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CROSSES BETWEEN *BETA VULGARIS* L. AND *BETA LOMATOGONA* F. ET M.

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SUMMARY

A population of $2x^{ms}$ sugar beets was crossed with 4x *Beta lomatogona* F. et M. The 3x F_1-plants were male sterile and were backcrossed with 2x and 4x sugar beets and multiplied without pollination as well. After the 1st backcross mainly 3x apomict types arose again and, among others, a small number of successful 4x backcrosses. After pollination by 4x sugar beets this 4x F_1B_1 produced, besides predominantly apomictically multiplied 4x plants, also about 7% haploid 2x hybrids. The latter probably possess 1 genome from *B. vulgaris* and 1 genome from *B. lomatogona*. In the meiosis of the PMC's a certain amount of homeology between a number of chromosomes of both species could be established. The amphihaploid hybrids can be used as breeding parents for the creation of types in which introgession can occur. During hybridization in addition to 2x and 4x *B. vulgaris* types a number of 2x-, 3x-, 5x- and 6x-hybrids arose. This is presumably caused by the presence of gametes with the somatic number of chromosomes and the occurrence sometimes of haploid apomictic multiplication.

The presence of large numbers of bolters in the F_1 and F_1B_1 suggests that the bolting tendency of both species is based on different genes.

INTRODUCTION

The wild beet species of the section *Corollinae* have a few qualities that are desirable for our cultivated beets. KRASOCHKIN (1959) gives a survey of the species and their interrelation. For *B. lomatogona* he mentions the presence of monogermity, resistance to drought and bolters and tolerance to some viruses. Undesirable characteristics are a hard coat of the clusters, slow juwenile development, ramification and lignification of roots and susceptibility to powdery mildew (*Erysiphe betae* (VANHA WELTZIEN). DALKE et al. (1971) crossed 4x sugar beets with 4x *B. corolliflora* ZOSS. and, after backcrossing with 2x and 4x sugar beets, they obtained in the 2nd and 3rd backcross a number of plants with resistance to Beta mosaic virus 2 and tolerance of yellow virosis.

According to BAROCKA (1959, 1966) the propagation of the polyploid species of the section *Corollinae* is apomictic. In Turkey WALTHER (1963) discovered natural 5x hybrids between 4x *B. lomatogona* and 6x *B. trigyna* W. et K. These 5x hybrids multiplied apomictically too.

MARGARA (1953) and MARGARA & OMETZ (1955) also mention successful hybridizations between 4x sugar beet and 4x *B. lomatogona*. In 1968 the 7th generation was demonstrated, but to our mind this was the F_1 still propagating apomictically. FILUTOWICZ & DALKE (1972), too, obtained hybrids between 4x sugar beets and 4x *B. lomatogona*. The 4x F_1 multiplied mainly apomictically; in a few cases backcrossing with 4x *B. vulgaris* proved to be possible. OLDEMEIJER & BREWBAKER (1956) were successful in getting hybrids between sugar beets and *B. lomatogona*. These hybrids were male sterile (ms) and after backcrossing with sugar beets a progeny developed in which besides a number of intermidates the parental types, too, were segregating. According to JASSEM (1969, 1972) and JASSEN & JASSEM (1969, 1970) the hybrids with 4x *B. lomatogona* are facultative apomictic. It is a case of adventitious embryony. In the meiosis no pairing was found between the chromosomes and, like BOSEMARK (1969), they conclude that there is no homology between the chromosomes of both species. Presumably this will be an explanation for the segregation of the parental types after backcrossing the ms F_1.

CLEIJ et al. (1968) described crosses between *B. vulgaris* L. and *B. intermedia* BUNGE. The latter species is closely related to *B. lomatogona*. A common feature of these species is the nearly exclusively apomictic propagation. With *B. intermedia* hybrids we have succeded in getting good results where production and tolerance of virus are concerned.

