callus survival			
No. of calli plated	23	21	T
No. of calli survived	13	12	MS_1
Callus survival (%)	56.5	57.1	MS_1
Plant organogenesis			
No. of embryoids obtained	10	8	MS_3§
No. of embryoids/plated callus	0.8	0.7	MS_3
No. of regenerated shoots/callus	4	2	MS_3
Per cent regeneration(%)	30.8	16.7	MS_3

†MS medium supplemented with 5mg/l of 2,4-D
‡MS_1 medium + 10 mg/l of culture filtrate (Threshold concentration)
§MS_3 medium + 0.5mg/l IAA + 1.0 mg/l BAP

Note: No. of regenerated shoots from plated calli were already treated with colchicine(0.04 %) in induction medium for three days at embryoid stage.

Table 2. Effect of different colchicine treatments on chromosome doubling in haploid embryoids/plants derived from wheat x maize crosses

Wheat F1's x maize cross	Cochicine treatment	No. of embryoids/ plantlets treated	Total no. of plants obtained	No. of DH plants obtained	Per cent DH plants
UP2338 x HD2285	C_1†	10	4	3	75.0
	C_2‡	19	19	11	57.9
WH533 x PBW343	C_1	8	2	2	100.0
	C_2	13	13	07	53.9

†Embryoids treated in induction medium with 0.04% colchicine for 3 days.
‡ Haploid seedlings treated in 0.05% colchicine for 1 day.

This reduction in obtaining regenerants could be due to colchicine treatment given in between. Barnabas et al. (1991) and Mentewab and Sarrafi (1997) reported similar findings. They observed that colchicine treatment applied in induction medium slightly decreased the frequency of embryoids and hence per cent regeneration.

Converting haploid to doubled haploids: The efficiency of colchicine in converting haploids to doubled haploids at embryoid stage and seedling stage is presented in Table 2. The efficiency of converting haploid seedlings was less than the efficiency of converting haploid calli having embryoids into doubled haploids. When embryoids and seedlings were treated with colchicine, in cross (UP2338 x HD2285) F_1 x maize 75.0 and 57.9 per cent DH plants were obtained, respectively. Similarly, in second cross i.e. (WH533 x PBW343) F_1 x maize 100 per cent doubled plants were obtained by treating the embryoids in induction medium, whereas, it was 53.9 per cent when seedlings were treated with colchicine solution. Results showed that for converting haploids into doubled haploids it was observed that embryoids plated on colchicine mediated medium give higher frequency of DH plants as compared to the whole seedlings treated with colchicine. These results are in accordance with that of Mentwab and Sarrafi (1997).

This investigation mainly embarked on validating *in vitro* methodology for development of DHs possibly tolerant to Karnal bunt using Indian wheat x maize crosses. Wheat x maize cross scheme as adopted in present investigation would be quite helpful in reducing the time of breeding programs aimed at developing disease resistant genotypes (DH) combining favorable gene constellations in homozygous state. Our studies indicated that culture filtrate of the fungi *Neovossia indica* can be used successfully under controlled environmental tissue culture conditions to pick up tolerant calli which can be regenerated to whole plant.

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Research article

Grain quality of durum wheat germplasm as affected by heat and drought stress at grain filling period

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Summary

Various physical and chemical screening tests can be used to predict pasta-cooking quality of the experimental lines in a durum wheat breeding program. In the present study, 300 durum wheat genotypes were evaluated for kernel characteristics contributing to end-product quality under irrigated field conditions. This study was conducted during 1998 and 1999 growing seasons at two sites of Abarkoh and Isfahan located in central of Iran. A combination of heat and drought stress occurred at Abarkoh region during grain filling period where air temperature ranged from 34 to 42°C which accompanied with a hot and dry wind. The maximum temperature exceeded 8-10°C from the long-term averages in May and June in this area during 1998 and 1999 growing seasons. The following criteria were used: test weight, grain hardiness, protein content, wet and dry gluten contents and sodium dodecyl sulfate (SDS)-sedimentation volume. Variation among genotypes for grain quality characteristics indicated and genotype responses to the environmental conditions varied. Statistical analysis using a paired t test indicated a significant differences ($P<0.01$) between two regions for all grain quality characteristics and the highest values were recorded where a combination of heat and drought stress occurred at grain filling period in Abarkoh region. The results also revealed that some genotypes have a similar protein content ranking under both heat and dry environment and optimal environment, while others have a substantially different ranking under optimal and stressed environments. Genotypes Osta/Gata, ICDW 7639 and PI40100 provide a good example of the former response, where their protein content ranked similarly in both of stressed and optimal environments.

