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Genetic Control of Seed Proteins in Wheat

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Summary. Electrophoretic profiles of crude protein extracts from seed of F_1 hybrids and reciprocal crosses among diploid, tetraploid and hexaploid wheats were compared with those of their respective parental species. The electrophoretic patterns within each of three pairs of reciprocal crosses, *T.boeoticum* × *T.urartu*, *T.monococcum* × *T.urartu* and *T.dicoccum* × *T.araraticum*, were different from one another but were identical with those of their respective maternal parents. Protein bands characteristic of the paternal parents were missing in F_1 hybrid seed suggesting that the major seed proteins in wheat were presumably regulated by genotype of the maternal parent rather than by the seed genotype. However, in another three pairs of reciprocal crosses, *T.boeoticum* × *T.durum*, *T.dicoccum* × *T.aestivum* and *T.zhukovskyi* × *T.aestivum*, protein bands attributable to the paternal parents were present in the F_1 hybrid seeds indicating that the seed proteins were not always exclusively regulated by the maternal genotype. The expression of paternal genomes is presumably determined by dosage and genetic affinity of the maternal and paternal genomes in the hybrid endosperm. The maternal regulation of seed protein content is probably accomplished through the maternal control over seed size. The seed protein quality may, however, depend upon the extent of expression of the paternal genome.

Key words: Seed proteins - Wheat hybrids - *Triticum* Crosses - Hybrid Endosperm - Maternal Regulation

Introduction

Seed protein content of F_1 hybrid seed in bread wheat (Singh and Nanda 1976), corn (Garwood and Lambert 1967) and soybean (Singh and Hadley 1972) is reported to be regulated by genotype of the maternal parent rather than by that of the hybrid endosperm or hybrid seed. Worzella (1934) reported that the seed quality of hybrid seed of wheat was essentially like that of the maternal variety. Bingham (1961), however, reported that the milling quality in wheat was determined by genotype of the endosperm rather than by that of the maternal parents. As expected in the case of maternal genotypic control of seed protein content and quality in wheat, F_2 seed within each of four pairs of reciprocal crosses did not differ from one another with respect to protein percentage, milling and baking quality (McNeal et al. 1968). Seed quality in wheat is principally determined by the storage proteins and other constituents of the endosperm which contains 75% of the total seed proteins. The endosperm is a triploid (3n) tissue comprising 2n chromosomes from the female (maternal) and n from the male (paternal) gamete.

The mechanism of control of seed protein content and seed quality of hybrid seed in wheat by the maternal genotype is not understood. This may be due to the failure of expression of the paternal genome in the hybrid endosperm. Alternatively, the maternal regulation of seed protein content and seed quality may be excercised indirectly through the regulation of seed size rather than by suppression of paternal genome. The seed protein content in wheat and other cereals is negatively correlated with seed size and often the hybrid seed resemble selfed seed with respect to their size and shape. In addition to these possibilities, the maternal control may also be attributed to cytoplasmic factors or extra dosages of the maternal genome (genes) in the hybrid endosperm. Information on the precise genetic control of seed proteins will be advantageous in handling segregating generations in the breeding programmes aimed at improvement of protein content and grain quality in cereals.

This paper deals with the results on protein electrophoretic patterns of six pairs of reciprocal F_1 hybrids among different wheat species with an endeavour to understand the genetic control of seed pro-

teins. The electrophoretic patterns of crude seed protein extracts were highly diagnostic for each of the species used. In the hybrid endosperms, the expression of the paternal genomes with respect to storage proteins was found to be variable. In some cases, the protein quality was regulated by the maternal genotype and not by the seed genotype. The maternal parent presumably regulated protein content of the hybrid seed either through its control over hybrid seed size or through its physiological support of the developing seed.