B. lomatogona has participated in the development of the cultivated beets. Therefore, it was investigated whether this species can be used to broaden the narrow genetic base of the latter.

MATERIAL AND METHODS

The main source of our experiment was WB5 of *B. lomatogona*, which had been obtained from the USDA Beltsville (USA) in 1960. The seed sample had been marked as seed originating from free pollination of diploid plants. Multiplication produced only tetraploid plants, which in the first year formed a rosette with dark, green, long, narrow leaves. In the 2nd, and sometimes in the 3rd year, seed stalks were produced with almost exclusively monogerm clusters. The plants are perennial and flower every year. Seed is produced apomictically.

Another source was WB23, which had been obtained from the Max-Planck-Institute at Ladenburg (BRD) in 1960. Though this seed sample was said to be of diploid origin, the progeny in Wageningen appeared to be tetraploid. The plants resembled somewhat *B. coroliflora* and at flowering the perianth leaves were turned back. Besides monogerm clusters, there were bi- and trigerm clusters.

Both sources were propagated a few times on isolation plots. In 1969 they were used as pollen donors of a group of ms diploid sugar beet families (IB–$2x^{ms}$) of West-European origin. All pollinations and multiplications took place in isolation plots in tree nurseries. The IB-$2x^{ms}$ families produced only a small number of F_1 seeds with bad germination capacity. For these reasons the F_1 seed was sown in the glasshouse in 1970 and the obtained seedlings were transplanted in pots and later transferred to a cold frame.

In 1971 the triploid F_1 plants were divided in 3 groups and pollinated in isolation plots by a diploid (IN-$2x^{m}$) and a tetraploid monogerm sugar beet (IN-$4x^{m}$). In addition they were propagated without pollination. Seed production was bad. In

1972 clusters of 12 families and 123 individual plants were sown again in the glasshouse and the seedlings were pricked out. Early in May the plants were transplanted into the field. The ploidy level was determined for a great number of plants both in the greenhouse and the field.

According to their degree of ploidy reserved plants were again pollinated in isolation plots in 1973 by the monogerm sugar beet families IN-2x^m, IN-4x^m add IK-2x^m. Again the harvested clusters of every individual plant were sown first in the glasshouse before transferring the seedlings to the field. An exception formed the *B. vulgaris* types separated in 1972, which produced seed with good germination capacity. The progenies of these plants were tested in yield trials and proved to be sugar beets. All the plants were scored individually for type, while for a great number the degree of ploidy was established.

In 1975 again, reserved beets were set out in isolation plots according to their degree of ploidy and pollinated by the monogerm sugar beet families IN-2x^m, IN-4x^m, IK-2x^m, B53–4x^m and B53–2x^m. At the same time a number of 2x hybrids were pollinated by the biennial *B. maritima* L. WB40 from the Netherlands and WB42 from USSR.

The level of ploidy was determined on the first small leaves of the hearts of the plants. Meiosis in 4x *B. lomatogona* and the most important types of hybrids was examined. Using the technique of MARTIN (1959), we also observed the development of the pollen tubes in the styles after pollination with pollen of *B. vulgaris*.

RESULTS

The seeds obtained from IB-2x^{ms} in 1969 produced about 700 plants. Some plants were true sugar beets and probably arose by apomixis or by open pollination. The species-hybrids looked like 4x *B. lomatogona* and were all triploid. Like BOSEMARK (1969), we found 9 bivalents and 9 univalents in metaphase I of the PMC's. The plants were very susceptible to powdery mildew (*Erysiphe betae* VANHA WELTZIEN). Both the pollinations by 2x and 4x sugar beets and the propagation without pollination of the ms 3x F_1 hybrid produced ms 3x plants mainly through apomixis (Table 1). Moreover, a few 5x and 6x hybrids were found. Also a number of 2x and 4x *B. vulgaris* plants segregated, which behaved like normal sugar beets after further multiplication. From the crosses also 80 aberrant 4x plants arose, which looked a little like *B. vulgaris*. Tables 1, 2 and 3 present the likely genome constitutions where V = one *B. vulgaris*-genome and L = one *B. lomatogona*-genome. A remarkable thing was that the hybrids showed a strong tendency to bolt whereas the parents were bolting resistant. In the middle of July more than half the plants were rogued on account of the high number of bolters. At harvest time in October only the true *B. vulgaris*-types were still vegetative. The rest of the plants all demonstrated a more or less strong tendency to produce stalks.

