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Paternal Regulation of Seed Development in Wheat Hybrids

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Summary. Diallel crosses among *Triticum boeoticum* (4 lines from different geographical areas), *T.urartu*, *Aegilops squarrosa* and *Ae.speltoides* exhibited reciprocal differences in hybrid seed morphology, endosperm development, and embryo viability. *T.urartu* and *Ae.squarrosa* as females with *T.boeoticum* and *Ae.speltoides* lead to shrivelled inviable seed. *T.boeoticum* accessions as female with *Ae.speltoides* also lead to shrivelled seeds. The reciprocal crosses produced plump seeds which either resembled the maternal parent or showed size differences. By altering the endospermic genome ratios, hybrid seeds with 1 (♀)/1 (♂) showed extreme shrivelling whereas those with 4 (♀)/1 (♂) were medium shrivelled to plump. Genetic experiments involving hybrids of *T.boeoticum*, *T.urartu* and *T.monococcum* showed that a factor is present in pollen or male gametes, which shows dosage effect and which, by interacting with the maternal genome, leads to endosperm abortion.

Key words: Paternal Regulation - Wheat Hybrids - Seed Development - Endosperm Abortion - Genome Unbalance

Introduction

There are many reports of differences in crossing compatibility and seed development in plant-species hybrids. Pronounced differences in seed morphology and development may be observed between the reciprocal crosses of two parental lines. In monocotyledonous plants, reciprocal differences may be manifest in one of four classes. 1) Endosperm and embryo development may be normal and result in viable seed. Hybrid seed morphology may either resemble the maternal parent or may show size differences. 2) The endosperm may develop abnormally leading to endosperm abortion and seed death. Here the embryo itself is viable but, since it is nursed by the endosperm, it dies as a result of the endosperm abortion. 3) Endosperm development is normal, but the hybrid embryo is lethal or semi-lethal. 4) Both the endosperm may abort and the embryo may be lethal. We have encountered all of the above reciprocal differences in *Triticum* L. and *Aegilops* L. crosses.

Boyes and Thompson (1937) studied seed development in reciprocal interspecific crosses among 14, 28 and 42 chromosome wheats. The endosperm of "low chromosome" and "high chromosome" crosses exhibited abnormal nuclei, curdy cytoplasm, delayed cellularization and, in some cases, early abortion, whereas the reciprocal crosses underwent normal endosperm and seed development. They attributed this reciprocal difference in seed development to the chromosomal unbalance in the endosperm. However, Kihara and Nishiyama (1932) from earlier studies in diploid and polyploid *Avena* crosses had stressed the role of the sperm nucleus in differential endosperm development. Beaudry (1951) performed similar studies in an *Elymus virginicus* L. × *Agropyron repens* (L.) Beauv. hybrid and concluded that *A.repens* pollen alters endosperm development by affecting antipodal cell physiology through a chemical stimulus. His hypothesis rests on the assumption that antipodals play a secretory role and supply nutrients during early endosperm development. Recently, Dhaliwal (1977) showed that cytoplasmic differences cannot account for the reciprocal difference between *Triticum urartu* Tum. and *T.boeoticum* Boiss. crosses, and he has supported the genome unbalance theory of endosperm abortion. Davies (1973, 1975) and Davies and Brewster (1975) reported on seed size, globulin protein, and ribosomal RNA differences between some reciprocal crosses in *Pisum sativum* L. They postulated that an inducer in the maternal parent may interact with repressors on the maternal and paternal genomes in the embryo. Depending on this interaction, maternal and paternal genes may or may not be expressed in the hybrid

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seed, and this thereby leads to reciprocal differences.

In this paper we report our results on seed morphology, endosperm development, and embryo viability in reciprocal crosses involving diploid *Triticum* and *Aegilops* species.

Materials and Methods

The genotypes used in reciprocal diallel matings are listed in Table 1. For hybrid-seed production, emasculations and pollinations were made under standard greenhouse conditions. Embryo culture was employed 12-18 days after pollination to rescue embryos in those crosses showing early endosperm abortion. The culture medium consisted of Murashige and Skoog medium supplemented with vitamins and amino acids (Murashige and Skoog, 1962).

For protein analysis, a gel electrofocusing method developed by LKB (Karlsson et al. 1973) was used. The flour from parental and hybrid seeds was extracted with 2M urea at room temperature for 10 minutes. The 4 × 8 mm 3M chromatography paper strips were soaked in the extractant and applied on the gels. Two molar urea polyacrylamide electrofocusing gels (pH range 3.5-9.5) were used for protein fractionation. The preparation of gels, electrofocusing, staining and destaining were carried out as in LKB application note (Karlsson et al. 1973). The technique allowed us to look at the albumin and gliadin fractions of seed proteins.