Materials and Methods

Six pairs of reciprocal crosses among diploid (*T.boeoticum*, *T.urartu* and *T.monococcum*), tetraploid (emmer and *timopheevii*) and hexaploid (*T.aestivum* and *T.zhukovskyi*) wheats were studied. The seed material used for making crosses, viz. *T.boeoticum* (G 1004, G 1916), *T.urartu* (G 1754, G 1834), *T. monococcum* (G 3371), emmer (*T.dicoccum* -G 497, *T.durum* cv. 'Produra'), *timopheevii* (*T.araraticum* - G 2507), *T.aestivum* (G 357) and *T.zhukovskyi* (G 986), was taken from the wheat collection maintained at University of California, Riverside. Reciprocal crosses, viz. *T.boeoticum* (G 1004) × *T. urartu* (G 1754), *T.monococcum* (G 3371) × *T.urartu* (G 1834), emmer (G 497) × *timopheevii* (G 2507), *T.boeoticum* (G 1916) × emmer ('Produra'), emmer (G 497) × *T.aestivum* (G 357) and *T.zhukovskyi* (G 986) × *T.aestivum* (G 357), were made in the green house in summer, 1975.

Proteins from ground seed of the parental lines, F_1 hybrids and their reciprocal crosses were extracted with 70% ethanol (1:3 w/v) for three hours of continuous shaking. The mixture was centrifuged at 10,000 × g for 30 min. The supernatant was dialysed against distilled water for 48 hrs at 4°C and the clear dialysate was freeze-dried. The protein extracts were electrophoresed on 14% polyacrylamide gels using β-alanine-acetic acid buffer (pH 4.3) following the method of Johnson et al. (1967). The gels were stained with amido black and destained electrophoretically.

Results and Discussion

The electrophoretic profiles of 70% ethanol seed protein extracts (Figs.1-3) can be arbitrarily divided into a slow moving series of bands (0 - 5.5 cm) commonly referred to as gliadins and a fast moving series of bands (5.0-10.7 cm) presumably comprising albumins. Discussion on the electrophoretic profiles will be restricted to the fast moving albumin bands as they are highly diagnostic for each of the wheat species used in this study. *Triticum boeoticum* (Figs. 1a, 2e) has none or occasionally one band at 9.0 cm

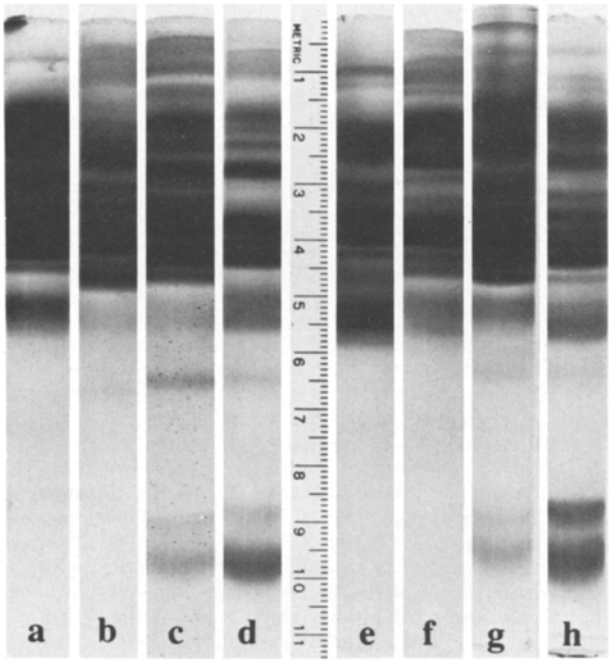

Fig.1. Electrophoretic patterns of crude seed proteins: a. *T.boeoticum* (G 1004), b. *T.boeoticum* (G 1004) × *T.urartu* (G 1754), c. *T.urartu* (G 1754) × *T.boeoticum* (G 1004), d. *T.urartu* (G 1754), e. *T.monococcum* (G 3371), f. *T.monococcum* (3371) × *T.urartu* (G 1834), g. *T.urartu* (1834) × *T.monococcum* (3371), h. *T.urartu* (G 1834)