The 80 tetraploid FIBI-plants all were male sterile. A cytological investigation of the PMC's showed a regular meiosis with 18 bivalents at metaphase. Anaphase and development of tetrads were also normal (Fig. 1).

Pollination by the monogerm 4x sugar beet IN-4x^m in 1973 produced mainly apomictic 4x hybrids (Table 2). Here too the material showed a strong tendency to bolt and before 1 August half of the plants had already been eliminated. Sometimes

Table 1. Results of crosses between $2x^{ms}$ sugar beet and 4x *Beta lomatogona* (S = seed, B = beet)

1969 : S		100 pl. IB-$2x^{ms}$ × 29 pl. 4x *Beta lomatogona* (LLLL) ↓ 557 F_1 plants		
1970 : B		190 pl. hybrids $3x^{ms}$ (VLL) ×	192 pl. hybrids $3x^{ms}$ (VLL) ×	175 pl. hybrids $3x^{ms}$ (VLL)
1971 : S		IN-$2x^{m}$ (VV)	IN-$4x^{m}$ (VVVV)	isolated increase
		Number of plants		
1972 : B	*B. vulgaris* type $2x^{mf}$ (VV)	14	23	15
	B. vulgaris type $4x^{ms\ mf}$ (VVVV)	7	2	3
	Hybrids $5x^{ms}$ (VVVLL)	1[2]	21[2]	0
	Hybrids $6x^{ms}$ (VVLLLL)	4[2]	3[2]	3[2]
	Hybrids $4x^{ms}$ (VVLL)	79[1]	1[1]	0
	Apomicts $3x^{ms}$ (VLL)	2316	1674	1413
	Total	2421	1724	1434

[1]See Table 2.
[2]See Table 3.
mf = male fertile; ms = male sterile; genome formalas assumed.

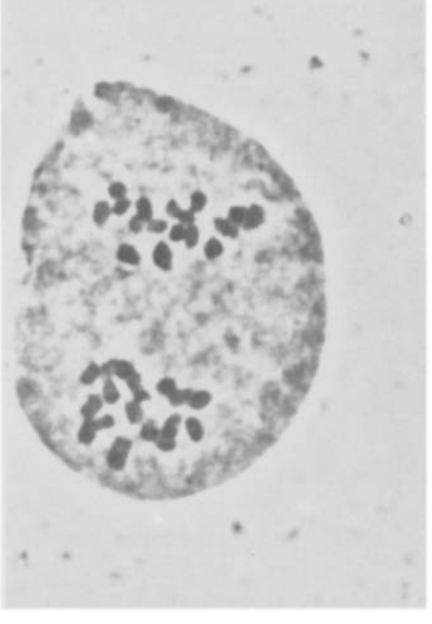

Fig. 1. Regular 18–18 distribution of the chromosomes in the 4x hybrid VVLL.

Fig. 2. Bivalent formation in metaphase I of the 2x hybrid VL.

Table 2. Pedigree of 4x hybrids from the backcross of $2x^{ms}$ sugar beet × 4x *Beta lomatogona* with 2x and 4x sugar beet.