Key words: durum wheat, *Triticum turgidum*, grain quality, protein content

Introduction

Durum wheat (*Triticum turgidum* L. var. *durum* Desf.) is the preferred class of wheat for the manufacture of pasta products. Assessing the cooking quality of durum wheat pasta is difficult because it is a perceived quality. Factors influencing cooking quality of spaghetti are complex and poorly understood, consequently various tests are performed to obtain reliable quality assessments (Kovacs 1985). The cooking test, however, is time consuming and requires a large sample size. Hence, developing acceptable quality cultivars at a successful breeding program in durum wheat depends on a number of physical and chemical tests used on experimental lines to predict the preferred cooking quality of durum wheat pasta (Dexter and Matsuo 1977; Matsuo and Dexter 1980; Kovacs 1985; D'Egidio et al. 1990).

Weight per unit volume, or test weight is used

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widely as a primary specification in wheat grading. Dexter et al. (1987) reported highly significant relationships between test weight and grain characteristics, milling performance and spaghetti quality in durum wheat. Differences in pasta cooking quality are believed to depend on both protein quantity and protein quality (D'Egidio et al. 1990). Durum wheat has greater grain hardiness than any other wheat classes. This is an advantage in the production of semolina, because the endosperm is less friable than that of softer wheats, allowing a higher yield of coarse-ground stock (semolina) and less durum flour (Finney et al. 1987). Hence, grain hardiness can be used to assess the semolina yield of experimental lines.

Protein content is the most important quality characteristic influencing cooking quality. Generally, as protein content increases, pasta becomes firmer, less sticky and its cooking score increases (Marchylo and Dexter 1997). Gluten content and gluten strength, which are related to protein quality, are universally acknowledged as the important prerequisites for production of quality pasta (Marchylo and Dexter 1997). The sodium dodecyl sulphate (SDS)-sedimentation volume (the Zeleny sedimentation test modified by addition of SDS), as a measure of gluten strength, is used when screening a large number of breeding lines in durum wheats (Dick and Quick 1983; Blanco et al. 1998). The sedimentation volume is well correlated with gluten strength and spaghetti cooking quality (Dexter and Matsuo 1980; Dick and Quick 1983).

Under field conditions, temperatures of 35 to 40°C are common in many wheat-producing areas of the world (Gusta and Chen 1987), some of which accompany with a hot and dry wind at grain filling period. The responses of wheat plants to high temperature stress at anthesis and grain filling period differ on at least one major account. Heat stress at anthesis causes both male and female sterility (Gusta and Chen 1987), whereas heat stress at grain filling period causes only grain shriveling (Arzani unpubl). The difference between heat stress and water stress at anthesis is that water stress causes only male sterility (Gusta and Chen 1987). Thus in the present study, grain set or grain number per spike was not reduced, but grain weight was drastically reduced (Arzani unpubl).

The objectives of this study were to evaluate grain quality characteristics of durum wheat germplasm with exotic and native origins grown under irrigated field conditions at two locations, and to determine the relationship of a combination of heat and drought stress at grain filling period to end-product quality.

Materials and methods

Plant materials and growing conditions: A total of 300 durum wheat genotypes comprising native cultivars and landraces as well as advanced lines and cultivars from International Maize and Wheat Improvement Center (CIMMYT) / International Center for Agricultural Research in the Dry Areas (ICARDA) were used in this study. The study was conducted in 1997-98 and 1998-99 growing seasons at two sites of Abarkoh (31° 08' N and 53° 17' E, 1510 m asl) and Isfahan (32° 32' N and 51° 23' E, 1630 m asl) located in central region of Iran. Abarkoh region is located in the marginal of Kavir desert and has always a warm and dry weather, particularly at grain filling period of autumn-grown cereal crops. An unreplicated trial with three-replicated-check cultivars (Shova, Altar84 and Aconchi89) was used at each of the locations. Fertilizer application was based on local practice of a rate of 40 kg ha^{-1} of N and 60 kg ha^{-1} of P_2O_5 at all locations. The seed rate was 400 seeds m^{-2}, and the harvested plot size was 6.25m^2 (5 rows, 5 m long, 25 cm apart).

Grain quality: Two grain samples were taken for each genotype from each of the successive years (1998 and 1999), combined into a single one and used in the present study. Hence, a total of 600 grain samples belonging to the two sites was evaluated for grain quality.

Weight per hectoliter (test weight), grain hardiness, protein content, wet and dry gluten contents and sodium dodecyl sulfate (SDS)-sedimentation volume were determined. Total grain nitrogen of flour was measured by the standard American Association of Cereal Chemists (AACC) Kjeldahl method (AACC 1962, 1983) and corrected to a 14% moisture basis. Grain hardiness was measured using Instron instrument.

