Effects of *Solanum demissum* chromosomes on crossability in the backcross progeny to *Solanum tuberosum*

Kazuyoshi Hosaka・Rena Sanetomo

K. Hosaka (corresponding author)・Rena Sanetomo

Potato Germplasm Enhancement Laboratory, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

E-mail: spudman@obihiro.ac.jp

Tel: +81-155-49-5494

Fax: +81-155-49-5577

Abstract A balance of maternal and paternal genetic factors, conceptually named the endosperm balance number (EBN), is required for normal endosperm development in interspecific crosses in potato. We previously found that *Solanum demissum* (D), a hexaploid wild species widely used in potato breeding, has a slightly lower EBN than *S. tuberosum* (T). To explore the genetic nature of the EBN, the berry-setting rate, seed number/berry, and seed weight were evaluated in BC_1 [(D × T) × T] plants, each possessing different portions of the *S. demissum* chromosomes, by reciprocal crosses with D and T, and a quantitative trait locus (QTL) analysis was performed. At least 99 *S. demissum*-derived QTLs were detected, of which 29 were associated with differential responses to D and T. Three QTLs were possibly co-localized on chromosomes 7A and 10D1, while the remaining 23 QTLs were independently located. The QTLs in the three *S. demissum* homoeologous chromosomes exhibited three types of interaction: 1) positive, 2) negative, and 3) one positive and one negative effect on the same trait. We found that several major genes, one of which was localized in the *S. demissum* chromosome 9A, and many minor genes controlled the crossability of BC_1 plants. The QTLs responsible for the differential responses to D and T were different between the BC_1 plants used as male and female parents, indicating that different genes control the male and female EBNs. Consequently, we conclude that the EBN is represented by the sum of various genetic effects controlled by a large number of genes.

Keywords *Solanum demissum*, backcrossing, crossability, endosperm balance number (EBN), genome duplication, potato

Introduction

Sexual reproduction in flowering plants depends on the delivery of pollen to a receptive stigma; pollen tubes grow through the stigma and style to the ovary. The process is often hampered in interspecific crosses by various factors classified as premating (pre-zygotic) and postmating (zygotic) barriers (Dobzhansky et al. 1977). Premating barriers include spatial, temporal, and mechanical barriers, as well as gametic isolation by pistil/pollen interactions affecting pollen germination rates and pollen tube growth rates (Camadro and Peloquin 1981; Bedinger et al. 2011). Postmating barriers include seed abortion, hybrid inviability, hybrid sterility, and hybrid breakdown (Molye and Graham 2005; Moyle and Nakazato 2008; Burkart-Waco et al. 2012).

The tuber-bearing *Solanum* species, which consist of the common potato (*Solanum tuberosum* L., 2*n*=4*x*=48) and related species, are broadly distributed in the Americas from the southwestern United States to the Southern Cone of South America (Hawkes and Hjerting 1969; Hawkes 1990; Spooner et al. 2014). Hawkes (1990) identified seven cultivated and 226 wild tuber-bearing *Solanum* species. Since 1990, intensive field collections in regions throughout the range of distribution and morphological and molecular studies have halved the number of species (Spooner et al. 2014). Most diploid tuber-bearing *Solanum* species are outcrossers with very similar floral structures, closely related cytogenetically, and interfertile within wide limits (Howard and Swaminathan 1952; Matsubayashi 1991). A considerable number of species (approximately 25%, according to Hawkes 1990) are polyploids or contain multiple polyploid cytotypes (Spooner et al. 2014).

Of the known reproductive barriers in the tuber-bearing *Solanum* species, such as gametophytic self-incompatibility (Pushkarnath 1942; Pandey 1962), unilateral incompatibility and incongruity (Abdalla and Hermsen 1972; Hermsen and Sawicka 1979), male sterility (Buck 1960; Gopal 1994), and cytoplasmic-genetic male sterility (Grun et al. 1977; Hanneman and Peloquin 1981), the endosperm balance number (EBN, Johnston et al. 1980) is considered the prominent postmating barrier. It is hypothesized that the EBN controls endosperm development in interspecific potato crosses (Ehlenfeldt and Ortiz 1995). According to this hypothesis, a 2:1 balance of maternal to paternal EBN dosage in the endosperm, independently of ploidy, is required for normal endosperm development (Johnston et al. 1980). EBN values have been identified in various potato species, including 2*x* (1EBN), 2*x* (2EBN), 4*x*

(2EBN), 4*x* (4EBN) and 6*x* (4EBN), based on the ease of crossability between standard testers, as the pollen parents, and the species in question (Hanneman 1994). If two species have the same EBN value, crosses between the species are successful even with different ploidy. This concept assumes that the same EBN-controlling gene(s) is/are functioning in the male and female gametophytes after fertilization for endosperm development. Two or three independent, additive loci have been proposed for the EBN-controlling genes (Ehlenfeldt and Hanneman 1988; Camadro and Masuelli 1995). Similar or identical EBN genes are fixed at the same loci and have been conserved in South American and Mexican species (Bamberg and Hanneman 1990; Bamberg 1994). Nishiyama and Yabuno (1978) proposed a similar biological concept, the polar-nuclei activation (PNA) hypothesis, to explain the diverse interspecific crosses in the genus *Avena* (Katsiotis et al. 1995). The degree of polar nuclei activation is expressed by the "activation index" (AI), which is the ratio of the "activating value" (AV) of the male gamete to the "response value" (RV) of the female gamete. In a self-pollinated plant, AV=RV, and AI = (AV/2RV) × 100 = 50%, which is the most balanced condition and results in normal endosperm development. Depending on the AI of the polar nuclei, the kernel type has the following outcomes: AI<20% − small inviable kernels, 20%<AI<30% − small viable kernels, 30%<AI<80% − normal viable kernels, and 80%<AI − large shriveled-empty inviable kernels (Nishiyama and Yabuno 1978). This hypothesis assumes that a few genes quantitatively control the activating value and the response value, but the controlling genes may or may not be identical for the two values.