1972 : B	80 plants $4x^{ms}$ hybrids (VVLL)	
	×	
1973 : S	IN-$4x^{m}$ (VVVV)	
1974 : B	6377 pl. $4x^{ms}$ apomicts (VVLL)	673 pl. $2x^{ms}$ haploids (VL)
	×	×
1975 : S	*B. corolliflora* (LLCC)	WB 40 (VV)
	IN-$4x^{m}$ (VVVV)	WB 42 (VV)
	B53–$4x^{m}$ (VVVV)	IN–$2x^{m}$ (VV)
		B53–$2x^{m}$ (VV)
		IK–$2x^{m}$ (VV)

complete progenies were rogued (Fig. 3). In 19 of the 72 progenies individuals were found which were somewhat smaller than the apomictic 4x plants and bolted somewhat later. They were behaving more like *B. vulgaris* (Fig. 4). Cytological investigation showed that all these (673) plants were 2x. The roots were highly branched and still appeared like *B. lomatogona* roots. These 2x plants were again male sterile in 1975. Besides univalents, cytological investigations also showed 2–6 bivalents at metaphase I (Fig. 2). After pollination by 2x sugar beets and *B. maritima* these amphihaploid plants produced mostly filled clusters. Pollination of two of these 2x ms plants with pollen of *B. procumbens* Chr. Sm gave a uniform apomictic 2x progeny. Sowing all the material will give a decisive answer in 1976 as to whether the rest of the crosses were successful or not.

Fig. 3. Bolting and vegetative 4x hybrids VVLL.

We had already observed in 1961 that 2x hybrids between sugar beet and *B. lomatogona* were often female sterile. This may explain the possible loss of the 2x hybrids in 1975. For that reason a number of apomictically propagating 4x hybrids had been reserved and were pollinated in 1975 by the two monogerm 4x sugar beet families IN-4x^m and B53-4x^m and an accession of 4x *B. corolliflora*. This time the 4x hybrids produced viable seed, and in 1976 it will become evident whether propagation occurred by way of apomixis, as a result of crossing or through haploidy. The 5x and 6x plants found in 1972 as well as a group of 3x apomictic hybrids were back-crossed with the monogerm sugar beet families IN-2x^m and IK-2x^m (Table 3).

The 22 5x^{ms} plants produced mainly again apomictic 5x^{ms} plants. These were all early bolting with the exception of one progeny of which all 11 plants remained vegetative. We further found 5 diploid, 2 triploid, 15 tetraploid and one hexaploid hybrids.

From the ten 6x^{ms} plants we obtained only 4 progenies with a total of 31 plants. These were all hybrids of the following ploidy level: 2x (4 plants), 3x (22 plants), 4x (2 plants) and 5x (3 plants).

After pollination in 1973 IK-2x^m, the 15 3x^{ms} hybrids produced mainly (184) 3x plants again. We also found three 4x^{ms} hybrids, which were pollinated again in 1975 by IN-4x^m. In one progeny we discovered three 2x *B. vulgaris*-types of which in 1975 two were male sterile and one male fertile.

Selfing of 4x *B. lomatogona* did not result in the growth of pollen tubes into the

Fig. 4. Small flat 2x hybrids VL between more erected 4x hybrids VVLL.

Table 3. Results of crosses of 5x and 6x hybrids and 3x apomicts from the cross $2x^{ms}$ sugar beet × 4x *Beta lomatogona* (see Table 1) and sugar beet.

1972 : B		22 pl. $5x^{ms}$ hybrids (VVVLL)	10 pl. $6x^{ms}$ hybrids (VVLLLL)	15 pl. $3x^{ms}$ apomicts (VLL)
		×	×	×
1973 : S		IN–$2x^{m}$ (VV)	IN–$2x^{m}$ (VV)	IK–$2x^{m}$ (VV)
		Number of plants		
1974 : B	*B. vulgaris* $2x^{ms\ mf}$ (VV)	0	0	3
	Hybrids $5x^{ms}$ (VVVLL)	0	3	0
	Apomicts $5x^{ms}$ (VVVLL)	562	0	0
	Hybrids $6x^{ms}$ (VVVVLL)	1	0	0
	Hybrids $4x^{ms}$ (VVLL)	15	2	3
	Hybrids $3x^{ms}$ (VVL)	2	0	0
	Haploids $3x^{ms}$ (VLL)	0	22	0
	Apomicts $3x^{ms}$ (VLL)	0	0	184
	Hybrids? $2x^{ms}$ (VL)	5	4	0

mf male fertile; ms – male sterile; genome formulas assumed.