The samples were tempered overnight to 16% moisture content and milled into semolina using a modified Bühler laboratory mill (Black and Bushuk 1967) with an extended mill flow to increase semolina yield. Semolina was milled into flour and the flour (10g) was mixed by hand for 20 s with deionized water to form dough ball. The dough ball was wrapped in an 80-μm silk sieve and hand washed under a flow of water for 50 s. The gluten ball was then worked between the fingers until it became sticky (about 5 min). Percent wet gluten content was determined by weighing the wet gluten ball after pressing it as dry as possible between the palms of hands for 30 s. Percent dry gluten was determined after drying the wet gluten ball by an oven at 70 °C for 48 h.

The sodium dodecyl sulfate (SDS)-sedimentation

volumes were determined according to Preston et al. (1982) with some modifications suggested by Kovacs (1985) for durum wheat.

Results and discussion

Table 1 presents mean, maximum and minimum, standard deviation (SD) and coefficient of variation (CV%) of grain quality traits of durum wheat genotypes grown in two sites and averaged on two years (1998–1999). A combination of heat and drought stress occurred at Abarkoh region during grain filling period where air temperature ranged from 34 to 42°C and accompanied with a hot and dry wind. The maximum temperature exceeded 8–10°C from the long-term averages in May and June in this area during 1998 and 1999 growing season. Annual precipitation during the two years of experiment (~25mm) was about half of the long term average of this region (50mm). Plant irrigated in a regular basis of 12d intervals as a common practice of Abarkoh's farmers despite of very low air humidity and low soil moisture content during the grain filling period. Statistical analysis using a paired Student's t test indicated a significant differences ($P<0.01$) between two regions for all grain quality characteristics and the highest values were recorded where a combination of heat and drought stress occurred at grain filling period in Abarkoh region (Table 1). This result is in agreement with that of Boggini et al. (1997) in durum wheat who reported that mean protein content and SDS sedimentation volume were lower in the environment with higher grain yield. Likewise in bread wheat, grain protein content was drastically increased by water stress, especially stress at anthesis (Jamal et al. 1996).

Coefficient of variation (CV%) of each grain quality characteristic as the quantitative traits indicates the effect of genetic diversity present in the germplasm. The highest variability was observed for protein content in both locations and for SDS-sedimentation volume in only Isfahan region (Table 1). Protein percentage had a range of 9.7–14.8 and a mean of 11.4 at Isfahan region, whereas these were 10.0–15.3 and 12.1 for Abarkoh region, respectively. A range of 24.3–68.0 with mean of 45.6 in Isfahan and a range of 30.8–68.9 with mean of 47.2 in Abarkoh were obtained for sedimentation volume. Likewise, Blanco et al. (1998) reported significant differences for protein content and SDS-sedimentation volume in the six environments examined. The sedimentation volume was found to be positively correlated ($P<0.01$) with protein content, wet gluten content, dry gluten content and grain hardiness in both locations. Protein content positively correlated at $P<0.01$ with wet and dry gluten contents at both environments.

Linear regression of protein content (%) of 15 durum wheat genotypes grown at Abarkoh region and

Table 1. Mean, maximum and minimum, standard deviation (SD) and coefficient of variation (CV%) of grain quality traits of durum wheat genotypes grown in two sites averaged on two years (1998-1999)

Site	Test weight (kg)	Grain hardiness	Protein content (%)	Wet gluten content (%)	Dry gluten content (%)	SDS-sed. volume (ml)
Isfahan						
Mean	79.8	470	11.4	25.4	8.9	45.6
Max.	84.5	668	14.8	40.8	14.0	68.0
Min.	77.2	300	9.7	10.3	4.3	24.3
S.D.	2.5	55	1.6	3.2	1.2	8.5
CV%	3.1	11.7	14.2	12.6	13.9	18.6
Abarkoh						
Mean	80.6	492	12.1	27.2	9.3	47.2
Max.	85.7	701	15.3	43.5	16.1	68.9
Min.	79.9	358	10.0	15.8	7.3	30.8
S.D.	4.35	47.7	2.1	2.9	0.7	5.1
CV%	5.4	9.7	17.4	10.6	7.7	10.8
t value	2.70**	5.23**	4.58**	7.20**	10.00**	2.80**

** Significant at $P<0.01$ (df=299).

Table 2. Comparison of protein content of 15 durum wheat genotypes in stressful field conditions with optimal field conditions