The EBN is useful for predicting the success or failure of a given interspecific cross in potatoes (Ortiz and Ehlenfeldt 1992; Hanneman 1999). If two species have different EBNs, chromosome-doubling of the lower EBN species should induce successful crosses (Johnston and Hanneman 1982). However, little is known regarding the genetic and molecular functions of the EBN. Polyploid species are all highly self-fertile and set abundant berries in nature, except for odd-polyploid species (Hawkes 1990). From an evolutionary perspective, an unanswered question is why do all these polyploid species have EBNs that are lower than the ploidy levels, for example, 4*x* (2EBN) and 6*x* (4EBN). Furthermore, it is not known how duplicated or homoeologous chromosomes function and express their EBNs in intra- and interspecific crosses.

Solanum demissum Lindl. is a wild Mexican hexaploid species (2*n*=6*x*=72) that is widely used

in potato breeding as a source of resistance to late blight (*Phytophthora infestans*) (Rudorf 1950; Ross 1986; Plaisted and Hoopes 1989), which is the most serious potato disease. Both the common potato (*S. tuberosum*) and *S. demissum* readily produced plump seeds when crossed with 4*x* (4EBN) testers and were assigned 4EBN (Johnston and Hanneman 1980; Hanneman 1994). Although *S. demissum* has an allohexaploid genome structure ($AADDD^dD^d$, Matsubayashi 1991), we can easily obtain pentaploid hybrids from *S. demissum* × *S. tuberosum* (AAA^tA^t, Matsubayashi 1991) when *S. demissum* is used as a female parent. The resultant pentaploid F_1 hybrids (AAA^tDD^d) produce abundant normal-looking pollen grains but are non-functional as males and usually produce seeds only if backcrossed with the pollen of *S. tuberosum* (Dionne 1961; Irikura 1968).

The EBNs assigned to potato are whole numbers because an interspecific cross either will or will not produce a hybrid seed. In contrast, according to the PNA hypothesis, activating values are usually integers because the hybrid seeds produced from an interspecific cross are classified into different categories. In our previous study (Sanetomo et al. 2011), we found that crosses of various accessions of *S. demissum* as female with a breeding clone Saikai 35 consistently produced abnormally large seeds (0.94 mg) than those produced by the reciprocal crosses (0.39 mg). We concluded that *S. tuberosum* has a slightly higher EBN than *S. demissum* and induces overproliferation of endosperm. Crossing behaviors in the reciprocal F_1 and BC_1 progenies, measured by berry-setting rates, seeds/berry and seed size, suggest that at least three genetic factors are involved in normal seed development: a cytoplasmic factor, 2) a nuclear genome-encoded factors function in female gametophyte and 3) a nuclear genome-encoded factors function in pollen. The latter two factors may be associated with the EBN. Therefore, these *S. demissum*-derived progenies are useful for exploring the relationship between the underlying mechanism of interspecific crossability and EBN (Sanetomo et al. 2011). In this context, we constructed a genetic map of *S. demissum* using 87 BC_1 plants generated from a pentaploid hybrid ($2n=5x=60$; *S. demissum* 5H109-5 × *S. tuberosum* breeding clone Saikai 35) crossed with the pollen of Saikai 35 (Ono et al. 2016). *S. demissum* is highly homozygous due to natural selfing. Thus, the F_1 hybrids with *S. tuberosum* possess the same genome constitution (AAA^tDD^d) with a relatively high homogeneity. The BC_1 plants, however, are all aneuploids ($2n=49-59$) with various degrees of *S. demissum* chromosomes or chromosomal segments from the *S. tuberosum* nuclear and cytoplasmic genome background.

In this study, the crossability of the BC_1 plants with *S. demissum* and *S. tuberosum* parents was evaluated, and a quantitative trait locus (QTL) analysis was performed, based on the association between *S. demissum*-specific markers and the crossability measurements. Because the BC_1 plants used had the same *S. demissum* cytoplasm, the observed differences in crossability are attributable solely to the *S. demissum* nuclear genome.

Materials and methods

Plant materials

The seeds of *S. demissum* (PI 186551) were obtained from the Potato Introduction Station (NRSP-6), Sturgeon Bay, Wisconsin, USA. One of the plants (5H109-5) was crossed as a female with the pollen of breeding clone Saikai 35 (Mori et al. 2012) of *S. tuberosum*, which generated the pentaploid hybrid 6H37-6 (2*n*=60, Sanetomo et al. 2011). This F_1 hybrid was backcrossed with the pollen of Saikai 35, generating a BC_1 population (7H2 family) consisting of 89 genotypes. This BC_1 population was previously used for a genetic map construction (Ono et al. 2016); in this study, it was used for a QTL analysis of crossability-related traits. These BC_1 plants were all aneuploids with somatic chromosome numbers ranging from 49 to 59 with an average of 53.5 (SD=2.06) (Ono 2008). Saikai 35, hereafter referred to as T, is a highly male and female fertile clone possessing a *S. phureja*-derived P type cytoplasm that avoids the cytoplasmic-genetic male sterility caused by *S. tuberosum*-specific T-type cytoplasm (Mori et al. 2012). Seedling plants from the selfed seeds of 5H109-5 are referred to collectively as D. Because *S. demissum* naturally sets abundant berries by selfing, the seedlings are considered genetically uniform and homozygous.

