Theor. Appl. Genet. 49 (1977) 283-286

Basis of Difference between Reciprocal Crosses Involving *Triticum boeoticum* and *T. urartu*

H.S. Dhaliwal
Department of Plant Sciences, University of California, Riverside, CA 92502

Summary. The *boeoticum* (♀) × *urartu* (♂) F_1 hybrids gave small, plump and viable seeds while the reciprocal crosses with *T. urartu* as the female parent had long, shrivelled and non-viable seeds. Reciprocal nuclear-substitution lines comprising the nucleus of one species into the cytoplasm of the other were developed through repeated backcrossing and were crossed as female parents with respective non-recurrent parents (the cytoplasm donors). The difference between the reciprocal crosses was presumably attributable to different *boeoticum:urartu* genomic ratios in the triploid endosperm rather than to the cytoplasmic difference between the diploid wheats. The endosperm with two doses of the *boeoticum* and one of the *urartu* genome resulted in small, plump and viable seed while the endosperm of the reciprocal crosses with two doses of the *urartu* and one of the *boeoticum* genome led to large but shrivelled and non-viable seeds irrespective of the cytoplasmic type. One dose of the paternal genome in the triploid endosperm is probably not expressed in the presence of two doses of the maternal genome thereby leading to the difference between the reciprocal crosses. The results reported here indicate that difference between reciprocal crosses may not always be attributed to cytoplasmic difference between the parental species.

Introduction

The difference between certain inter- and intra-specific reciprocal crosses is usually attributed to the cytoplasmic difference between the parents. Novel phenotypes, different from that of either parent, occasionally occur in certain crosses as a result of unfavourable nucleo-cytoplasmic relationships (Michaelis 1954). The F_1 hybrid seeds of reciprocal crosses involving *T. boeoticum* and *T. urartu* were different from one another and from those of either parent (Johnson and Dhaliwal 1976). The F_1 hybrid with *T. boeoticum* as the female parent gave extremely reduced but plump and viable seeds (Fig. 1c) while the reciprocal cross with *T. urartu* as the female had long, shrivelled and non-viable seeds (Fig. 1d). The difference between the phenotypes of the reciprocal crosses was tentatively attributed to the cytoplasmic difference between the diploid wheats (Johnson and Dhaliwal 1976). The reciprocal crosses, however, also differed with respect to genomic composition of their endosperms. Substitution of the *urartu* nucleus into the *boeoticum* cytoplasm gave male sterile plants, indicating that the *boeoticum* cytoplasm was different from that of *urartu* (Dhaliwal 1976). The basis of the difference between the reciprocal crosses, whether due to the cytoplasmic difference or different genomic composition of the endosperms, remains to be resolved. The cytoplasmic difference, if responsible for the reciprocal crosses difference, can be verified by crossing reciprocal nuclear substitution lines with the parental species.

This paper reports that the difference between the reciprocal crosses involving *T. boeoticum* and *T. urartu* was presumably due to their different endosperm constitutions rather than the cytoplasmic differences between the parental diploid wheats.

Material and Methods

Reciprocal crosses involving several lines of *T. boeoticum* and *T. urartu* (Fig. 1c, 1d) were made and the observations were recorded from the mature F_1 hybrid seeds as described by Johnson and Dhaliwal (1976). Substitution of the nucleus of one species into the cytoplasm of the other was carried out as reported by Dhaliwal (1976). The substitution of the *urartu* nucleus into the *boeoticum* cytoplasm was carried out up to the 5th backcross generation (Fig. 1e) while that of the *boeoticum* nucleus into the *urartu* cytoplasm up to the 2nd backcross (Fig. 1f). The reciprocal nuclear substitution lines were crossed as the female parents with several lines of each of *T. boeoticum* and *T. urartu* as the male parents. Observations on the morphology and germination were made from the