Table 1. Plant material used in hybrid crosses

Taxon	Accession and Source
Triticum boeoticum Boiss.	558 (*T.aegilopoides* var. *pseudo-boeoticum*) Armenia; 1814, 1912 & 1918, Turkey; 2520, Iran; 3116, Lebanon.
T.monococcum L.	3327 (PI 289599) & 3371 (CI 13961)
T.urartu Tum.	1785 & 1841, Turkey
Aegilops speltoides Tausch	1272 & 1316, Israel
Ae.squarrosa L.	0-445 (32.2, Sears, Missouri)
T.boeoticum (4x)	Colchicine induced tetraploid of F_1 642 (Thrace) × 640 (Beysehir) Turkey. Made by D. Barnhart

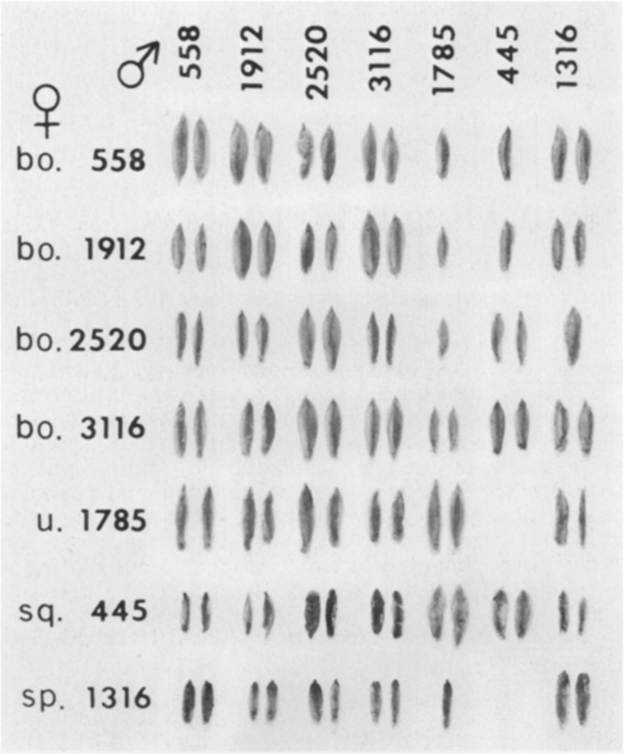

Fig.1. Selfed-parental, and F_1-hybrid seed from reciprocal diallel crosses among *Triticum boeoticum* (558, 1912, 2520 and 3116); *T.urartu* (1785); *Aegilops squarrosa* (445) and *Ae.speltoides* (1316). See Results, section I "Seed morphology and endosperm development" for details

Results

I. Seed Morphology and Endosperm Development

The morphologies of selfed-parental and most reciprocal hybrid seeds of the seven accessions are illustrated in Fig.1 and will be discussed with respect to the 4 classes of seed development outlined in the introduction. All selfed seeds are viable, and their size, shape and plumpness due to normal endosperm development are taken as control. They provide examples of class 1 development. In intraspecific *T.boeoticum* crosses among lines 558, 1912, 2520 and 3116, seeds are mostly well formed and plump, but in some hybrid combinations there is moderate shrivelling and size reduction, these combinations forming a separate subclass. Accession 558 as male parent, and acc. 2520 as female, lead to small hybrid seed with some shrivelling, the seeds of reciprocal crosses are normal.

Interspecific crosses with *boeoticum* lines 558, 1912, 2520, and 3116, as females with *speltoides* as a male parent; and *urartu* 1785 as a female with *boeoticum* lines 558, 1912, 2520 and 3116 or *speltoides* 1316 as male parents provide examples of class 2 development. The hybrid endosperm aborts;

there is extreme endosperm shrivelling followed, in most cases, by embryo death. F_1 seeds with *Ae.squarrosa* 445 as female with *boeoticum* lines 558, 1912, 2520, and 3116 or *speltoides* 1316 as males are extremely shrivelled and the embryo is lethal (class 4). A notable exception to this is the F_1 seed of cross *squarrosa* 445 (♀) × *urartu* 1785 (♂) where seed is plump and almost twice the size of the selfed seed, but smaller than that of the 1785 parent (class 3). Moreover 1785 pollen in crosses with all *beoeticum* accessions results in plump seed, but greatly reduced in size. The F_1 seed of *speltoides* as a female with *urartu* and all *boeoticum* accessions as males; and all *boeoticum* accessions as females with *squarrosa* 445 as a male are viable and more closely resemble the female parent. Many attempts were made to obtain F_1 seeds of interspecific crosses *urartu* (♀) × *squarrosa* (♂) and *speltoides* (♀) × *squarrosa* (♂) but were unsuccessful.