Crossing

The pentaploid F_1 (*S. demissum* × *S. tuberosum*) hybrids, including 6H37-6, were all self-infertile (Sanetomo et al. 2011). 6H37-6 was crossed reciprocally with D and T. Then, each of the BC_1 genotypes was crossed reciprocally with D (BC_1 × D and D × BC_1, in the order of female first) and T

(BC_1 × T and T × BC_1). The plants were grown in pots in an insect-free screenhouse under a 16-hr-day condition with supplementary lights. The flowers were emasculated one day before anthesis and immediately pollinated with fresh pollen. To minimize environmental effects each cross combination was completed with crosses performed on several different days. The berries were collected one month after pollination and matured for another month before the seeds were extracted. The matured berries were macerated and squeezed in a water-filled beaker to extract the seeds. Since the aborted seeds were collapsed and severely damaged, it was technically impossible to use the collection procedures described by Ehlenfeldt and Hanneman (1988). Therefore, only plump seeds were collected. The extracted seeds were dried first at room temperature for a week before being placed in a container with silica gels in a refrigerator for several weeks and then counted and weighed.

Measurement of traits

The berry-setting rate (%) was calculated by dividing the number of berries by the number of pollinated flowers. Only genotypes that were pollinated to at least ten flowers were used to calculate the berry-setting rate (%). The seed number/berry (no.) was calculated by dividing the total number of seeds by the number of berries obtained from the cross. The seed weight (mg) was the total weight of the seeds divided by the number of seeds. In self-crosses of Saikai 35 and *S. demissum*, the berry-setting rate was 68.1 and 92.8%, the seed number/berry was 219.5 and 166.8, and the seed weight was 0.49 and 0.41 mg, respectively (Sanetomo et al. 2011).

Genetic map

A genetic map of the *S. demissum* 5H109-5 genome was previously constructed using 87 BC_1 plants (Ono et al. 2016). Using the AFLP, SSR, STS and CAPS marker systems, 581 *S. demissum*-specific simplex markers (D-markers) were mapped to 539 map positions of 38 linkage groups, of which 37 were assigned to 36 potato chromosomes. Three homoeologous chromosomes were distinguishable, and the genomic origin (either the A or D/D^d genomes) could be estimated for 10 of the 12 potato chromosomes (Ono et al. 2016). This map was used in this study with the following modifications: 1) chromosomes

that could be from either the A or D/D^d genomes were designated A or D1/D2 (D1 or D2 was arbitrarily assigned), respectively; 2) since the genomic origin of chromosomes 6 and 11 was unknown, three homoeologous chromosomes were designated a, b, and c as in the work by Ono et al. (2016); 3) the previously designated 8a and 8d linkage groups were aligned to "a" and "b" in the 8D1 chromosome because the two linkage groups were hypothesized to be chromosomal segments of chromosome 8 (Ono et al. 2016); 4) the previous chromosome maps of 8a and 12a were represented upside-down and designated chromosomes 8D1a and 12D1, respectively; and 5) the unlinked linkage group previously designated chromosome 13 was unchanged.

QTL analysis

The QTL analyses were carried out using MapQTL 6 (Van Ooijen 2009). The nonparametric Kruskal-Wallis test was used to analyze single markers in the trait measurements. An interval mapping method with a maximum likelihood mixture model was also used. For the interval mapping, the significance threshold (P=0.05) of the LOD scores was obtained by performing permutation tests with 10,000 iterations of each linkage group.

Results

F_1 hybrid crosses

In the crosses of the F_1 hybrid 6H37-6 as a female and the bulked pollen of *S. demissum* PI 186551 (F_1 × D), 49 pollinations produced 16 berries, containing 1,172 seeds (32.7% berry-setting rate, 73.3 seeds/berry, and a seed weight of 0.58 mg). When the F_1 hybrid was crossed with the pollen of Saikai 35 (F_1 × T), 52 pollinations produced nine berries containing a total of 288 seeds (17.3% berry-setting rate, 32.0 seeds/berry). These seeds were unusually large, with a weight of 0.94 mg. In the cross D × F_1, 50 pollinations produced six berries containing a total of 121 seeds (12.0% berry-setting rate, 20.2 seeds/berry, and a seed weight of 0.49 mg). In the cross T × F_1, 38 pollinations were all unsuccessful.

BC_1 progeny crosses

In total, 1,297, 1,410, 1,619, and 1,452 flowers were pollinated in the crosses BC_1 × D, BC_1 × T, D × BC_1, and T × BC_1, respectively (all crossing data are available in Online Resources 1−4). Eighty-nine BC_1 genotypes were grown for the crossing experiments, but one genotype that never flowered was discarded and not used for further analysis. Furthermore, some genotypes rarely flowered and could not be pollinated to at least 10 flowers. Thus, the number of genotypes obtained for analysis of the berry-setting rates varied from 72 to 82, depending on the cross combinations (Fig. 1). The seed weight (mg) and seeds/berry (no.) measurements were obtained only from the genotypes that set berries (Figs. 2, 3).

The berry-setting rate in the crosses of the BC_1 genotypes as a female showed a large variation, ranging from 0 to 93.8% in BC_1 × D and from 0 to 100% in BC_1 × T. Both crosses exhibited bi-modal distributions or, if the 0−10% peak was considered, tri-modal distributions (Fig. 1). In 67 BC_1 plants, the Pearson's pairwise correlation coefficient between the BC_1 × D and BC_1 × T crosses was calculated for the berry-setting rates (Table 1), which indicated a significant positive correlation (r=0.682, P<0.0001). The mean berry-setting rate in BC_1 × D was 33.2%, which was almost similar to the 32.7% in the cross F_1 × D. The mean berry-setting rate in the cross BC_1 × T was 40.7%, which was higher than that in F_1 × T (17.3%). When the BC_1 genotypes were used as a male, 49 of the 82 D × BC_1 and 67 of the 74 T × BC_1 crosses failed (0% berry-setting rate) (Fig. 1). The berry-setting rates of 33 successful D × BC_1 crosses ranged from 5.0 to 81.8% with an average of 27.4%, while those of the seven successful T × BC_1 crosses ranged from 4.8 to 20.0% with an average of 11.9%. Including the failed crosses, the mean berry-setting rates were almost as those in the F_1 parent (Fig. 1). There was also a positive correlation between the D × BC_1 and T × BC_1 crosses (r=0.354, P=0.0025, Table 1). These results may indicate that certain identical major genes responded to D and T in the BC_1 females with bi- or tri-modal distributions, and a few identical gene(s) responded to D and T in the pollen of BC_1 plants, leading to successful crosses. Although BC_1 × D was not correlated with D × BC_1, BC_1 × T was significantly correlated with T × BC_1 (r=0.366, P=0.0025), which may indicate that the same genes responsible for the berry-setting rate functioned in the male and female gametophytes.

