ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 52, nr 2: 121-129
1983

Embryo and endosperm development in caryopses of hybrids from crosses between tetraploid wheats and their alloplasmic lines with rye

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(Received: July 29, 1982)

Abstract

Data concerning the embryo and endosperm development in twenty-day-old caryopses of hybrids obtained as the result of pollination with rye pollen of tetraploid wheats (*Triticum dicoccoides, T. dicoccum, T. durum* and *T. polonicum*), their alloplasmic lines with *T. timopheevi* plasma and alloplasmic *T. timopheevi* lines with cytoplasma of the above mentioned tetraploid wheats and hexaploid wheat (*T. macha*) were analysed. A high variability was noted between the tetraploid wheats as regards the degree of development of the embryo and of the endosperm in the hybrid caryopses and a decisive influence of the wheat genotype on these characters. The data for alloplasmic lines showed that the cytoplasm may have a modifying effect on the expression of these genotype characters.

INTRODUCTION

The specific inhibition reaction in remote crossing conditions both fertilisation and the further development of the embryo. Investigations on wheat-rye hybrids demonstrated that the ability of wheats to cross with rye is genetically conditioned (Lein 1943), and the observed variability depends on the distribution of the genes Kr_1 and Kr_2 determining it. These genes in dominant form greatly limit the number of hybrid seeds set (Riley and Chapman 1967, Krolow 1970). Investigations show, however, that the germination capacity of the seeds obtained is not related to the ability of seed setting by the given variety (Vettel 1961, Rigin 1964, Shulyndin and Naumova 1965, Krolow 1970, Łapiński et al. 1980). The reduced germination capacity is caused by disturbances in the development of the embryo and endosperm. These disturbances are one of the main factors limiting the extension of the *Triticale* genetic pool by way of synthesis of new amphiploids, what would allow a wider utilisation of the genetic variability

occurring within the wheat and the rye parent. Owing to disturbances in caryopse development, in some crossing combinations obtention of hybrid seeds capable of germination and development is not possible. Culture *in vitro* of immature hybrid embryos practiced with succes for some years gives positive results owing to the circumvention of the barriers connected with endosperm development. This method, does not, however, ensure positive results if the underdevelopment of the caryopse is due to major disturbances in embryo development.

Change of the wheat cytoplasm exerts a strong influence on the functioning of the genome, especially as regards characters involving the physiology of the alloplasmic plant. Among others Ogura and Tsunewaki (1974) demonstrated that change of the cytoplasm may raise the frequency of induction of the androgenesis process under *in vitro* conditions, whereas studies on wheat and rye amphiploids showed that the cytoplasm also conditions endosperm development (filling and mass of seeds) in wheat-rye hybrids (Jenkins 1974, Nalepa 1980).

In the present investigations it was attempted to establish the influence of cytoplasm on the development of hybrid caryopses obtained from crosses of tetraploid wheats with rye. For this purpose the development of the embryo and endosperm was followed in caryopses obtained after pollinating with rye pollen various tetraploid wheat varieties and their alloplasmic lines with *T. timopheevi* cytoplasm and alloplasmic lines of *T. timopheevi* with cytoplasm of various tetraploid wheats and the hexaploid wheat *T. macha*.

MATERIAL AND METHODS

Four tetraploid wheats: *T. dicoccoides, T. dicoccum, T. durum* and *T. polonicum* and their alloplasmic lines with *T. timopheevi* cytoplasm and the wheat *T. timopheevi* and its alloplasmic lines with the cytoplasm of the above named four tetraploid wheats and the wild hexaploid wheat *T. macha* (Table 1) were used for the experiments. The enumerated wheat forms were pollinated with diploid rye pollen (cv. Dańkowskie Złote) and 20 days after the moment of pollination the development of the embryo and endosperm in 100 hybrid caryopses was examined from each cross. The presence and degree of development of the endosperm and the presence and degree of development of the embryo were determined with the use of a preparation magnifying glass. Four development stages were distinguished on the basis of microscopic analysis. To the first developmental group were classified embryos of tear-like shape which had not yet reached the stage of differentiation of the scutellum. To the second group belonged embryos with scutellum primordia. At this stage usually initiation of functioning of shoot and root meristems was observed. The third group comprised embryos with

developed scutellum in which it was, however, difficult to distinguish macroscopically the polar separation of the shoot part from the root part. The fourth group consisted of fully developed embryos which did not show any macroscopically noticeable developmental defects. The results were elaborated by the chi-square test.