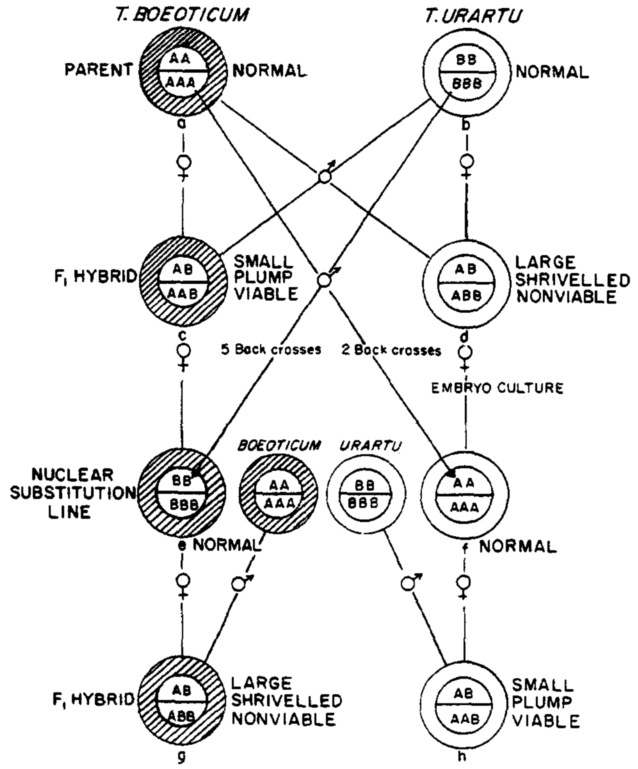

Fig. 1. Genomic constitution of nucleus or embryo (upper half of the inner circle) and endosperm (lower half of the inner circle) of *T. boeoticum* and *T. urartu* (top row), their reciprocal F_1 hybrids (2nd row), reciprocal nuclear substitution lines (3rd row) and F_1 hybrids involving the nuclear substitution lines and *T. boeoticum* and *T. urartu* (bottom row). The cytoplasm of *T. boeoticum* is represented by the shaded area between the outer and inner circle while that of *T. urartu* by the clear area between the circles

mature F_1 hybrid seeds (Fig. 1g, 1h). In Fig. 1, the genomes of *T. boeoticum* and *T. urartu* are represented by letters A and B, respectively. These genomic symbols, however, do not necessarily correspond to the A and B genome of the tetraploid wheats which are presumed to have evolved from *boeoticum-urartu* amphiploids (Johnson 1975; Dhaliwal and Johnson 1976). The identity of the parental genomes was lost due to modifications at the tetraploid level.

Results and Discussion

Morphological characteristics of the seed of *T. boeoticum*, *T. urartu*, their reciprocal F_1 hybrids, reciprocal nuclear substitution lines and their F_1 hybrids with *T. boeoticum* and *T. urartu* are summarized in Fig. 1. The genomic constitution of the nucleus/embryo (upper half of the inner circle) and endosperm (lower half of the inner circle) of the different types of seeds and their cytoplasm donor are also shown in Fig. 1. The cytoplasm of *T. boeoticum* is distinguished from that of *T. urartu* by the shaded area enclosed by the inner and outer circles (Fig. 1).

The difference between F_1 hybrid seeds from the reciprocal crosses involving *T. boeoticum* and *T. urartu* (Fig. 1c, 1d) may be attributed to the different genomic constitution of their endosperm or to the cytoplasmic difference between the parental species. Endosperm in wheat, like other monocots, is a triploid (3n) tissue comprising 2n complement from the maternal parent and n from the paternal parent. The endosperm of the F_1 hybrid seed with *T. boeoticum* as the female parent (Fig. 1c) had genomic constitution AAB with two doses of the *boeoticum* genome and one of that of the *urartu*. The endosperm of the reciprocal cross with *T. urartu* as the female had genomic constitution ABB comprised of two doses of the *urartu* and one of the *boeoticum* genome (Fig. 1d). Assuming two doses of one genome dominant over one dose of the other, the paternal genomes B and A, respectively, in the endosperms of the reciprocal crosses would not be expressed resulting in the difference between the reciprocal crosses. Electrophoretic profiles of the 70 % ethanol seed protein extracts of F_1 hybrid seed of the reciprocal crosses involving *T. boeoticum* and *T. urartu* resembled completely the maternal parent indicating that the paternal genome was indeed suppressed in the hybrids (Dhaliwal, unpublished). The hybrid endosperms with different genomic constitutions, although identical with the maternal endosperms with respect to their chemical composition, showed the specific interactions only with the hybrid embryos, as the selfed seed of both the parents were normal with the endosperms having all the three doses of the respective parental genomes (Fig. 1a, 1b). Two genomes in the hybrid embryos presumably required species specific metabolites which were not furnished by the hybrid endosperms due to suppression of the paternal genome.