Genotype	Origin	Optimal conditions		Heat and drought stress	
		Protein content (%)	Rank	Protein content (%)	Rank
Ghalavandi	Iran	11.2	12	13.4	6
Shahsavandi	Iran	10.6	14	12.9	9
Zardak	Iran	12.0	6	14.1	4
Khoday	Iran	11.9	8	14.5	3
Shova	Iran	11.5	9	12.2	13
Symarah	Iran	10.9	14	12.3	12
Altar 84	CIMMYT	11.0	13	11.8	14
Yavarous 79	CIMMYT	12.4	4	12.7	10
Massara 1	ICARDA	12.4	4	13.6	5
Aconchi 89	CIMMYT	11.5	9	12.2	13
Chen/Altar 84	CIMMYT	12.0	6	12.4	11
Mexical 75	CIMMYT	12.5	3	13.1	8
Osta/Gata	CIMMYT	13.8	1	14.9	1
ICDW 7639	CIMMYT	13.2	2	14.6	2
PI 40100	CIMMYT	12.1	5	13.8	5

suffered with heat and drought stress on their protein content (%) of non-stressful conditions at Isfahan region was shown in Fig. 1. This result revealed that some genotypes have a similar protein content ranking under both a combination of stressed and optimal environments, while others have a substantially different ranking under optimal and stressed environments. Genotypes Osta/Gata, ICDW 7639 and PI40100 provide a good example of the former response, where their protein contents ranked similarly in both of stressed and optimal environments (Table 2). On the other hands, other genotypes listed in Table 2 provide a good example of the latter response, since their protein contents tend to rank considerably higher in stressed conditions than in optimal conditions.

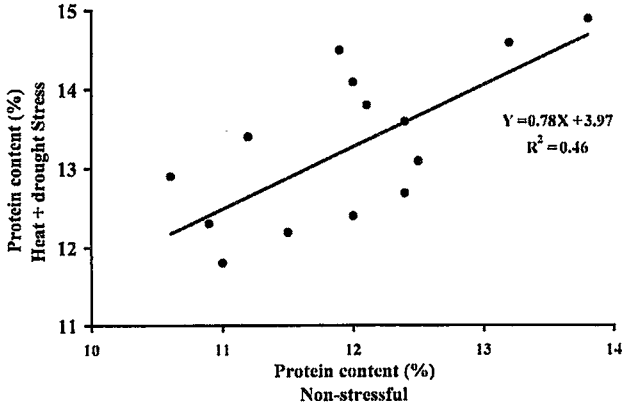

Fig. 1. Linear regression of protein content (%) of durum wheat genotypes at Abarkoh region with heat and drought stress on their protein content (%) of non-stressful conditions at Isfahan region.

Under field conditions, temperatures of 35 to 40°C are common in many wheat-producing areas of the world (Gusta and Chen 1987), some of which like those of Abarkoh region accompany with a hot and dry wind at grain filling period. The responses of wheat plants to high temperature stress at anthesis and grain filling period differ on at least one major account. Heat stress at anthesis causes both male and female sterility (Gusta and Chen 1987), whereas heat stress at grain filling period causes only grain shriveling (Arzani unpubl).

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Research article

Gene system governing grain yield per spike in macaroni wheat

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Summary

To study the nature and magnitude of gene systems governing grain yield per spike in three crosses of macaroni wheat, analysis of gene effects was done using means of twelve generations viz., P_1, P_2, F_1, F_2, B_1, B_2, B_{1S}, B_{2S}, B_{11}, B_{12}, B_{21} and B_{22} under normal and late sown environments, separately. Three, six and ten parameter models were employed and found adequate in different crosses as well as in different environments. The additive, dominance and epistatic effects were found responsible in controlling the inheritance of this trait, however, trigenic and digenic non-allelic interactions were contributed more. Duplicate type of epistasis was observed frequently under late sown condition. Non-fixable gene effects were much higher than the fixable in almost all cases in both the environments. Epistatic interactions, particularly trigenic non-allelic interactions and dominance (h) contributed maximum towards significant and positive heterosis. Dissipation of epistatic effects involving dominance in F_2 generation causes significant inbreeding depression. In the cross HI8062 x JNK-4W-128 and Raj911 x DWL5002, appropriate suitable environment can provide better opportunities for improvement through single seed descent and bulk method of breeding. However, in other cases hybridization systems, such as, restricted recurrent selections and / or diallel selective mating methods, which exploit both additive and non-additive gene effects, simultaneously, could be useful in the genetic improvement of the grain yield per spike in macaroni wheat.