Table 1

Development of the endosperm of hybrid seeds obtained from crosses of tetraploid wheats and their alloplasmatic lines with diploid rye cv. Dańkowskie Złote (20 days after pollination)

Cytoplasm	Genom	Seeds no. with abundant endosperm	Seeds no. with poor endosperm	Seeds no. without endosperm
Dicoccoides	*dicoccoides*	0	0	100
Timopheevi	*dicoccoides*	0	3	97
Dicoccum	*dicoccum*	0	3	97
Timopheevi	*dicoccum*	0	0	100
Durum	*durum*	26	17	57
Timopheevi	*durum*	29	10	61
Polonicum	*polonicum*	30	27	43
Timopheevi	*polonicum*	45	25	30
Timopheevi	*timopheevi*	23	9	68
Dicoccoides	*timopheevi*	23	24	53
Dicoccum	*timopheevi*	25	23	52
Durum	*timopheevi*	26	15	59
Polonicum	*timopheevi*	24	22	54
Macha	*timopheevi*	30	28	42

For performing a fuller embryological analysis of anomalies in embryo development permanent slides were prepared with embryos derived from pollination with rye pollen of the wheats *T. durum* and *T. timopheevi* and their alloplasmic lines and the line *T. timopheevi* with cytoplasm of the hexaploid wheat *T. macha*. The embryos of randomly chosen ears from plants of each of the above mentioned cross combinations were fixed in FAA, embedded in paraffin and sections were cut 10 nm thick. The preparations were stained with crystal violet and G orange or iron haematoxylin and fast green.

RESULTS

DEGREE OF DEVELOPMENT OF ENDOSPERM AND EMBRYO IN HYBRID CARYOPSES

The results concerning endosperm development in the hybrid caryopses are shown in Table 1. The caryopses obtained by pollination with rye of various tetraploid wheats differed in the degree of endosperm development. *T. dicoccoides* and *T. dicoccum* did not produce endosperm

in the hybrid caryopses at all or only sporadically its slight traces could be noted after 20 days of development of the caryopse.

T. durum, T. polonicum and *T. timopheevi* behave differently, about 20 per cent of the caryopses form profuse though highly hydrated endosperm. Such caryopses 20 days after pollination were large and vividly green without any traces of drying. Transfer of genomes of *T. dicoccoides* and *T. dicoccum* as well as *T. durum* and *T. polonicum* to the cytoplasm of *T. timopheevi* did not cause any changes in the above mentioned differences, thus indicating that they are conditioned by genetic differences specific to the genomes of these species. Neither did transfer of the *T. timopheevi* genotype to the cytoplasm of the four different tetraploid wheats affect noticeably the possibility of endosperm formation in the hybrid caryopses after pollination with rye pollen, although in all the alloplasmic lines the number of caryopses completely deprived of endosperm was somewhat lower than when the autoplasmic line *T. timopheevi* was pollinated and these differences were statistically significant at the confidence level $p = 0.05$. The difference was, however, distinct in the case of hybrid caryopses of the alloplasmic line *T. timopheevi* with *T. macha* cytoplasm where the endosperm developed better as compared with that in the control line *T. timopheevi* with its own cytoplasm. The number of caryopses possessing, endosperm was higher by about 25 per cent, this being a statistically significant difference at the confidence level $p = 0.01$.