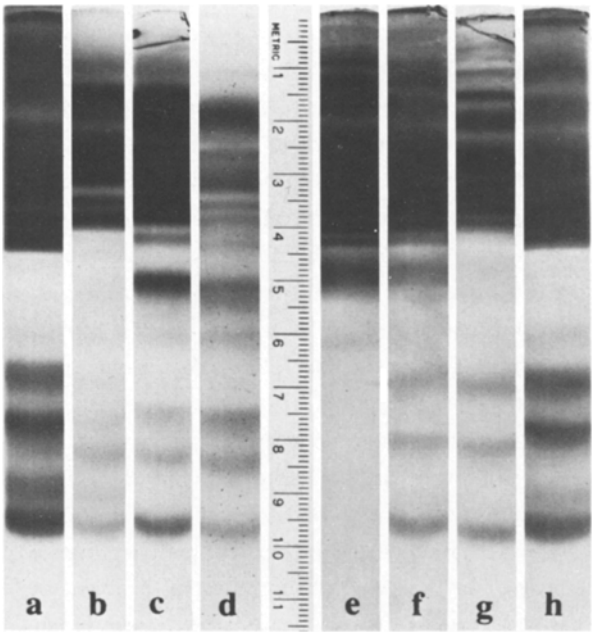

Fig.2. Electrophoretic patterns of crude seed proteins: a. *T.dicoccum* (G 497), b. *T.dicoccum* × *T.araraticum* (G 2507), c. *T.araraticum* (G 2507) × *T.dicoccum* (G 497), d. *T.araraticum* (G 2507), e. *T. boeoticum* (G 1916), f. *T.boeoticum* (G 1916) × *T.durum* cv. 'Produra', g. *T.durum* × *T.boeoticum* (G 1916), h. *T.durum* cv. 'Produra'

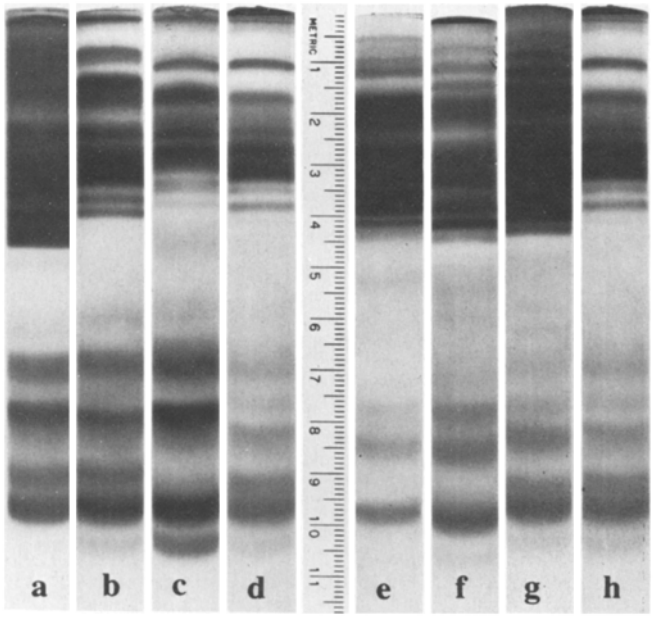

Fig.3. Electrophoretic patterns of crude seed proteins: a. *T.dicoccum* (G 497), b. *T.cicoccum* (G 497) × *T.eastivum* (G 357), c. *T.aestivum* (G 357) × *T.dicoccum* (G 497), d. *T.aestivum* (G 357), e. *T.zhukovskyi* (G 986), f. *T.zhukovskyi* (G 986) × *T.aestivum* (G 357), g. *T.aestivum* (G 357) × *T.zhukovskyi* (G 986), h. *T.aestivum* (G 357)

whereas *T.monococcum* (Fig.1e) possesses no band in the diagnostic part of the profile. *Triticum urartu* has three bands at 6.5, 8.5 and 9.7 cm (Figs.1d, 1h). Tetraploid emmer (Figs.2a, 3a) and *timopheevii* (Fig.2d) with 6-8 albumin bands differ from one another with respect to a band at 9.0 cm which is present in emmer but absent in *timopheevii*. *Triticum zhukovskyi* (Fig.3c) has a pattern essentially similar to that of the *timopheevii* tetraploids. *Triticum aestivum* (Figs.3d, 3h) has a conspicuous band at 10.3-10.7 cm contributed by *Aegilops squarrosa*, the D genome parent.