style. Nor did pollen of 2x- and 4x- sugar beets germinate on the stigmata of *B. lomatogona* and on the $3x^{ms}$ hybrids with the constitution VLL. In the $4x^{ms}$ hybrids the pollen tubes of 4x sugar beets sometimes showed weak development.

DISCUSSION

The results reported in Table 1 show that crosses between IB-2x^{ms} and 4x *B. lomatogona* easily produce 3x hybrids. Just like 4x *B. lomatogona*, these 3x^{ms} hybrids propagate apomictically. Sexual reproduction is not yet completely excluded and backcrossing with 2x sugar beets produces about 3% (79 out of 2421) of the desired 4x F_1B_1 descendants. Like MARGARA & OMETZ (1955) we found 18 bivalents at metaphase I of meiosis in these 4x plants and at anaphase a normal 18–18 distribution of the chromosomes (Fig. 1). From this, we can conclude that the plants have an amphidiploid genome composition (VVLL) and that according to the expectation pairing of the chromosomes is autosyndetic. SAVITSKY (1969) found the same with the 4x F_1B_1 hybrids between 4x sugar beet and 4x *B. corolliflora*.

As a rule these 4x F_1B_1 hybrids propagate apomictically again after pollination by 4x *B. vulgaris* but about 7% of the descendants (673 out of 7050) appear to possess 18 chromosomes. It may be assumed that these plants arose parthenogenetically and can be considered as amphihaploids (VL).

The lack of development of pollen tubes after selfing of 4x *B. lomatogona* points to self-incompatibility of the wild species. The weak development of pollen tubes of 4x sugar beets on the stigmata of the 4x^{ms} F_1B_1 hybrids indicates a certain affinity between the plants.

The amphihaploid 2x descendants of the 4x F_1B_1 are male sterile. The occurrence of bivalents in the meiosis of these plants is indicative of a certain amount of homeology between the chromosomes of both species (Fig. 2). Perhaps this will offer a possibility of introgression as found by SAVITSKY (1975) with hybrids between sugar beets and *B. procumbens*. Pollination with 2x sugar beets will take care of the conservation of the amphihaploids. The identification of potential introgression products will not be easy because there is not a clear merker gene like in the *B. procumbens* hybrids e.g. nematode resistance. Close observation of the plants may help, as was the case with the *B. intermedia*-hybrids (CLEIJ et al., 1968).

Another difficulty can be also the strong tendency towards apomictic propagation in the hybrids. On the other hand apomixis may serve as consolidation of a favourable genotype.

During the crossings different degrees of ploidy appeared, which are based presumably on the presence of gametes with the somatic number of chromosomes. Also, the development of haploid 3x plants from 6x hybrids was already known in the *B. intermedia* hybrids (CLEIJ et al., 1968).

Sugar beet types arising from the 3x F_1 both with and without pollination by *B. vulgaris*, indicates that in these cytotypes little or no association will be present between the chromosomes of both species.

Contrary to the case of the crosses with *B. intermedia* (CLEIJ et al., 1968) only a few aneuploids were found in the *B. lomatogona*-hybrids and only in a few cases plants were found with 35 of 37 chromosomes.

Bolting tendency is governed presumably in both species by different genes considering the large numbers of bolters both in the F_1 and F_1B_1. Perhaps cryptomeres or complementary genes may play a part. However, it has been ultimately possible to obtain hybrids, both 2x and 4x, which remained vegetative in the 1st year.