A similar picture of interspecific differences and of effects of substituting cytoplasm were obtained in studies on hybrid embryo development with the exception that *T. timopheevi* and its alloplasmic lines with cytoplasm of tetraploid wheats were similar as regards frequency of embryo formation to the wheats *T. dicoccoides* and *T. dicoccum* (Table 2). The latter wheats produced a large number of caryopses containing embryos (about 80%) and markedly differed in this respect from *T. durum* and *T. polonicum* in which the number of such caryopses was found in not much more than one half of the analysed ones. Investigation of the alloplasmic lines with cytoplasm of tetraploid wheats confirmed these differences, but they did not show any influence of foreign cytoplasm on the frequency of embryo formation in hybrid caryopses. Neither does the degree of development of embryos in the alloplasmic lines seem to differ from the situation in normal autoplasmic forms. An exception, like in the case of endosperm formation, was the alloplasmic line *T. timopheevi* with *T. macha* cytoplasm. The frequency of embryo formation was in this line much lower amounting barely to about 55 per cent of the frequency of embryo presence in the *T. timopheevi* line with own cytoplasm and in the alloplasmic lines *T. timopheevi* with cytoplasm of tetraploid wheats.

Table 2

Development of the embryo of hybrid seeds obtained from crosses of tetraploid wheats and their alloplasmatic lines with diploid rye cv. Dańkowskie Złote (20 days after pollination)

Cytoplasm	Genom	No. of studied seeds	Seeds no. without embryo	Seeds no. with embryo	Stage of the embryo development				
					I	II	III	IV	total
Dicoccoides	*dicoccoides*	100	15	85	19	12	2	3	36
Timopheevi	*dicoccoides*	100	24	76	12	9	10	11	42
Dicoccum	*diccocum*	100	33	67	21	15	0	0	36
Timopheevi	*dicoccum*	100	21	79	8	16	0	0	24
Durum	*durum**	100	41	59	13	8	0	4	25
Timopheevi	*durum**	100	44	56	5	1	0	9	15
Polonicum	*polonicum*	100	48	52	15	6	9	21	51
Timopheevi	*polonicum*	100	45	55	13	7	10	15	45
Timopheevi	*timopheevi**	100	23	77	9	7	9	11	36
Dicoccoides	*timopheevi*	100	29	71	11	3	5	6	25
Dicoccum	*timopheevi*	100	22	78	13	9	11	8	41
Durum	*timopheevi**	100	21	79	15	6	7	13	41
Polonicum	*timopheevi*	100	18	82	10	5	8	3	26
Macha	*timopheevi**	100	58	42	6	2	6	7	21

* — embryos were analized by paraffin method.

In view of the ability of both embryo and endosperm formation, the incrossability barrier with rye due to disturbances in the development of hybrid caryopses is least pronounced in the case of *T. timopheevi*. Since endosperm forms in a large proportion of seeds and the frequency of embryo formation is high, the possibillity of appearance of caryopses containing simultaneously endosperm and a developed embryo is here highest. Such caryopses were observed also in the course of preparation with a frequency of about 2 per cent and their germination capacity was confirmed by the obtention of *T. timopheevi* × *S. cereale* hybrids without the aid of embryo cultures, whereas this did not succeed in relation to any of the remaining tetraploid wheat species used. Although part of the *T. polonicum* and *T. durum* caryopses had watery endosperm, they did not as a rule contain an embryo. All well developed embryos arising from pollination of these species with rye pollen were found in caryopses deprived of endosperm.