The protein electrophoretic patterns of the F_1 hybrid seed of three pairs of reciprocal crosses, viz. *T.boeoticum* × *T.urartu* (Figs.1a-d), *T.monococcum* × *T.urartu* (Figs.1e-h) and *T.dicoccum* × *T.araraticum* (Figs.2a-d), resembled those of their respective maternal parents with complete absence of the diagnostic bands from the paternal parents. The F_1 hybrid seed of crosses *T.boeoticum* (♀) × *T.urartu* (♂) (Fig.1b) and *T.monococcum* (♀) × *T.urartu* (♂) (Fig. 1f) did not possess any of the diagnostic *urartu* bands between 8.5-9.7 cm. A band of *T.urartu* at 6.5 cm is, however, expressed to some extent (Fig.1b). The possibility that the seeds might not be genuine hybrid seed is eliminated as the seed of crosses with *T.urartu* as the male parent were extremely reduced in size (Johnson and Dhaliwal 1976) as compared to the selfed seed of the maternal parents. The characteristic band of *T.dicoccum* at 9.0 cm (Fig.2a) was also not expressed in the reciprocal cross, *T.araraticum* (♀) × *T.dicoccum* (♂) (Fig.2c). The absence of characteristic paternal bands in the F_1 hybrid seed of each of three crosses suggests that the paternal genome was presumably suppressed in the hybrid seed. The findings of Singh and Nanda (1976) and Garwood and Lanbert (1967) that the protein content in wheat and maize, respectivley, was regulated by the maternal rather than by the hybrid seed genotype, may be explained on the basis of lack of expression of the paternal genome. The regulation of seed quality or protein quality by the maternal parent may also be due to a similar phenomenon. Recently, Yang, Sorenson and Scandalios (1977) have reported that the isozymes of malate dehydrogenase, peculiar to the paternal parents, were expressed in the hybrid endosperm suggesting that the paternal genome may not be entirely suppressed in the hybrid endosperm. Only the genes for storage proteins may be sensitive to such suppression.

Electrophoretic profiles of another three sets of reciprocal crosses, viz. *T.boeoticum* × emmer (Figs. 2e-h), emmer × *T.aestivum* (Figs.3a-d) and *T.zhukovskyi* × *T.aestivum* (Figs.3e-h), showed clearly the presence of characteristic bands of the paternal parents indicating that the paternal genome is not always completely suppressed in the hybrid endosperm. Diagnostic bands of emmer at 7, 8 and 9.7 cm (Fig. 2h) are expressed in the F_1 hybrid *T.boeoticum* (♀)× emmer (♂) (Fig.2f). The fast moving band of *T.aestivum* at 10.3 cm (Figs.3d, 3h) is expressed in the crosses emmer (♀)× *T.aestivum* (♂) (Fig.3b) and *T.zhukovskyi* (♀) × *T.aestivum* (♂) (Fig.3f). Results from the electrophoretic profiles suggest that the seed protein content, protein quality and grain quality may not always be regulated entirely by the maternal genotype. The contribution of the paternal genome appears to be, however, highly variable.

As stated earlier, endosperm in wheat and other cereals is a triploid tissue containing 2n chromo-

somes from the maternal and n from the paternal parent. If the maternal and paternal genomes contribute to the endosperm proteins proportionately, proteins of the paternal origin will have half the concentration compared to that of the maternal proteins in the protein extracts. Therefore, in electrophoretic profiles, proteins of the paternal origin may not be detected at the usual concentration of proteins per column. In the reciprocal crosses, where the characteristic paternal bands were absent, electrophoretic patterns at twice the normal amount of proteins per column did not show the characteristic paternal bands. This suggests that the failure to detect the paternal proteins in those crosses was not due to their low concentration in the endosperm but presumably due to suppression of the paternal genes. Furthermore, most of the seed proteins in soybean are concentrated in diploid cotyledons where, theoretically, the maternal and paternal genomes should contribute equally to the seed protein content. The presence of strong maternal effects on protein content in soybean (Singh and Hadley 1972) suggests that the paternal genome was also probably not expressed in soybean. Extra dosages of the maternal genome in the triploid endosperm, therefore, do not seem to be important for maternal genotypic control of seed proteins.