Alternatively, the difference between the reciprocal crosses may be attributed to cytoplasmic differences between *T. boeoticum* and *T. urartu* (Fig. 1c, 1d). Different cytoplasms, presumably, interacted differently with the identical hybrid nuclei in the reciprocal crosses. Substitution of the *T. urartu* nucleus into the *boeoticum* cytoplasm (Fig.1e) resulted in male sterile plants indicating that the diploid wheats had different cytoplasms (Dhaliwal 1976). Characteristics purely under cytoplasmic control usually follow maternal inheritance and are conserved even if the nucleus of a particular species is substituted by that of another. In this situation, reciprocal nuclear substitution lines would be helpful to decide the basis of the difference between the reciprocal crosses.

The nuclear substitution lines on crossing with the parental as well as other lines of the nuclear-donor species gave essentially normal seed (Fig.1e, 1f) indicating that the cytoplasm of either species did not interact unfavorably with the intact nucleus from the other species. It may also be inferred from this that the cytoplasms also did not react unfavorably with the foreign genomes in the reciprocal F_1 hybrids.

The F_1 seeds from the crosses involving the substitution line comprising the *urartu* nucleus into the *boeoticum* cytoplasm as the female parent and *T. boeoticum* as the male were large, shrivelled and non-viable (Fig.1g), and were identical to these of the F_1 hybrid seed with *T. urartu* as the female (Fig.1d). Both F_1 hybrids (Fig.1d, 1g) had identical embryo and endosperm genomic constitutions, but differed with respect to cytoplasm. Similarly, the F_1 hybrid involving the substitution line consisting of the *boeoticum* nucleus in *urartu* cytoplasm with *T. urartu* gave small, plump and viable seeds (Fig.1h) like that of the *boeoticum* (♀) × *urartu* (♂) hybrid (Fig.1c) irrespective of the cytoplasmic difference between them. This clearly demonstrates that the *boeoticum* × *urartu* reciprocal crosses difference is due to different genomic constitution of the endosperms rather than to the cytoplasmic difference between the parents. The behavior of the reciprocal nuclear-substitution lines similar to that of the nuclear-donor species, however, may be attributed to two other phenomena.

(i) The cytoplasmic determinants responsible for the reciprocal crosses difference might have been transmitted through the pollen during the substitution of the *urartu* nucleus into the cytoplasm of *T. boeoticum* (Fig.1e) and vice-versa (Fig.1f). A low frequency of pollen transmission of certain cytoplasmic organelles such as plastids has been reported in *Oenothera* (Renner 1936) and *Epilobium* (Michaelis 1935). Even if it occurs at such a low rate in wheat, the abundant maternal cytoplasmic determinants still would be predominant after several generations of backcrossing with the nuclear-donor species unless the maternal determinants are selectively eliminated or suppressed.

(ii) The cytoplasmic determinants responsible for the difference between the reciprocal crosses might be under the nuclear control or selective influence of the nucleus. With the substitution of the nucleus of one species into the cytoplasm of the other, the cytoplasmic determinants are also modified in accordance with the nucleus. If one or both of these highly unlikely phenomena are prevelant in wheat, the cytoplasmic difference could still be held responsible for the reciprocal crosses difference.