SOMATIC DISTURBANCES IN THE DEVELOPMENT OF EMBRYOS OBTAINED FROM WHEAT-RYE CROSSES

Embryos isolated from caryopses after pollination of *T. durum* with rye pollen did not on the 20th day after pollination differ in their structure those characteristic for wheat, although most of them were not fully developed. The embryos most advanced in development consisted

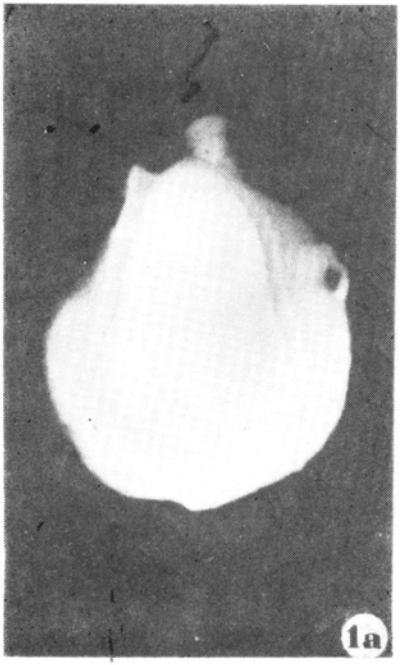
of the main axis, scutellum and epiblast. In the main axis the coleoptilar and coleorhizal poles could be distinguished, between them lay the mesocotyl (Fig. 1). The coleoptile covered the primordia of leaves I, II and III and the shoot apex. The primordium of the third leaf surrounded the apex collar-like. At the pole opposite to the coleoptile the coleorhiza surrounded the main embryonal root, the radicule. Two horizontally arranged primordia of side roots lay above the radicule. The mesocotyl — the scutellum node — was built of parenchymatous cells and conducting elements. The latter joined the vascular-sieve system of the scutellum. The scutellar conducting bundle passed on its whole length through regularly arranged layers of parenchyma cells (Fig. 1b).

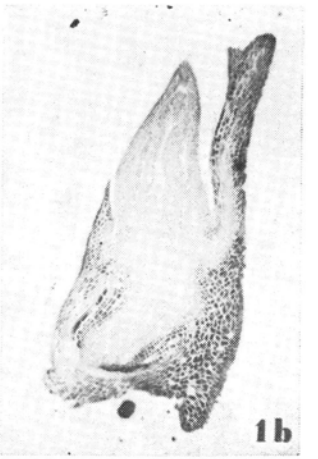
Embryos formed as the result of pollination of *T. timopheevi* with rye pollen were delayed in the development of primordia of the shoot elements. Only primordia of leaf I were found. The coleorhizal pole was more advanced in development. Of the three primordia of seed roots the two lateral ones were relatively well developed (Fig. 2a). The scutellum showed hyperplasia of parenchymatous tissue in its thickness and shape (Fig. 2).

Embryos derived from pollination with rye pollen of the alloplasmic line *T. timopheevi* with *T. durum* cytoplasm, although they were classified to developmental stage III and IV showed a high variability of size and shape (Fig. 3). The diverse shapes and excessive thickness of the scutellum were due to the unequal number of parenchymatous cell layers along its whole length. This organ formed the surrounding structures and in some cases even closing apical parts of the coleoptile and shoot primordia. Figure 3d shows the structure surrounding the primordia of the shoot elements in the shape as if of a cap. In this combination some embryos had almost undifferentiated primordia of leaf I, whereas others had well developed primordia of the successive three leaves. In all the embryos examined a well developed radicule was noted, while the development of the lateral seed roots was unnormal or they were absent.

A similar picture was seen when examining embryos from pollination of the second alloplasmic *T. timopheevi* line containing *T. macha* cytoplasm (Fig. 4). In embryos from this cross a retardation of radicule development was found as compared with the development of shoot primordia.

Embryos from caryopses of the alloplasmic *T. durum* line with *T. timopheevi* cytoplasm formed a polymorphous cell mass and only microscopic analysis allowed to distinguish primordia of shoot and root elements (Fig. 5). Disturbances were also observed in the symmetry of the embryo. As compared with the expansion of scutellar and coleorhizal cells the coleoptile was relatively poorly developed. The coleorhiza, however, exhibited hyperplasia in the direction of the base.