The extremely shrivelled seeds were tested for endosperm development by assaying for storage proteins in mature hybrid seeds of the crosses *urartu* 1785 × *boeoticum* 1912, *boeoticum* 3116 × *speltoides* 1316, *squarrosa* 445 × *speltoides* 1272, which represent a sample of the abnormal classes in the diallel set. The shrivelled seeds from the first two crosses did yield some storage proteins, but none were recovered from seeds of the third cross. In this cross lack of storage proteins may result from total inhibition of endosperm development, or the endosperm may at first develop and later be degraded. In the non-germinable combinations, the amount of endosperm development varies with the degree of seed shrivelling.

II. Embryo Viability

All crosses in the diallel set that produced good seed also produced viable plants, except *squarrosa* 445 × *urartu* 1785 seed, which provides an example of class 3 development. This latter combination is always lethal or semi-lethal. The lethal embryos die early during seed development. The semi-lethal embryos may die very early during seed germination or later at the seedling stage. Of 15 cultured embryos of the 445 × 1785 cross, four were lethal, five semi-lethal early, and six semi-lethal late. Of these six semi-lethal late plants, one produced two tillers with very small ears and then died (Table 2).

The incompatibility of *squarrosa* with *boeoticum* is so strong that embryos are dead as early as 12-14 days after pollination. They provide an example of class 4 development. All attempts at embryo culture have failed except in the case of one weak seedling obtained with *boeoticum* 558. Of ten embryos tested of the *squarrosa* × *speltoides* crosses, all were of the semi-lethal early type. Diploid *squarrosa* (♀) × tetraploid *boeoticum* (♂) produced vigorous seedlings which may turn out to be viable (Table 2). These seedlings are under observation. Morphologies of some of the parental and hybrid embryos are shown in Fig.2.

No germination was obtained in other highly shrivelled seeds with *urartu* 1785 as female parent, or *speltoides* 1316 as male parent in crosses with other lines in the diallel set, with the exception of one seed of 1785 × 1912, which produced a small seedling (1 cm long) that promptly died. Viable plants were obtained, however, in the above category of crosses by embryo culture at 14-18 days after pollination.

III. Altered Genome Ratios and Endosperm Development

Tetraploid *boeoticum* (2n = 4x = 28) was crossed either as female or as male with diploid *urartu* (2n = 2x = 14), *squarrosa* (2n = 2x = 14) or *speltoides* (2n = 2x = 14). Many crosses of *boeoticum* (4x) (♀) with *urartu* (2x) or *squarrosa* (2x) (♂); and *speltoides* (2x) (♀)

Table 2. Seedling survival in cultured embryos of F_1 crosses of *Aegilops squarrosa* with 2x and 4x *Triticum boeoticum*, *T.urartu* and *Ae.speltoides*

Cross		Lethal	Semi-lethal		Normal
♀	♂		early	late	
Ae.squarrosa	× *T.boeoticum* (2x)	40	1	0	0
Ae.squarrosa	× *T.boeoticum* (4x)	0	1	0	2
Ae.squarrosa	× *T.urartu*	4	5	6	0
Ae.squarrosa	× *Ae.speltoides*	0	10	0	0

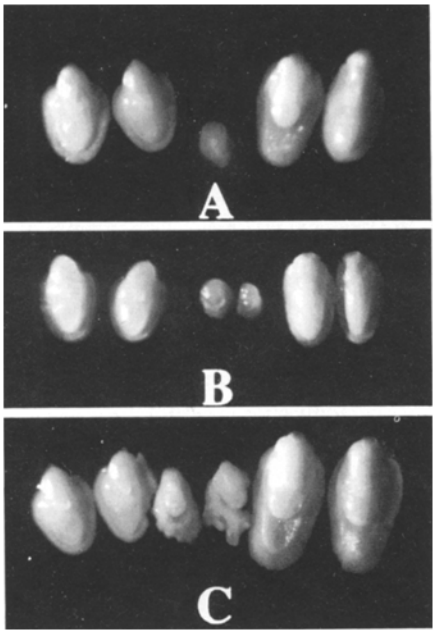

Fig.2. Morphologies of parental and hybrid embryos dissected out at days 17-21 following pollination