Notably, since one of the successful T × BC_1 crosses produced only one berry, which was seedless,

the number of seeds/berry was obtained from seven genotypes (Fig. 3), whereas the seed weight was obtained from six of the seven genotypes (Fig. 2).

The seed weight in crosses of BC_1 × D and BC_1 × T were normally distributed (Fig. 2). The mean seed weight of BC_1 × D (0.44 mg) was significantly lower than that of BC_1 × T (0.77 mg) by *t*-test ($P<0.0001$), and the respective means were lower than the seed weight obtained using the F_1 parent (Fig. 2). The seed weight in crosses of BC_1 × D was significantly correlated with BC_1 × T ($r=0.607$, $P<0.0001$, Table 1). These results confirmed that the D pollen and T pollen have different EBN values, to which each BC_1 plant responded in the same manner. The seed weight in the D × BC_1 crosses exhibited a bi-modal distribution (Fig. 2) and was not correlated with the BC_1 × D cross (Table 1). Apparently, different gene(s) were involved in the seed weight distribution in the male and female gametophytes. The D × BC_1 crosses produced heavier seeds (0.63 mg) than the BC_1 × D crosses (0.44 mg), while the T × BC_1 crosses produced lighter seeds (0.59 mg) than the BC_1 × T crosses (0.77 mg). The mean seed weight in the D × BC_1 crosses varied little from that in the T × BC_1 crosses, and the two measurements were not correlated (n=4) partly because the sample size for T × BC_1 was too small to detect a statistical significance.

The seed number/berry in crosses of BC_1 × D and BC_1 × T showed wide and nearly normal distributions (Fig. 3), and the former crosses produced significantly more seeds (83.6) than the latter crosses (55.9) by *t*-test ($P<0.0001$). The mean seed numbers in the BC_1 crosses were higher than those in the F_1 crosses (Fig. 3). The seed number/berry in the BC_1 × D crosses were positively correlated with that in the BC_1 × T crosses ($r=0.626$, $P<0.0001$, Table 1). When the BC_1 genotypes were crossed as males, the mean seed number/berry in the D × BC_1 crosses was slightly lower, but not significantly ($P=0.1905$), than that in the T × BC_1 crosses, and the two measurements were not correlated (n=5) partly because the sample size for T × BC_1 was too small to detect a statistical significance. The D × BC_1 and T × BC_1 crosses produced lower mean seed numbers/berry than the BC_1 × D and BC_1 × T crosses.

The somatic chromosome numbers in the BC_1 plants were not correlated with any traits, except for those in the cross D × BC_1, where the correlations were negative with the berry-setting rate ($r=-0.263$, $P<0.05$) and seed number/berry ($r=-0.440$, $P<0.05$) and positive with the seed weight ($r=0.475$, $P<0.01$).

Differential response of the BC_1 genotypes to D and T

Some BC_1 genotypes tended to produce large seeds, while others tended to produce small seeds regardless of the crossing with D or T. However, the BC_1 genotypes often showed drastic differences between crosses with D and T. For instance, of the 67 BC_1 genotypes crossed with both D and T pollen, seven did not set berries with either one, 20 set berries at higher rates with D pollen, and 40 set berries at higher rates with T pollen. To evaluate the degree of this "differential response" to D and T in each BC_1 plant, the trait F(D−T), indicating the differential response of the BC_1 plant when used as a female, was calculated by $(BC_1 \times D)-(BC_1 \times T)$, while the trait M(D−T), indicating the differential response of the BC_1 plant used as a male, was calculated by $(D \times BC_1)-(T \times BC_1)$. F(D−T) and M(D−T) were considered measurements of the effect of the different EBN values in this study.

For the berry-setting rates, F(D−T) showed much larger variability than M(D−T) (Fig. 1), and neither correlated with the other (r=−0.0588, P=0.6473, n=63), indicating that F(D−T) and M(D−T) were independent characteristics. Regarding the mean seed weight, F(D−T) exhibited a normal distribution with all negative values because the seed weight in the $BC_1 \times D$ crosses was smaller than that in the $BC_1 \times T$ crosses in each of the genotypes (Fig. 2). The differential responses of the BC_1 females in the seed number/berry also exhibited a normal and wide distribution with an average of 28.1 (Fig. 3).

Since the $T \times BC_1$ crosses were successful in a small number of genotypes as described above, the differential responses of the BC_1 males in the seed weight and seed number/berry were observed in only four and five genotypes, respectively (Figs. 2 and 3). Therefore, these genotypes were not included in the following QTL analysis.