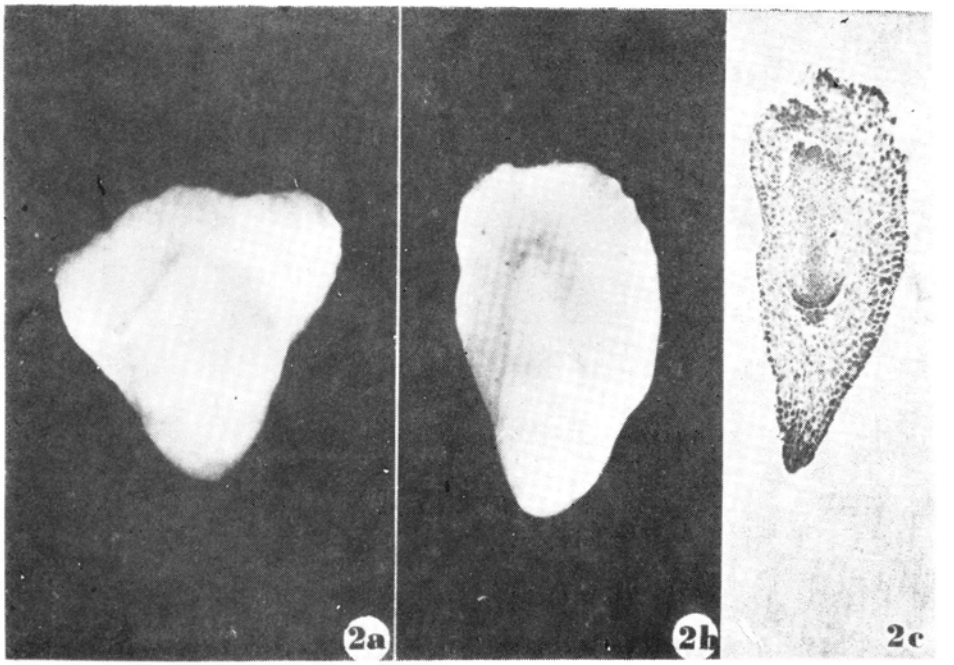


Fig. 1. Hybrid embryos from *Triticum durum* × *Secale cereale* (2n). a — embryo after 20 days of development, ×19; b — lateral longitudinal section, ×19

Fig. 2. Embryos of hybrid *Triticum timopheevi* × *Secale cereale* (2n).. a, b — 20-day-old embryos, × 19; c — frontal longitudinal section, × 22