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

Introduction

Macaroni wheat (*Triticum durum* Desf.) is the second important wheat species of India but the exploitation of genetic information has not been very well demonstrated as compared to bread wheat. Durum (macaroni) wheat is highly valued for production of semolina and pasta products like macaroni, spaghetti, vermicelli, couscous, burghul and frekeh. These preparations are popular in North America, Europe particularly and African countries and thus impart bright prospects to durum wheat from the export point of view (Tandon 1994). In recent years in India there has been a renewed interest in durum cultivation because of newly devolved dwarf, rust resistant durums for irrigated areas. However, earliness, high yield potential and superior grain quality (bold, golden, hard and lustrous grains) are yet to be explored for higher profitability. This necessitated acceleration of improvement in this species. An efficient breeding program is needed to break down the present plateau of productivity through improvement of durum wheat varieties. Direct components of grain yield in wheat were reported to be tillers per plant, grain yield per spike and kernel weight (Srivastava et al. 1982; Singh and Rana 1989; Joshi 1997). By improving these direct and some other indirect components, grain yield can be improved in durums. The knowledge about the nature and magnitude of gene effects may greatly help in formulating plant breeding program, since such a knowledge not only tells about the relative importance

of different kinds of gene effects (additive, dominance and epistatic) in the control of characters but also provides information about the cause(s) of heterosis (Jinks 1955; Hayman 1958; Brim and Co-ckerham 1961; Gamble 1962; Hill 1966; Matinzinger 1968; Stuber and Moll 1974). In the present study, an attempt has been made to estimate various kinds of gene effects through generation mean analysis and to know the relative importance of these gene effects in the control of grain yield per spike in durum wheat under normal and late sown environment. The information based on the nature and magnitude of gene action controlling inheritance of character like grain yield per spike which is ultimately related to productivity would aid in the choice of effective and efficient breeding methods and thus, accelerate the pace of its genetic improvement for grain yield in durums. Present study deals with such endeavour.

Materials and methods

The experimental material generated from six diverse parents, comprised three crosses namely, Cocorit71 x A-9-30-1, HI8062 x JNK-4W-128 and Raj911 x DWL 5002. Twelve basic generations viz. two parents, F_1 and F_2, first backcross generations with both parents (BC_1 and BC_2), Where BC_1 was the cross between F_1 x female parent and BC_2 was F_1 x male parent, their selfed progenies (BC_1F_2, BC_2F_2) and second backcross generations i.e. the BC_1 and BC_2 plants again crossed with both original parents (BC_1 x female parent; BC_1 x male parent and BC_2 x female parent; BC_2 x male parent). All these populations were raised together in randomized block design with three replications at 30cm x 15cm spacing under normal and late sown environments in the same cropping seasons at Research farm of Rajasthan Agricultural University, Agricultural Research Station, Durgapura, Jaipur. Each parent and F_1 generations was sown in 2 rows, each backcross generation in 4 rows and F_2 and the second cycle of backcrosses in 6 rows of 5 m length. Grain yield per spike (g) of the main tiller was recorded on 15 random plants in each parent and F_1, 30 plants in each backcross generations and 60 plants in each F_2 and second backcross generations in both environments.

The data of each population in both environments were analyzed separately by joint scaling test of Cavalli (1952) to determine the nature of gene action. Components of heterosis in the presence of digenic interactions were calculated as suggested by Jinks and Jones (1958) and trigenic interactions were calculated as suggested by Hill (1966).

Results and discussion

Significant differences were observed among generation means for grain yield per spike in all the three crosses in both the environments, which revealed the presence of genetic diversity for this attribute in the material. The generation mean analysis results revealed that 3-parameter model was adequate in the cross HI8062 x JNK-4W-128 and Raj 911 x DWL5002 under normal and late sown condition, respectively. The additive (d) gene effects were consistently significant in both the cases. However, the dominance (h) gene effect was only significant in the former cross. In other cases 10-parameter model under normal sown and 6-parameter model under late sown condition was found adequate to account genetic variation among the generation means, indicated that epistatic interactions had a greater role than additive (d) and dominance (h) gene effects in governing the inheritance of the trait. The additive (d) and dominance (h) gene effects were frequently observed significant.

Among digenic interactions additive x additive (i) and dominance x dominance (l) were contributed maximum towards in controlling the inheritance of this trait under normal and late sown environments, respectively. Among trigenic interactions most of the interactions were found significant in the cross of Cocorit71 x A-9-30-1, whereas only additive x dominance 3K dominance (y) found significant in the cross Raj911 x DWL 5002 under normal sown condition (Table 1). Absolute totals of epistatic effects revealed that epistatic effects were much higher than the main effects in all the cases except where additive-dominance model was fitted to data. These results further confirmed that the non-allelic interactions, such as first order and second order had somehow more important though the additive (d) and dominance (h) were also contributed significantly in controlling the inheritance of trait studied (Table 2). Duplicate type of epistasis was observed in the cross Cocorit71 x A-9-30-1 and HI8062 x JNK-4W-128 under late sown conditions only.

Results of the study further revealed that the absolute totals of the non-fixable effects were higher than the fixables in all the cases except in the cross Raj 911 x DWL 5002 in late sown condition signifying the greater importance of non-additive gene effects, a major portion of which was shared by epistatic effects (Table 2). Earlier, Widner and Lebsock (1973), Ram et al. (1977), Bhatia et al. (1979) and Srivastava et al. (1982) in durum Wheat and Singh and Rana (1989) and Joshi, (1997) in bread wheat reported that both

additive and non-additive gene effects were important in the inheritance of grain yield per spike.