A. *Aegilops squarrosa* selfed (left 2 at days 21), *squarrosa* × *monococcum* hybrid (middle 1 at days 17) and *Triticum monococcum* selfed (right 2 at days 21) embryos, respectively. The hybrid embryo suffers extreme retardation in growth and is lethal

B. *Ae.squarrosa* selfed (left 2 at days 21), *squarrosa* × *urartu* hybrid (middle 2 at days 18) and *T. urartu* selfed (right 2 at days 21) embryos, respectively. The hybrid embryo is again extremely retarded but may grow into a tiny seedling when cultured on appropriate media

C. *Ae.squarrosa* selfed (left 2 at days 21), *squarrosa* (2x) × *boeoticum* (4x) hybrid (middle 2 at days 19) and *T.boeoticum* (4x) selfed (right 2 at days 21) embryos, respectively. The scutellum of *squarrosa* selfed embryo (no. 2 from left) was injured during the dissection procedure. Of the hybrid embryos, one showed normal but reduced growth. The second embyo had malformed scutellum. The hybrid embryos grew into vigorous seedlings and produced normal plants

with *boeoticum* (4x) (♂) were attempted, but were unsuccessful. The results of other crosses are presented in Table 3. The F_1 hybrid seeds of *urartu* (2x) × *boeoticum* (4x) and *squarrosa* (2x) × *boeoticum* (4x), where the endosperm genome ratio is 2 (♀) : 2 (♂), showed extreme shrivelling, more so than the corresponding diploid × diploid seeds, which have a 2 (♀) : 1 (♂) endosperm genome ratio. However, the cross *boeoticum* (4x) × *speltoides* (2x), where the endosperm genome ratio is 4 (♀) : 1 (♂), produced medium shrivelled to plump seed.

These experiments rule out endosperm genome unbalance as the cause of reciprocal differences in seed development in these hybrids. Furthermore, the results are consistent with the interpretation that a pollen factor(s) which shows dosage effect, and for which maternal genome sites compete, may be responsible for endosperm abortion. When there are two copies of this pollen factor for which two maternal genomes are competing, the endosperm is completely aborted. However, if there is only one copy of the pollen factor, for which four maternal genome copies are competing, seeds are well formed. The following genetic experiments were undertaken to further characterize this pollen factor.

IV. Genetic Studies

F_1 seed of *urartu* (♀) × *boeoticum* (♂) is extremely shrivelled, whereas *urartu* (♀) × *monococcum* (♂) F_1 seed is partially shrivelled. *Boeoticum* × *monococcum* crosses produce viable, plump seed. We exploited this situation to study the genetics of seed development. Embryo cultured F_1 plants of *urartu* × *boeoticum*,

Table 3. Effect of altered genome ratios on endosperm development in F_1 seed of diploid *Triticum urartu*, *Aegilops squarrosa*, and *Ae.speltoides* with autotetraploid *T.boeoticum*

Cross		Genome ratio (♀/♂)		Seed*		
♀	♂	endosperm	embryo	+	+-	-
T.urartu ×	*T.boeoticum* (4x)	2/2	1/2	0	0	3
Ae.squarrosa ×	*T.boeoticum* (4x)	2/2	1/2	0	0	3
T.boeoticum (4x) ×	*Ae.speltoides*	4/1	2/1	0	2†	0

* +, +-, - denote normal, medium shrivelled and shrivelled seed, respectively
† seed reduced in size

Table 4. Seed development in BC_1 of F_1 *Triticum urartu* × *T.boeoticum* with *T.boeoticum* or *T.urartu*, and cross of *T.urartu* pollinated with F_1 *T.boeoticum* × *T.monococcum*

Cross		Seed*		
♀	♂	+	+-	-
F_1 (*urartu* × *boeoticum*) ×	*boeoticum*	34	1	1
F_1 (*urartu* × *boeoticum*) ×	*urartu*	3	0	0
urartu ×	F_1 (*boeoticum* × *monococcum*)	0	12	13

* +, +-, - denote normal, medium shrivelled and shrivelled seed, respectively

which are completely male sterile, were backcrossed to either *boeoticum* or *urartu* as males. Of 39 F_1 seeds that were obtained, 37 were plump, one partially shrivelled and one completely shrivelled. These results indicate that essentially no segregation for seed development took place in the female gametes (Table 4).