QTLs detected by a single-marker analysis and their chromosomal distribution

As depicted in Figs. 1−3, nine traits obtained from the BC_1 females [$BC_1 \times D$ (%), $BC_1 \times T$ (%), F(D−T) (%), $BC_1 \times D$ (mg), $BC_1 \times T$ (mg), F(D−T) (mg), $BC_1 \times D$ (no.), $BC_1 \times T$ (no.), and F(D−T) (no.)] and five traits obtained from the BC_1 males [$D \times BC_1$ (%), $T \times BC_1$ (%), M(D−T) (%), D×BC1 (mg), and $D \times BC_1$ (no.)] were tested. The association between the trait measurements and the *S. demissum*-specific markers (D-markers) was investigated in the 37 linkage groups covering all 36 *S. demissum* chromosomes and one unassigned linkage group. First, we conducted interval mapping using MapQTL, which

disclosed 41 QTLs (Online Resource 5). However, if the locus order is obscure, as occurred with the present map, trait-associated loci are sometimes falsely interrupted, and the interval mapping is unable to detect the QTLs; thus, a single-marker analysis was conducted by a Kruskal-Wallis test using MapQTL. The results are displayed in a "trait evaluation array" for each map position in Fig. 4. Loci showing significantly "higher" or "lower" mean values between the presence and absence of D-markers are colored in the trait evaluation array in red or blue, respectively, with different densities depending on the significance levels (Fig. 4). Only loci with significant effect(s) are shown in the trait evaluation array. For example, in chromosome 12A, the significant loci for BC_1×D (%) were interrupted by non-significant loci, indicating the presence of two separate QTLs, but more likely, one QTL was interrupted by incorrectly ordered loci. Thus, QTLs could only show approximate locations in this study.

A single-marker analysis using the Kruskal-Wallis test disclosed 172 QTLs, which included all 41 QTLs discovered by the interval mapping (Fig. 4). Of the 172 QTLs, 99 were supported by either multiple markers with $P<0.05$ or single markers with $P<0.005$. These 99 QTLs were analyzed in further detail (Table 2). Two QTLs were found for the same trait with opposite effects (increase and decrease) in chromosomes 1D2 and 2D1 (Table 2, Fig. 4). All other QTLs were single QTLs per chromosome for respective traits. The largest number of QTLs was found in chromosomes 2D1, 7A, 9A and 10D1 (seven QTLs in each), followed by chromosome 5D1 (six QTLs). Chromosomes 2D2 and 9D2 were not associated with any traits (Fig. 4). Consequently, there was no preferential distribution of the QTLs among the three homoeologous chromosomes.

Ten QTLs were associated with BC_1×D (%) and seven were associated with BC_1×T (%) (Table 2), of which the QTLs in chromosomes 5D1 and 6c were co-localized and associated with reduced berry-setting rates (Fig. 4). For BC_1×D (mg) and BC_1×T (mg), six and seven QTLs, respectively, were found, of which one in chromosome 7A was co-localized and associated with increased seed weights (Fig. 4). For BC_1×D (no.) and BC_1×T (no.), six and nine QTLs, respectively, were found, of which one in chromosome 6b was co-localized and associated with the reduced seed numbers (Fig. 4). For D×BC_1 (%) and T×BC_1 (%), seven and six QTLs, respectively, were found, of which one in chromosome 9A was co-localized and associated with the increased berry-setting rates (Fig. 4).

The loci in chromosome 9A had a significant effect on the berry-setting rates (Fig. 4). In all cross combinations of T×BC_1 (%), D×BC_1 (%), BC_1×T (%), and BC_1×D (%), the presence of D-markers was

associated with increased berry-setting rates. In particular, in the BC_1×T crosses, which are an ordinary backcrosses of *S. demissum* used in potato breeding, 28.9% of the variance was explained by this QTL (Online Resource 5). Furthermore, D-markers in chromosome 9A were also associated with the reduced seed weight in BC_1×T (mg) and the increased seed weight in D×BC_1 (mg) (Table 2, Fig. 4). However, as shown in Fig. 4, the QTL associated with the berry-setting rate was likely not co-localized in chromosome 9A with the QTL associated with the seed weight, and their locations were slightly below STI57 and the upper terminal end, respectively.

QTLs for the "differential response"

Regarding the differential responses of the BC_1 plants as females, nine QTLs for F(D−T) (%), five QTLs for F(D−T) (mg), and eight QTLs for F(D−T) (no.) were detected (Table 2). Among them, two QTLs in chromosome 1D2 showed opposite effects (increase and decrease) on F(D−T) (no.) and two QTLs in chromosome 2D1 showed opposite effects on F(D−T) (%) (Table 2, Fig. 4). When the BC_1 plants were crossed as males, the differential response was tested only for the berry-setting rate. Seven QTLs were unambiguously associated with M(D−T) (%) (Table 2, Fig. 4). In total, 29 of the 99 crossability-related QTLs were associated with differential responses to T and D. Three QTLs in chromosome 7A and three QTLs in chromosome 10D1 appeared to be located at the same positions in the respective chromosomes, while the remaining 23 QTLs were independently located in different chromosomes (Table 2, Fig. 4). Regarding the three QTLs in chromosome 7A, the D-markers were associated with the increased F(D−T) (%) and reduced F(D−T) (mg) and M(D−T) (%). In contrast, the D-markers in chromosome 10D1 were associated with the reduced F(D−T) (%) and F(D−T) (no.) and increased M(D−T) (%).

QTLs for the same trait in homoeologous chromosomes

Each of the 12 QTL pairs for the same traits was found at approximately the same position in two homoeologous chromosomes. These QTLs could be classified into the three types (Table 2, Fig. 4) described below.

1) The presence of D-markers in both QTLs showed positive effects: BC_1×D (mg) in chromosomes 1A

and 1D2, BC_1×T (no.) in chromosomes 5A and 5D2, and D×BC_1 (mg) in chromosomes 9A and 9D1. 2) The presence of D-markers in both QTLs showed negative effects: BC_1×D (no.) in chromosomes 2A and 2D1 and BC_1×T (no.) in chromosomes 6b and 6c. 3) The presence of D-markers was associated with a positive effect in one QTL and a negative effect in another QTL in the homoeologous chromosomes: F(D−T) (%) in chromosomes 2A and 2D1, F(D−T) (%) in chromosomes 4D2 and 4A, and M(D−T) (%) in chromosomes 10D1 and 10A. This indicates that the differential responses in the berry-setting rates were partially controlled by the contrasting effects of the homoeologous chromosomes. A third type was also found for BC_1×D (mg) in chromosomes 5D1 and 5D2, D×BC_1 (%) in chromosomes 10D1 and 10A, T×BC_1 (%) in chromosomes 9A and 9D1, and D×BC_1 (mg) in chromosomes 11b and 11a. In addition, the QTLs in the three homoeologous chromosomes of chromosome 7 were all associated with BC_1×T (no.); D-markers were associated with negative effects in chromosomes 7A and 7D1 and positive effect in chromosome 7D2 (Table 2).