Analysis of components of heterosis revealed that epistatic interactions had important role to cause significant and positive heterosis in the cross Cocorit 71 x A-9-30-1 whereas non-additive gene effect causes

Table 1. Results of joint scaling test and gene effects for grain yield per spike

Effects	Cocorit71 x A-9-30-1		HI8062 x JNK-4W-128		Raj911 x DWL 5002	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
m	1.63**	2.12**	2.44**	2.31**	2.48**	2.17**
	±0.18	±0.15	±0.05	±0.17	±0.16	±0.04
(d)	0.21	0.62**	-0.17**	-0.39**	0.32**	0.41**
	±1.16	±0.07	±0.05	±0.04	±0.11	±0.04
(h)	1.57**	0.58**	0.71**	0.55*	0.78**	0.15
	±0.31	±0.22	±0.11	±0.23	±0.23	±0.08
(i)	2.34**	0.25		0.16	1.51**	
	±0.44	±0.21		±0.24	±0.44	
(j)	-0.12	0.79**		-0.43	0.98*	
	±0.65	±0.20		±0.22	±0.48	
(l)	1.75	-0.88*		-0.80*	-0.89	
	±1.68	±0.41		±0.33	±1.58	
(w)	1.03				-0.14	
	±0.64				±0.46	
(x)	5.71**				1.58	
	±1.98				±1.97	
(y)	-4.47**				2.72**	
	±1.58				±0.82	
(z)	-5.85*				-0.66	
	±2.62				±2.41	
χ^2 for 10 parameter model	0.34(2)†	2.49(6)	16.09(9)	8.430(6)	34.79(2)	12.19(9)

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

†Degree of freedom for χ^2

Table 2. Absolute totals of epistatic effects, fixable and non-fixable gene effects for grain yield per spike

Cross	Environment	Main effects		Epistatic effects†		Tatal gene effects‡	
		(d)	(h)	I order	II order	Fixable	Non-fixable
Cocorit71 x	Normal	0.21	1.57	4.22	17.06	3.58	19.48
A-9-30-1	Late	0.62	0.58	1.92	-	0.86	2.25
HI8062 x	Normal	-0.17	0.71	-	-	0.17	0.71
JNK-4W-128	Late	-0.39	0.55	1.38	-	0.54	1.77
Raj911 x	Normal	0.32	0.78	3.38	5.10	1.97	7.61
DWL5002	Late	0.41	0.15	-	-	0.41	0.15

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

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

Table 3. Components of heterosis for grain yield per spike

Effects	Cocorit 71 x A-9-30-1		HI 8062 x JNK-4W-128		Raj 911 x DWL 5002	
	Normal sown	Late sown	Normal sown	Late sown	Normal sown	Late sown
(h)	1.57	0.58	0.71	0.55	0.78	0.15
-(i)	-2.34	-0.25	-	-0.16	-1.51	-
1/2(x)	2.86	-	-	-	0.79	-
1/4(Z)	-1.46	-	-	-	-0.16	-
-(d)	-0.21	-0.62	0.17	0.39	-0.32	-0.41
1/2(j)	-0.06	0.40	-	-0.21	0.49	-
-(w)	-1.03	-	-	-	0.14	-
-1/4(y)	1.12	-	-	-	-0.68	-
Heterosis (%)	17.89*	10.97	23.32**	14.51	-5.26	-5.51
Inbreeding depression (%)	16.55*	3.95	16.67**	7.84	11.40**	13.33

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

heterosis in the Cross HI8062 x JNK-4W-128 under normal sown condition. Most of the trigenic interactions [(x), (y) and (z)] and only additive x additive (i) digenic interaction in the former cross and dominance (h) component in latter cross, contributed maximum towards significant heterosis. Absence of significant heterosis in remaining cases could be explained due to the internal cancellation of heterosis components (Table 3). Significant inbreeding depression in all the three crosses in normal sown condition, showed reduction in F_2 over F_1 due to the dissipation of non-additive dominance effects or epistatic effects involving dominance in F_2. Thus, it is confirmed that non-additive genes had a significant importance in durum wheat. Earlier, Sharma et al. (1986) and Dasgupta and Mondal (1988) reported that non-additive gene actions were the responsible for significant heterosis and inbreeding depression in grain yield per spike.

This study further revealed that varied results were recorded in different crosses and environments regarding the relative importance of gene action in controlling the inheritance of grain yield per spike in durum wheat. Such variations may arise due to the differences in the parental material of investigation and the differences caused by sowing environments.