Next we crossed *urartu* (♀) × (*boeoticum* × *monococcum* F_1) (♂). Out of 25 seeds, 12 showed medium shrivelling and 13 were completely shrivelled (Table 4). These results indicate a 1:1 segregation for an embryo abortion factor that is active in the haploid male gametophyte. This factor does not induce shrivelling in selfed seed, thereby indicating that a specific site on the maternal chromosome is essential for its expression. The mechanism by which this factor causes seed shrivelling is unclear.

The fact that *T.boeoticum* pollen does not induce seed shrivelling on backcrossing with the F, *urartu* × *boeoticum* hybrid means that the *boeoticum* factor for normal development on the female side is dominant and controlled by the maternal parent genotype. It also follows that on crossing with *T.boeoticum* pollen, progeny of BC_1 (*urartu* × *boeoticum*) × *boeoticum* should not segregate and BC_1 (*urartu* × *boeoticum*) × × *urartu* should segregate for endosperm development.

Discussion

The experiments reported here are consistent with the interpretation for paternal regulation of seed development in reciprocal crosses of diploid *Triticum* and *Aegilops*. Briefly, paternal regulation is mediated by a factor(s) which is active only in the pollen, which shows dosage effect, and which affects endosperm development by interacting with the maternal genes in the hybrid seed. The various manifestations of paternal regulation include reduced or enlarged seed size when compared to selfed seed, and partial or complete seed shrivelling in reciprocal crosses.

We do not know if the same factor is active in the pollen of different species, or if the variation observed with respect to seed morphology and endosperm development is the result of novel interactions with different maternal genes. Alternatively, there could be a variety of factors that are active in the pollen of different species and also many kinds of maternal genes with which they interact. These preliminary results indicate that wheat hybrids offer a good model for differential gene expression and regulation studies in higher plants.

Many other reports on investigations of reciprocal differences in species crosses in diploid wheats (Dhaliwal 1977), peas (Davies 1973; Davies 1975; and Davies and Brewster 1975) and cowpeas *Vigna unguiculata* (L.) Walp. (Rawal et al. 1976) may also be explained by paternal regulation of seed development. Dhaliwal's (1977) hypothesis that genome unbalance in the endosperm may be the cause of reciprocal difference in diploid wheat hybrids is not supported by our experimental evidence. Rawal et al. (1976) reported reduction in seed size when cultivated cowpeas were crossed to two lines of wild cowpeas as males. Pollen factors, which affect hybrid seed size, may be involved in such crosses. Evidence for paternal regulation of seed development may also come from the work of Pfahler and Linskens (1977). Other examples where seed size is under maternal control would indicate lack of paternal regulation, for example, *boeoticum* (♀) × *squarrosa* (♂) and *speltoides* (♀) × *boeoticum* (♂) where F_1 seed resembled the selfed female parent seeds.

The reciprocal difference leading to seed death may be assumed as a step towards continuing reproductive isolation in evolving species. Sears (1944) reported one or two mutation differences which lead to inviability of hybrids between *T.monococcum* and *Ae.umbellulata* Zhuk. His results indicate, as our results also do, that one or a few factors can lead to total reproductive isolation in nature. Also, some *Triticum* and *Aegilops* chromosomes are sufficiently differentiated to form an additional barrier to chromosome pairing and gene exchange between species. Which of these barriers is more likely to be first established in natural populations is an open question. It would seem that at least in some species barriers to crossability arise first. In the *T.boeoticum* × *T.urartu* hybrids the anthers do not open, and in reciprocal hybrids the seeds are inviable, even though in both groups of hybrids the chromosomes pair and form seven ring bivalents (Johnson and Dhaliwal 1976).

The lethality of hybrid embryos *Ae.squarrosa* (♀) with *Triticum* species or *Ae.speltoides* as (♂) is most plausibly explained by cytoplasmic-nuclear interactions; as is well known in many other cases (Maan 1976). The reciprocal crosses involving these species produce normal plants. The unfavorable cytoplasmic-nuclear interactions in such hybrids can range from complete lethality (embryo dies during seed development) to semi-lethality (seedling dies) to male sterility of the mature hybrid plants. Are these varying manifestations due to the same basic phenomenon, or do they involve different cytoplasmic-genetic factors? The evidence for the former interpretation comes from the work of Maan (1976). These and other questions of hybrid breakdown between species and genera are of fundamental concern to geneticists and evolutionists who seek to understand the biochemical basis of isolating mechanisms and the perpetuation of these phenomena in populations. Wide hybridization is also of importance to plant breeders seeking exotic genes in crop improvement.

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