Discussion

Dissected *S. demissum* genomes and crossing behaviors of the BC_1 plants

S. demissum ($AADDD^dD^d$) is highly homozygous; thus, the pentaploid F_1 hybrids with *S. tuberosum* (AAA^tA^t) are relatively homogeneous with the same genome constitution (AAA^tDD^d). In this study, the three different *S. demissum* genomes in the F_1 hybrid were dissected by crossing with *S. tuberosum* into aneuploid BC_1 plants. The BC_1 plants possessing different portions of the *S. demissum* chromosomes were investigated by reciprocally crossing with the parental *S. demissum* or *S. tuberosum*. In these crosses, other than the genes directly related to crossability, many factors, such as meiotic irregularity and abnormal gene expression due to aneuploidy, could affect the crossability of the BC_1 plants. These combined effects on the crossability are important to investigate because backcrossing is generally used to incorporate *S. demissum* germplasm in breeding (Dionne 1961; Ross 1986; Plaisted and Hoopes 1989).

According to the EBN (Johnston et al. 1980) and PNA (Nishiyama and Yabuno 1978) hypotheses, a paternal factor (paternal EBN, or activating value) that is higher than the maternal factor (maternal EBN,

or response value) produces seeds that are heavier than normal seeds, via overproliferation of endosperms. The apparent seed size difference between D × T (0.94 mg) and T × D (0.39 mg) suggests that *S. demissum* has a slightly lower EBN than *S. tuberosum*, although the two species are grouped in the same 4 EBN category (Sanetomo et al. 2011). Compared with these seed weights, the mean seed weights of F_1 × T (0.94 mg), F_1 × D (0.58 mg), and D × F_1 (0.49 mg) indicate that the pentaploid F_1 is somewhat similar to the D parent. Considering the mean seed weights observed in the BC_1 crosses [D × BC_1 (0.63 mg), BC_1 × D (0.44 mg), T × BC_1 (0.59 mg), and BC_1 × T (0.77 mg), Fig. 2], the EBN values could be D ≈ F_1 < BC_1 < T. This relationship among F_1, BC_1 and T is expected because EBN has been hypothesized to have additive effects (Ehlenfeldt and Hanneman 1988; Camadro and Masuelli 1995). However, this is only a comparison of the means. BC_1 plants have different somatic chromosome numbers, ranging from 49 to 59 (Ono 2008), and show broad variations in crossability-related traits (Figs. 1−3). Approximately 50% of the D-markers were transmitted from F_1 to the BC_1 plants (Ono et al. 2016). This verifies that the *S. demissum* genomes in F_1 are dissected and segregated in the BC_1 plants, suggesting that the observed phenotypic changes most likely accounted for the dissection or reduction of the *S. demissum* genome, although the genetic effects of *S. tuberosum* cannot be completely excluded.

Crossability is controlled by several major genes and many minor genes

The obtained data for crosses of the F_1 and BC_1 plants with D and T were almost comparable to our previous results using smaller numbers of BC_1 plants and pollinations (Sanetomo et al. 2011) but are more comprehensive and sufficient for conducting a QTL analysis. We found at least 99 QTLs localized in almost all *S. demissum* chromosomes, including an unassigned linkage group in chromosome 13. This suggests that the crossability of BC_1 plants is controlled by many loci. However, bi- or tri-modal distributions were observed in BC_1×D (%), BC_1×T (%), and D×BC_1 (mg), indicating the presence of a few major genes that control these traits (Figs. 1 and 2). The significant positive correlations found between BC_1×D (%) and BC_1×T (%), between D×BC_1 (%) and T×BC_1 (%), and between BC_1×T (%) and T×BC_1 (%) further suggest that common major genes are involved in the successful BC_1 female and BC_1 male crosses. One of these genes is definitely a QTL found in chromosome 9A of *S. demissum* and exhibited prominently positive effects on the berry-setting rates in all four cross combinations.

The S-locus was mapped to potato chromosome 1 with the reference markers GP128 and CP100 (Gebhardt et al. 1991). According to the Sol Genomics Network (https://solgenomics.net), these reference markers are located near STI31 in the present map (Fig. 4). We could not detect any significant effect of this locus on the berry-setting rate, which is an immediate reflection of cross-incompatibility (Fig. 4). Thus, we cannot consider S-locus one of the major genes controlling crossability in the *S. demissum* backcross progenies.

Maternal and paternal QTLs exhibiting differential responses

Ehlenfeldt and Hanneman (1988) proposed a genetic model in which the EBN is controlled by three unlinked, additive loci in a threshold-like system. Alternatively, Camadro and Masuelli (1995) proposed a model in which the EBN is controlled by two independent loci with two homozygous alleles per genome. Both hypotheses seem to postulate that the same set of independent loci in the male and female gametes contribute to the balance for normal endosperm development. In the present study, the EBN-controlling genes of *S. demissum* were dissected and segregated among the BC_1 plants. The degree of EBN differences was measured by assessing differential responses in crosses with D and T. We found 29 QTLs associated with the differential responses, of which five were associated with seed size and eight were associated with seed number/berry; all were found in the BC_1 females (differential responses of these traits in the BC_1 males were not examined in this study). Regarding the differential responses in the berry-setting rate, nine QTLs in the BC_1 females and seven QTLs in the BC_1 males were found. Among them, the QTLs for F(D−T) (%), M(D−T) (%) and F(D−T) (mg) were co-localized in chromosome 7A, while the QTLs for F(D−T) (%), M(D−T) (%) and F(D−T) (no.) were co-localized in chromosome 10D1 (Table 2). Although these QTLs have an effect similar to that of the EBN, they are not additive and have opposite effects (Table 2). None of the other differential response-related QTLs were co-localized (Fig. 4). This suggests that the QTLs responsible for differential responses are different between male and female gametes, such as those described by Nishiyama and Yabuno (1978) as activating and responsive values. We further discovered that the gene actions are controlled in an additive manner by the suppression/promotion of different and/or homoeologous chromosomes. The existence of different maternal and paternal QTLs may imply the existence of a genetic system similar to