REFERENCES

BAROCKA, K. H., 1959. Die 'einzelfrüchtigen' Arten der Gattung *Beta* L. im Hinblick auf ihre mögliche Verwendung zur Einkreuzung in *Beta vulgaris* L. subsp. *vulgaris* (Zucker- and Futterrübe). Züchter 29: 193–203.

BAROCKA, K. H., 1966. Die Sektion *Corolinae* der Gattung *Beta* (Tournef.) L. Z. Pflanzenzüchtung 56: 379–388.

BOSEMARK, N. O., 1969. Interspecific hybridization in *Beta* L.; prospects and value in sugar beet breeding. Journal I.I.R.B. 4: 112–122.

CLEIJ, G., T. S. M. DE BOCK & B. LEKKERKERKER, 1968. Crosses between *Beta intermedia* BUNGE and *B. vulgaris* L. Euphytica 17: 11–20.

DALKE, L., A. FILUTOWICZ & K. PAWELSKA-KOZINSKA, 1971. Studies on hybrids of tetraploid monogerm sugar beet with *Beta corolliflora* 2n - 36. Biuletyn I.H.A.R. 6: 3–7. (Polish).

FILUTOWICZ, A. & L. DALKE, 1972. Hybrids from crosses between sugar beet (*Beta vulgaris* L.) and *Beta lomatogona* F. et M. Hodowla roślin aklimatyzacja i nassiennictwo 16: 85–94 (Polish).

JASSEM, B., 1969. Apomixis in tetraploid hybrids between multigerm sugar beet and *Beta lomatogona* F. et M. Hodowla roślin aklimatyzacja i nassiennictwo 13: 245–255 (Polish).

JASSEM, B. & M. JASSEM, 1969. Embryology of tetraploid hybrids between monogerm sugar beet and *Beta lomatogona* F. et M. Hodowla roślin aklimatyzacja i nassiennictwo 13: 257–265 (Polish).

JASSEM, B. & M. JASSEM, 1970. Embryological investigations into the polyploid *Beta* species of the *Corollinae* section. Genetica Polonica 11: 367–377.

JASSEM, B., 1972. Embryological investigations on sugar beet x *Beta lomatogona* F. et M. hybrids. Part III. Hodowla roślin aklimatyzacja i nassiennictwo 16: 549–558 (Polish).

KRASOCHKIN, V. T., 1959. Obzor vodov roda *Beta*. Trudy prikl. Bot. Genet. Selek. 32 (3): 3–36 (Russian).

MARGARA, J., 1953. Sur l'obtention d'un hybride amphidiploide entre *B. vulgaris* et *B. lomatogona* F. et M. C. R. Acad. Sci (Paris) 237: 863–868.

MARGARA, J. & S. OMETZ, 1955. Les hybridations interspécifiques chez la betterave. Étude cytologique et morphologique d'un hybride entre betterave sucrière et *Beta lomatogona* F. et M. Ann. Amél. Plant. Serie B, 5: 445–462.

MARTIN, F. W., 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technology 34: 125–128.

OLDEMEIJER, R. K. & H. E. BREWBAKER, 1956. Interspecific hybrids in the genus *Beta*. J. Am. Soc. Sug. Beet Technol. 9: 15–18.

SAVITSKY, H., 1969. Meiosis in hybrids between *Beta vulgaris* and *Beta corolliflora* and transmission of resistance to curly top virus. Can. J. Genet. Cytol. 11: 514–521.

SAVITSKY, H., 1975. Hybridization between *Beta vulgaris* and *B. procumbens* and transmission of nematode (*Heterodera schachtii*) resistance to sugar beet. Can. J. Genet. Cytol. 17: 197–209.

WALTHER, F., 1963. Untersuchungen an Wildrüben in der Turkei; Cytologische Untersuchungen an Wildrüben im Gebiet von Eskisehir. Z. Pfl. Zücht. 49: (173–180).