However, in view of high magnitude of inter-allelic interactions of digenic and trigenic level for most of the cases, it was suggested that wheat breeder should follow such methods, which can mop-up the genes, to form superior gene constellations interacting in a favorable manner. The breeding methods suggested to achieve this objective are restricted recurrent selection (Joshi 1979) and / or diallel selective matting (Jensen 1978). The presence of considerable additive variation with or without fixable gene effects for this trait (HI8062 x JNK-4W-128 and Raj911 x DWL 5002 under normal and late sown condition, respectively) indicated that simple procedures like single seed descent, bulk method of breeding, and their various modifications should be rewarding in obtaining superior recombinant pure breeding lines in durum wheat. Thus, it is clear from the study that suitable environment should be selected for rewarding superior recombinant in a favorable environment with suitable material for desired improvement in this trait for further tangible advancement of grain yield in macaroni wheat in future.

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Research information

Breeding behavior and inheritance of genic male-sterility in hexaploid wheat

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In wheat, breeders have utilized cytoplasmic male-sterility (CMS) and chemical hybridizing agents (CMS) to develop hybrids. Genic male-sterility (g-mst) has not been utilized extensively, although in other crops it has been used very successfully. The reason could be non availability of appropriate kind of genic male-sterility.

In the year, 1994, male sterile plants with open florets were detected among F_3 progenies derived from a cross between selection 212 (wheat-rye recombinant possessing resistance to leaf and stem rusts of wheat) and HD2009, an Indian rust susceptible wheat. Out of these, one plant showing resistance to leaf and stem rusts produced 7 seeds from 10 selfed spikes. Four viable plants were obtained from these seeds.

The expression of genic male-sterility in the present case was caused due to the conversion of anthers into fully fertile ovaries. Since the phenomenon of anther conversion into ovaries was incomplete, therefore male-sterility was partial, thus it was designated as partial genic male-sterility (p-mst). Genic male sterility has been reported in *Triticum aestivum* formerly by Pugsley and Oram (1959), Lupton and Bingham (1966-1967), Krupnov (1968), Lemekh et al. (1971) and Jan and Qualset (1977). The p-mst strain, stabilized for six generations was considered appropriate to study its inheritance.

To study the inheritance of p-mst trait, it was crossed with an Indian semi-dwarf wheat variety Kundan. At appropriate stage of anthesis, some spikes from p-mst plants were emasculated to make sure that no pollen grain remained in the maternal floret. After 24 hours of emasculation, all the florets were pollinated with fresh pollen grains taken from var. Kundan and sufficient number of crossed seeds were obtained. Parents, F_1's and F_2 plants, were planted together at IARI, New Delhi. Observations on dominance/recessive behavior of p-mst trait in F_1 and pattern of segregation in F_2 were recorded. Chi-square test was applied to test the goodness of fit for the assumed segregation ratio.

Breeding behavior of genic male-sterility trait was studied for 6 generations (1994-1999). During this period, selfed p-mst spikes produced 6.1 seeds per spike, which was much lower than 40.4 seeds per spike observed in Sel. 212 (one of the parent). The observed range of seed set was 0 to 15 seeds per spike in p-mst. In terms of sterility p-mst plants exhibited 85% to 100% male-sterility (Table 1).

The p-mst plants were late in maturity and fully resistant to leaf and stem rusts of wheat (resistance from rye). Anthers in most of the florets in p-mst plants were modified ovaries (Fig. 1). In some frorets, anthers appeared little normal and produced normal pollen grains. The seeds formed on p-mst plants were partially shrivelled (sub-normal development of endosperm).

In p-mst plants the meiosis proceeded normally. Their female fertility was normal, including the modified ovaries which could produce two to three seeds by cross pollination.

As revealed by the Table 2, variety Kundan produced 43.3 seeds per spike while p-mst produced only 6.5 seeds per spike. It clearly indicated the stability of male-sterility. All the F_1 hybrids on the other hand produced 45.1 seeds per spike, comparable to fully fertile parent variety Kundan, demonstrating the dominance of fertility trait over the sterility. In the F_2 generation, a segregation ratio of 3 fertile and 1 sterile was observed (Table 2).

Table 1. Breeding behaviour of male sterile hexaploid wheat stock observed for 6 generations.