that found in the interspecific and inter-ploidy crosses of *Arabidopsis*. Endosperm growth in *Arabidopsis* is controlled by a balance of maternally contributed Polycomb repressive complex proteins and paternally contributed AGAMOUS-LIKE Type-1 MADS domain transcription factors in a dosage-dependent manner (Dilkes and Comai 2004; Josefsson et al. 2006; Walia et al. 2009; Köhler et al. 2010).

In all the traits measured in this study, the BC_1 plants showed larger variances when used as females than as males, resulting in a much larger variability in F(D−T) than in M(D−T) (Figs. 1−3). In addition, the BC_1 females tended to produce more seeds than the BC_1 males (Fig. 3). Gametes produced from the parental *S. demissum* and *S. tuberosum* have predominantly 36 and 24 chromosomes, respectively, and hold their species specificity and uniformity. In contrast, the F_1 and each BC_1 plant produced mixtures of various aneuploid gametes or gametophytes genetically segregated in the presence or absence of the *S. demissum* alleles from the parental F_1 and BC_1 plants. Thus, highly intense competition occurred among the aneuploid gametes or gametophytes to fertilize and form normal seeds. Based on the present observations, we suggest that the maternal QTLs are more generous and tolerant of variability than paternal QTLs.

Functionally sterile BC_1 pollen in backcrosses to *S. tuberosum*

As demonstrated previously (Sanetomo et al. 2011) and in this study, the pollen of F_1 and the backcross hybrids were male fertile when crossed to *S. demissum*. Nevertheless, F_1 and the following backcross hybrids are usually used as females for successful backcrossing to *S. tuberosum* (Dionne 1961; Irikura 1968). The functional male sterility of the backcrossed progeny is caused by the interaction between the *S. demissum*-derived cytoplasmic genome and the nuclear factors contributed by *S. tuberosum* (Dionne 1961; Phumichai et al. 2006). However, three of 43 (Sanetomo et al. 2011) or six of 74 T × BC_1 crosses (this study) successfully produced hybrid seeds. Genetic-cytoplasmic male sterility has been observed in *S. tuberosum* cytoplasm interacting with various nuclear genes (Grun 1979). In this cytoplasm, a restorer gene (*Rt*) is known (Iwanaga et al. 1991). Likewise, there may be a similar restorer gene in the *S. demissum* cytoplasm. We detected nine QTLs for T×BC_1 (%), of which seven were negatively associated with D-markers (Table 2). This finding indicates that reducing the *S. demissum* chromosomes by backcrossing will increase T×BC_1 (%). Of the seven QTLs, however, the QTL with the largest effect

was detected in chromosome 1D1, which explained only 11.0% of the variance (Online Resource 5). Thus, successful T × BC_1 crosses likely resulted from the loss of multiple portions of the *S. demissum* genome. Alternatively, a restorer gene(s) might be introduced from *S. tuberosum*. Since reducing the *S. demissum* genome and increasing the *S. tuberosum* genome could be quickly accomplished by repeated backcrossing, male fertile pollen could be produced. However, this prediction is inconsistent with empirical observations that male sterility in the backcrossed progeny persists after ten or more successive generations of backcrossing (Dionne 1961). Compared with the T × (DT)T crosses generated in this study (1.1% berry-setting rate), a much higher berry-setting rate (14.7%) has been reported in T × (TD)T crosses (Sanetomo et al. 2011), suggesting a third possibility in which a cytoplasmic factor in the pollen was involved. Previously, we found that both female gametophytes and pollen having the *S. demissum* cytoplasm produced lower berry-setting rates than those having the *S. tuberosum* cytoplasm in any *S. demissum* F_1 and backcross progenies (Sanetomo et al. 2011). However, because the present BC_1 pollen had the *S. demissum* cytoplasm the successful T × BC_1 crosses cannot be fully explained by the cytoplasm alone. A fourth possibility is that some of these QTLs for T×BC_1 (%) restored a balanced condition in the pollen that resulted in successful berry-setting. According to this hypothesis, the balance once restored will be disrupted quickly by further backcrossing. Consequently, the unilateral cross-compatibility in the *S. demissum* backcross progenies remains unexplained. A further survey in BC_2 and the following backcross progenies is necessary.

Crossability is altered by genome duplication

We found that the crossability-related QTLs were not localized in chromosomes in a specific genome of *S. demissum* but were distributed among homoeologous chromosomes over three sets of genomes. Thirteen homoeologous chromosome pairs had QTLs for the same traits, in which five homoeologous chromosome pairs functioned similarly either to increase or decrease the trait, suggesting they have additive effects. Eight homoeologous chromosome pairs showed opposite effects, suggesting that one of the pair functioned as a suppressor. Therefore, we suggest that duplicated genomes not only function additively on crossability but also alter the crossing ability by suppressing or promoting the gene actions.