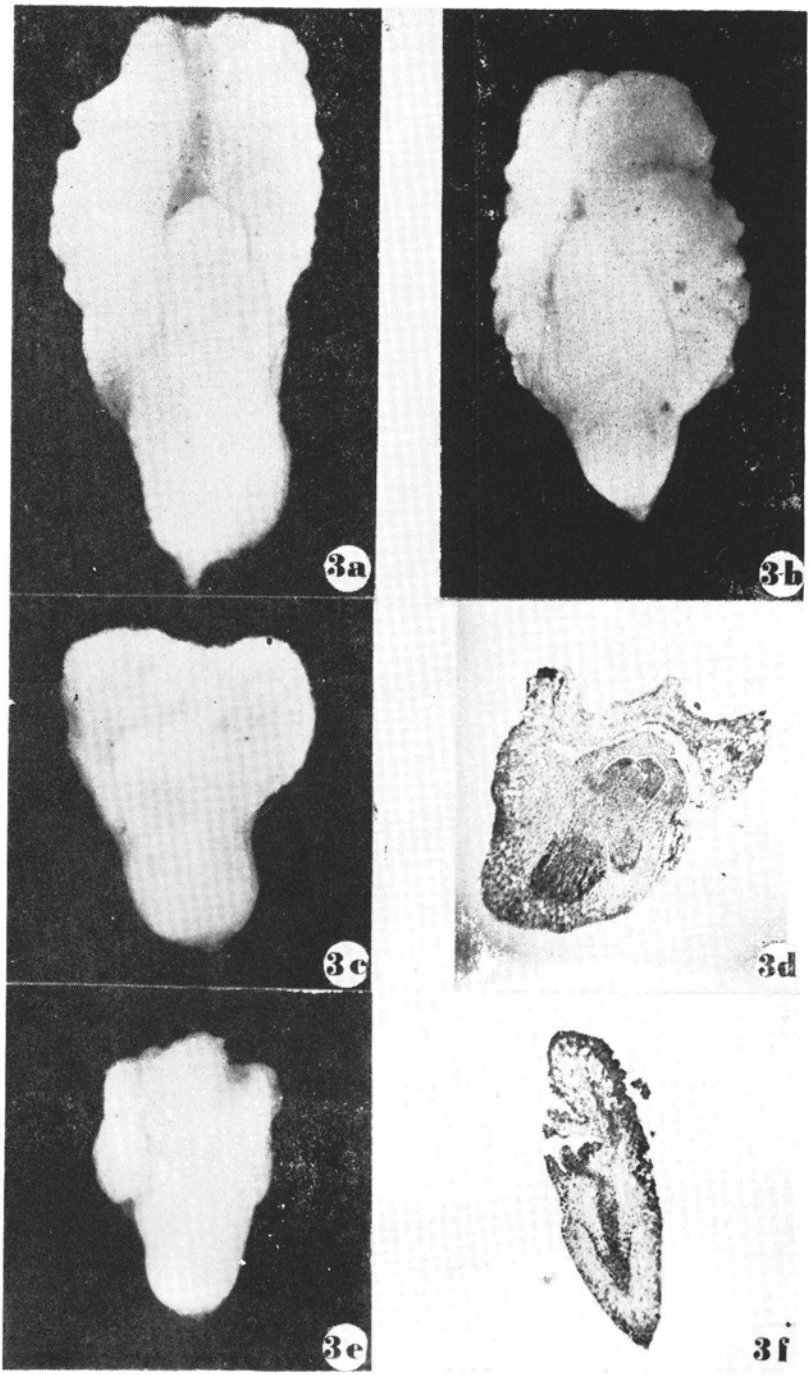


Fig. 3. Hybrid embryos after pollination with rye pollen of alloplasmic *Triticum timopheevi* line with cytoplasm of *T. durum*. a, b, c, e, — embryos 20 days after pollination, × 19; d — frontal longitudinal section, × 19; f — lateral longitudinal section, × 22