Year of selfing	No. of spikes selfed	No. of seeds obtained	Seeds per spike
1993-94	10	7	0.70
1994-95	17	98	5.76
1995-96	293	1806	6. 16
1996-97	447	2812	6.29
1997-98	230	1543	6.70
1998-99	324	1841	5.68
Total	1321	8107	6.13
Sel. 212 (fertile)	16	647	40.43

Table 2. F_2 segregation for partial male-sterility from a cross between p-mst and var. Kundan (seeds from first 3 spikes in each plant)

Parents	Fertile type			Sterile type			Chi-square 3: 1	"P" value between
	No.of plants	No.of seeds	Seeds /spike	No.of plants	No.of seeds	Seeds /spike		
Var. Kundan	30 (90)	3897	43.3					
P-mst				120 (360)	2340	6.5		
F_1 (p-mst x Kundan)	15 (45)	2030	45.1					
F_2	282 (846)	39170	46.3 (324)	108	2527	7.8	1.51	0.2-0.3

Figures in parenthesis are the number of spikes

On the basis of these observations it is suggested that the male-sterility trait in p-mst strain, is controlled by a recessive gene. In the past, the control of genic male-sterility by single recessive genes has been reported by Pugsley and Oram (1959), Latypov (1974), Driscoll and Barlow (1976), Kleijer and Fossati (1976), Jan and Qualset (1977) and Sasakuma et al. (1978).

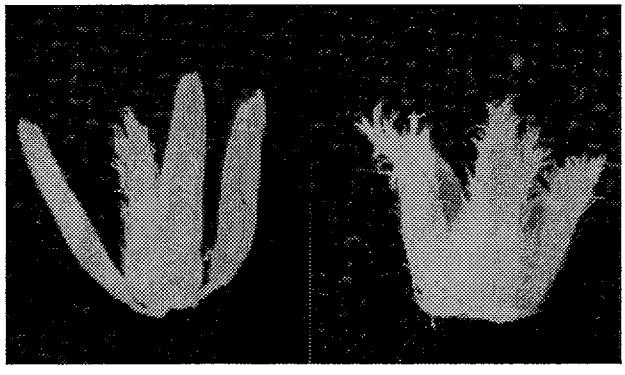

Fig. 1. Normal anthers with one ovary (left), and modified ovaries with one normal (right)

The aneuploid analysis of genic male-sterility in p-mst has revealed the involvement of two recessive genes located on chromosomes 4A and 6B (Singh unpub). The variation in the results thus could be due to the use of variable parents in the studies. In the present study the p-mst was crossed with a recently released Indian hexaploid wheat var. Kundan while in the aneuploid study cv. Chinese Spring and its monosomic lines were used. It is, therefore, likely that for normal anther development var. Kundan carry one gene (located on chromosome 4A) while cv. Chinese Spring carry two genes (on 4A and 6B) respectively. The gene located on chromosome 6B in var. Kundan may be in recessive form.

Wheat, basically a self-pollinated crop, exhibits 30-70% positive mid-parent heterosis for grain yield. Thus development of hybrid wheat is in the priority

areas of modern agriculture. The p-mst under report is easy to maintain (10-12% selfed seeds), easily transferrable (simple inheritance), least damage from rust diseases (it possesses rust resistances from rye genome) expected enhanced cross pollination (the outer glume of the florets are forced to remain open by the modified ovaries), no adverse effect on female fertility. It may, therefore, serve as an additional tool to develop wheat hybrids.

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Editorial remarks

Due to delay of editorial business, No.94 of Wheat Information Service contains less pages than usual. This is not because of reduction of number of submitted articles, but because of the increased level of reviewing. Consequently, a considerable number of contributed article are under reversion. Since WIS is wide-open for information exchange, articles for **Research information** (articles without reviewing) are very much welcome.

New fields of biological science are going to be opened. Accumulation of cDNA clones, genetical and physical mapping of genes and markers, micro- and macro-arrays, *in silico* or bioinformatics, and so on. If these ideas and techniques will be jointed and combined to the genetic materials, studies on evolution, genetic diversity, and breeding would be on new stages. Already, an international cooperation for 'wheat genome project' has been proposed, and discussion about the subjects is going on at the meeting of International Triticeae Mapping Initiative held in Winnipeg on June, 2002. The next meeting of International Wheat Genetics Symposium will be one of the places for information exchange on these subjects, which is planed to be held in Italy, 2003. On these advances, it is never-the-less to say that materials or genetic stocks are critical for any kinds of study with genotype or phenotype information. WIS hopes to accumulate and exchange the information.

June, 2002

Editorial Board
Tetsuo SASAKUMA, Secretary

Kihara Memorial
Yokohama Foundation for the Advancement of Life Sciences

The Kihara Memorial Foundation (KMF) was established in 1985 in memory of the late Dr. Hitoshi Kihara, a world famous geneticist and evolutionary scientist. The activities of the KMF are promotion of life science by supporting symposia, workshops, and technical courses for researchers, enlightenment of scientific information to citizens, awarding of 'KMF Prize' and 'Child Scientist Prize', and publication of journals such as 'Wheat Information Service'.

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The recent economic condition in Japan is limiting our support of these KMF activities. KMF is, therefore, taking up subscriptions from colleagues who approve of the activities of KMF. We would appreciate receiving from you inquiries about this matter, thank you.

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