Reciprocal crosses involving *T. urartu* and the tetraploid wheats *T. dicoccoides* or *T. araraticum* behave similarly to the *boeoticum* × *urartu* reciprocal crosses. Hybrids involving *T. urartu* as the female and the tetraploids as male had large, shrivelled and non-viable seeds while the reciprocal cross had small, plump and viable seed (Johnson and Dhaliwal 1976). However, the reciprocal crosses involving *T. boeoticum* and the tetraploids had almost normal seed in either direction. The nuclear substitution line consisting of the *urartu* nucleus in the *boeoticum* cytoplasm when crossed as female with two lines of each of the tetraploids gave large shrivelled and non-viable seed, further confirming the previous findings that the difference between the reciprocal crosses was not attributable to the cytoplasmic difference. Otherwise, the nuclear substitution line with the *boeoticum* cytoplasm on crossing with the tetraploids should have had normal seed like that of the *boeoticum* (♀) × tetraploid (♂) hybrid.

The tetraploid wheats (AABB) were presumably evolved from the *boeoticum*-*urartu* amphiploids (Johnson 1975; Dhaliwal and Johnson 1976). As mentioned previously, the F_1 hybrid seeds of the reciprocal crosses involving *T. boeoticum* and the tetraploids AA (♀) × AABB (♂) and AABB (♀) × AA (♂) were normal with the endosperm genomic constitution AAAB and AAABB, respectively. However, the reciprocal crosses involving *T. urartu* and the tetraploids BB(♀) × AABB (♂) and AABB (♀) × BB (♂) with the endosperm genomic constitution ABBB and AABBB, respectively, were different from each other as explained earlier. The information on the phenotypes and endospermic genomic constitution from the reciprocal crosses between *T. boeoticum* and *T. urartu* and between the diploid and tetraploid wheats suggests that no simple relationship exists between the endosperm *boeoticum: urartu* genomic ratios and the phenotypes of the hybrid seeds. The hybrid seeds were long, shrivelled and non-viable as long as the endosperm *boeoticum: urartu* genome ratio was less than or equal to 0.5, while the seeds were normal or at the most reduced in size when the ratio was greater than 0.5. The tetraploid wheats and *boeoticum-urartu* amphiploids with the endosperm (AAABBB) *boeoticum:urartu* genomic ratio 1 had perfectly normal seeds.

Acknowledgement

I am indebted to Dr. B.L. Johnson, University of California, Riverside for the plant materials and research facilities. This work was supported in part by a D.F. Jones post-doctoral fellowship to the author.

Literature

Dhaliwal, H.S.: Cytoplasmic relationship between *Triticum boeoticum* and *T. urartu*. Wheat Inf. Ser. 41-42, 1-2 (1976)

Dhaliwal, H.S.; Johnson, B.L.: Anther morphology and the origin of the tetraploid wheats. Amer. J. Bot. 63, 363-368 (1976)

Johnson, B.L.: Identification of the apparent B genome donor of wheat. Can. J. Genet. Cytol. 17, 21-39 (1975)

Johnson, B.L.; Dhaliwal, H.S.: Reproductive isolation of *Triticum boeoticum* and *Triticum urartu* and the origin of the tetraploid wheats. Amer. J. Bot. 63, 1088-1094 (1976)

Michaelis, P.: Entwicklungsgeschichtlich genetische Untersuchungen an *Eiplobium*. III. Zur Frage der Übertragung von Pollen Schlauchplasma in die Eizelle und ihre Bedeutung für die Plasmavererbung. Planta 23, 486-500 (1935)

Michaelis, P.: Cytoplasmic inheritance in *Epilobium* and its theoretical significance. Adv. Genet. 6, 287-401 (1954)

Renner, O.: Zur Kenntnis der nichtmendelnden Buntheit der Laubblätter. Flora (Jena) 30, 218-290 (1936)

Received October 13, 1976
Communicated by H.F. Linskens

Dr. Harcharan S. Dhaliwal
Friedrich-Miescher-Institut
P.O. Box 273
Ch-4002 Basel
Switzerland