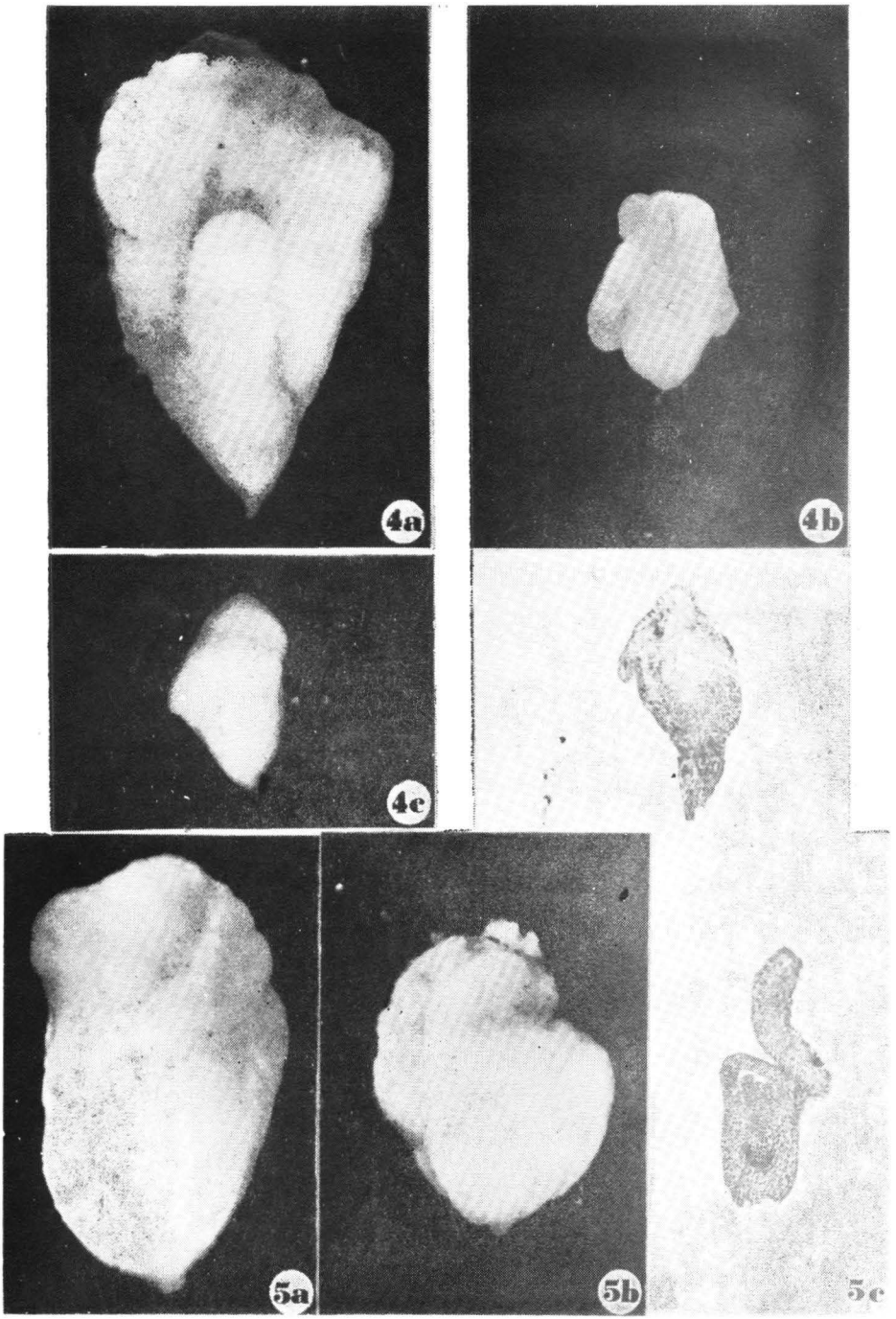


Fig. 4. Hybrid embryos after pollination with rye pollen of alloplasmic *Triticum timopheevi* line with cytoplasm of *T. macha*. a, b, c — embryos 20 days after pollination, × 19; d — lateral longitudinal section, × 22

Fig. 5. Hybrid embryos after pollination with rye pollen of alloplasmic *Triticum durum* line with cytoplasm of *T. timopheevi*. a, b, — 20-day-old embryos, × 19; c — frontal longitudinal section, × 19

Thus, embryos formed by pollination of *T. durum* exhibited a harmonious development of both parts of the embryonal axis. In embryos from the alloplasmic line of this variety with *T. timopheevi* cytoplasm retardation of growth of the coleoptilar part of the embryo was noted as was the case for the pure *T. timopheevi* line, whereas embryos from the *T. timopheevi* line with foreign cytoplasm, both *T. durum* and *T. macha* — showed a prevalent development of the coleoptilar part of the embryos.

Detailed investigations on the development of embryos seem, therefore, to indicate that a change of the cytoplasm of the maternal species may exert an influence on the synchronisation and normal development of the embryo.

DISCUSSION

The results obtained in crosses with rye of various cytoplasmic genotypic combinations of wheats point above all to the significant role of the wheat genotype for the ability of forming both hybrid embryos and endosperm development. Noteworthy is the variability among tetraploid wheats as regards the pertinent properties. *T. dicoccoides* and *T. dicoccum* produced caryopses deprived of endosperm most of which contained embryos inhibited in early stages of growth. A large part of caryopses of the remaining three wheat species contained abundant endosperm. From among these wheats embryos were most numerous in *T. timopheevi*, however, they were most advanced in development in the caryopses of *T. polonicum* and least advanced in *T. durum*.