Maternal genotypic control of seed proteins content may also be visualized if either the seed proteins are synthesized in maternal tissues and subsequently translocated to the developing seeds or controlled by cytoplasmic organelles. It is, however, established in corn that zein, the major storage protein, is largely synthesized in the membrane-bound polysomes of the developing kernels (Larkins et al. 1976). Furthermore, reciprocal nuclear substitution lines of *T.boeoticum* and *T.urartu* (Dhaliwal 1977), in the cytoplasm of one another, gave protein patterns identical to that of the nuclear-donor species (H.S. Dhaliwal, unpublished) suggesting that the cytoplasms of the diploid wheats did not regulate their seed proteins. This may also be true for other wheat species.

Most of the previous conclusions about the maternal genotypic regulation of the seed proteins were based on comparisons of protein content in the reciprocal F_1 hybrid seeds with one another and with that of the selfed seed of the parental lines. Irrespective of the fact whether paternal genome was expressed or not, protein content of F_1 hybrid seed was approximately the same as that of the selfed seed (H.S. Dhaliwal, unpublished). Therefore, similar protein content of the F_1 hybrid seed and selfed seed of its maternal parent may not be taken as the evidence that the seed proteins are regulated by the maternal genotype and not by the seed genotype. Protein content may be regulated rather indirectly either through seed size or physiological support of the developing seeds by the maternal parent.

Expression of paternal genome with respect to protein synthesis appears to be sensitive to the genome dosages in the endosperm and genetic affinities between the maternal and paternal genomes. Tetraploid emmer *(AABB)* and *timopheevii (AABB)* wheats probably originated as an amphiploid between *T.boeoticum* and *T.urartu* (Dhaliwal and Johnson 1976; Dhaliwal 1976). A band at 9.7 cm in the tetraploid patterns (Figs. 2a, 2d) was presumably derived from *T.urartu*. That band of *T.urartu* did not express in crosses of *T.urartu* as the male parent, with *T.boeoticum* or *T.monococcum* (Figs. 1b, 1f), but it was expressed when tetraploid emmer (with the *urartu* genome) was used as the male parent in crosses with *T.boeoticum*. The endosperms in the two cases had different doses of the *boeoticum* and *urartu* genomes. Furthermore, the genes from closely related paternal genomes are not expressed while the ones from distantly related genomes are expressed. The D genome of *T.aestivum (AABBDD)* was derived from *Aegilops squarrosa*. A band at 10.3 cm in *T. aestivum*, attributable to the D genome, was expressed (Figs. 3b, 3f) when *T.eastivum* was used as the male parent in crosses with *T.dicoccum (AABB)* and *T.zhukovskyi (AAAABB)*. The protein bands of *T.urartu* are not expressed when it is used as the paternal parent in crosses with other closely related species *T.boeoticum* and *T.monococcum* (Figs. 1b, 1f). The expression of genes from a distantly related paternal genome may be due to its distinct structural and regulatory genes for protein synthesis.

The results reported here suggest that a breeder interested in improving protein content and quality of cereals and legumes must know the genetic control of protein content and the role of individual polypeptides

in amino-acid composition and seed quality in his set of breeding lines. Such information will be crucial in planning selection strategy in the segregation generations. If the seed protein content and protein quality are regulated by maternal and not by the seed genotype, seed borne on any heterozygous plant such as F_1 will not segregate for protein characters. Protein and amino-acid analyses of whole or part of individual F_2 seed, therefore, will be useless in this situation. Selection for such traits will have to be deferred until F_3 seed is obtained. The F_3 seed from a given F_2 plant, although genetically segregating, will be homogeneous with respect to protein traits. Quality tests requiring larger seed samples can be performed using F_3 seed lots. Based on F_3 seed analysis, only desirable F_2 progenies may be carried forward. The same procedure will have to be followed until the desirable characters are fixed.

With the electrophoretic system used in this study, only a small fraction of the genomes coding for the storage proteins could be analysed. Two-dimensional fractionation through combination of electrophoresis and iso-electric focussing may be useful to study the regulation of more polypeptides. Nutritionally important protein fractions, viz. albumins and globulins, may also be included in such studies. By the use of various combinations of diploid genomes of *Triticum* and *Aegilops* and available aneuploid series in *T. aestivum* it would be possible to understand thoroughly the regulation of seed proteins in the polyploid wheats.

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