Taira and Larter (1977) and Taira et al. (1978) demonstrated that the degree of development of the endosperm and embryo in crosses of tetraploid wheats with rye changes also when various forms of rye are used. Thomas and Anderson (1978) compared these data with their own obserwations about differences of endosperm development in *Triticale* lines different in rye parental form used and with other data indicating a cytological similarity between the shrivelling of opses from crosses of wheat with rye (Bennett 1977, Darvey 1973, Kaltsikes et al. 1975, Moss 1970). They believe that the degree of development of hybrid caryopses F_1 might be indicative (or even a basis for evaluation) of the influence of the particular genotypes on the development of caryopses of the wheat-rye alloploid obtained with the participation of the given rye genotype. If this assumption is correct, the properties of the wheat parent as regards hybrid seed development may be of practical importance for the formation of the alloploid.

Ogura and Tsunewaki (1974) in studies on induction of androgenesis in alloplasmic *T. aestivum* lines found that *T. timopheevi* and

Aegilops ovata cytoplasm enhance significantly the frequency of callus formation as compared with normal cytoplasm of the hexaploid wheat Chinese Spring. Jenkins (1974) noted that in hexaploid *Triticale T. timopheevi* cytoplasm has a favourable effect on fertility and caryopse development. Nalepa (1980) while studying dwarf *Triticale* demonstrated that introduction of *T. timopheevi* cytoplasm influences favourably the 1000-grain weight.

In investigations on hybrid caryopse development the alloplasmic lines of four tetraploid wheats with *T. timopheevi* cytoplasm showed no essential changes both as regards caryopse development and the possibility of endosperm formation. Neither did the alloplasmic lines of *T. timopheevi* with cytoplasm of the tetraploid wheats used differ in embryo formation significantly from the original form of *T. timopheevi*. Certain changes were observed only in synchronisation of the shoot and root parts development of the embryo. These lines, however, exbibited a certain significant tendency to formation of a smaller number of caryopses completely deprived of endosperm. The line of *T. timopheevi* with cytoplasm of the hexaploid wheat *T. macha* differed markedly from the remaining ones containing cytoplasm of tetraploid wheat and from the autoplasmic line of *T. timopheevi* by a large number of caryopses with more or less developed endosperm and by a lower frequency of presence of embryos, the differences in these respects were statistically significant.

It would seem, therefore, that the cytoplasm may have a modifying effect on these characters and that these differences between the cytoplasm of hexaploid and tetraploid wheats are wider than those within the latter.

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Rozwój zarodka i bielma w ziarniakach mieszańcowych pochodzących z krzyżowania pszenic tetraploidalnych i ich linii alloplazmatycznych z żytem

Streszczenie

Analizowano dane dotyczące rozwoju zarodka i bielma w dwudziestodniowych ziarniakach mieszańcowych otrzymanych w wyniku zapylenia pyłkiem żyta pszenic tetraploidalnych (*Triticum dicoccoides, T. dicoccum, T. durum, T. polonicum*) i ich linii alloplazmatycznych z cytoplazmą *T. timopheevi* oraz linii alloplazmatycznych *T. timopheevi* z cytoplazmami wymienionych pszenic tetraploidalnych i heksaploidalnej pszenicy *T. macha*. Stwierdzono znaczną zmienność wśród pszenic tetraploidalnych pod względem stopnia rozwoju zarodka i wykształcenia bielma w ziarniakach mieszańcowych oraz decydujący wpływ genotypu pszenicy na przejawianie tych cech. Analiza danych dotyczących linii alloplazmatycznych wykazała, że cytoplazma może w pewnym stopniu wpływać modyfikująco na ekspresję tych właściwości genotypu.