Although alterations of ploidy caused subtle expression changes in a substantial percentage of genes

in the potato genome (Stupar et al. 2007), silencing or promoting the unequal expression of duplicated genes has been documented in established allopolyploids (Adams et al. 2003; Bottley et al. 2006; Udall et al. 2006; Bardil et al. 2011). The duplicated homoeologous genes in allopolyploids might be undergoing subfunctionalization (partitioning of function and/or expression patterns between duplicated copies), allowing the allopolyploid to differentially use the homoeologous genes for different or variable responses to an array of stressful conditions (Adams 2007; Bardil et al. 2011; Madlung 2013). *S. demissum* seems to be a successful wild species because it is an extremely widespread, highly self-fertile species (Hawkes 1990; Spooner et al. 2004), and natural and artificial hybrids of *S. demissum* with various species have been reported (Howard and Swaminathan 1952; Marks 1965; Hawkes 1990). The species specificity of *S. demissum* and the feasibility or plasticity to form interspecific hybrids might be acquired by fine-altering the system of crossability embedded in homoeologous chromosomes, which is apparently one of advantages of polyploidy in the survival of the species (Comai 2005).

Conclusion

It has been hypothesized that EBN is controlled by a small number of genes that function similarly in male and female gametophytes (Ehlenfeldt and Hanneman 1988; Camadro and Masuelli 1995). In this study, *S. demissum* genomes and EBN-controlling genes were dissected in BC_1 plants. We found that the crossability of BC_1 plants of *S. demissum* was controlled by several major genes and many minor genes. The QTLs detected when the BC_1 plants were used as males and those used as females were not necessarily co-localized. Duplicated loci among homoeologous chromosomes likely altered the crossability by suppressing or promoting the gene actions. Therefore, we conclude that the EBN is represented by the sum of various genetic effects controlled by many genes that function differently in male and female gametes. Through the process of backcrossing, rapid gene silencing and/or subfunctionalization might be induced by hybridization (Comai et al. 2000; Adams et al. 2003; Adams 2007), and other genetic abnormalities might occur by aneuploidy. Thus, the dissected *S. demissum* chromosomes in the BC_1 plants might not function similarly in the original *S. demissum*. However, the present study demonstrated a dynamic and complex gene network in the BC_1 plants, which should be considered to better understand the genetic nature of the EBN and the backcrossing process of *S.*

demissum.

Acknowledgments

We would like to thank Shizuka Souma and Eri Kakuta for technical assistance. This research was supported by Calbee Inc., Hokkaido Potato Growers Association, Kewpie Corp., KENKO Mayonnaise Co., Ltd., and Japan Snack Cereal Foods Association.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Table 1 Pearson's correlation coefficients (*r*) between traits

Trait A	Trait B	n[1)]	Correlation (*r*)
BC_1×D (%)	BC_1×T (%)	67	0.682***
BC_1×D (%)	D×BC_1 (%)	73	0.104
BC_1×T (%)	T×BC_1 (%)	66	0.366***
D×BC_1 (%)	T×BC_1 (%)	71	0.354***
BC_1×D (mg)	BC_1×T (mg)	60	0.607***
BC_1×D (mg)	D×BC_1 (mg)	32	−0.141
BC_1×T (mg)	T×BC_1 (mg)	5	−0.458
D×BC_1 (mg)	T×BC_1 (mg)	4	0.108
BC_1×D (no.)	BC_1×T (no.)	60	0.626***
BC_1×D (no.)	D×BC_1 (no.)	32	0.143
BC_1×T (no.)	T×BC_1 (no.)	6	−0.744
D×BC_1 (no.)	T×BC_1 (no.)	5	0.005

[1)] The number of BC_1 plants compared

*P<0.05, **P<0.01, or ***P<0.005

Table 2 The number of QTLs and the chromosomal distribution of QTLs supported by either multiple markers with P<0.05 or single markers with P<0.005

Trait	No. of QTLs[1)]			Chromosomal distribution													
	+	−	Total	1A	1D1	1D2	2A	2D1	2D2	3A	3D1	3D2	4A	4D1	4D2	5A	5D1	5D2
BC_1×D (%)	4/7	6/12	10/18		−			−			−						−	
BC_1×T (%)	3/7	4/8	7/15			+		−					+				−	
F(D−T) (%)	5/7	4/6	9/12				+	+/−					−		+			
BC_1×D (mg)	5/8	1/3	6/11	+		+		+									+	−
BC_1×T (mg)	5/5	2/8	7/13			+						+		+	−		+	
F(D−T) (mg)	2/4	3/4	5/8		−									−				
BC_1×D (no.)	0/4	6/8	6/12				−	−						−				
BC_1×T (no.)	3/4	6/8	9/12												−	+		+
F(D−T) (no.)	2/3	6/8	8/10			+/−		−						−				
D×BC_1 (%)	2/2	5/9	7/11	−													−	
T×BC_1 (%)	1/2	5/7	6/9		−													
M(D−T) (%)	1/2	6/8	7/10	−													−	
D×BC_1 (mg)	6/11	2/6	8/17									−						
D×BC_1 (no.)	0/1	4/13	4/14															

[1)] (No. of QTLs supported by either multiple markers with P<0.05 or single markers with P<0.005) / (no. of detected QTLs)

+ or – indicates significant increase or decrease for the trait, respectively, when the *S. demissum*-specific marker(s) was present

Table 2 Continued

Trait	Chromosomal distribution																				
	6a	6b	6c	7A	7D1	7D2	8A	8D1	8D2	9A	9D1	9D2	10A	10D1	10D2	11a	11b	11c	12A	12D1	12D2	13
BC_1×D (%)	+		−						+	+				−					+			
BC_1×T (%)			−							+			−									
F(D−T) (%)				+		−								−			+					
BC_1×D (mg)				+																		
BC_1×T (mg)				+						−												
F(D−T) (mg)				−						+											+	
BC_1×D (no.)		−												−						−		
BC_1×T (no.)		−	−	−	−	+															−	
F(D−T) (no.)		−			+			−						−								
D×BC_1 (%)				−						+			−	+			−					
T×BC_1 (%)									−	+	−				−					−		
M(D−T) (%)	−			−			−						−	+								
D×BC_1 (mg)							+			+	+			+		−	+					+
D×BC_1 (no.)		−						−												−		−

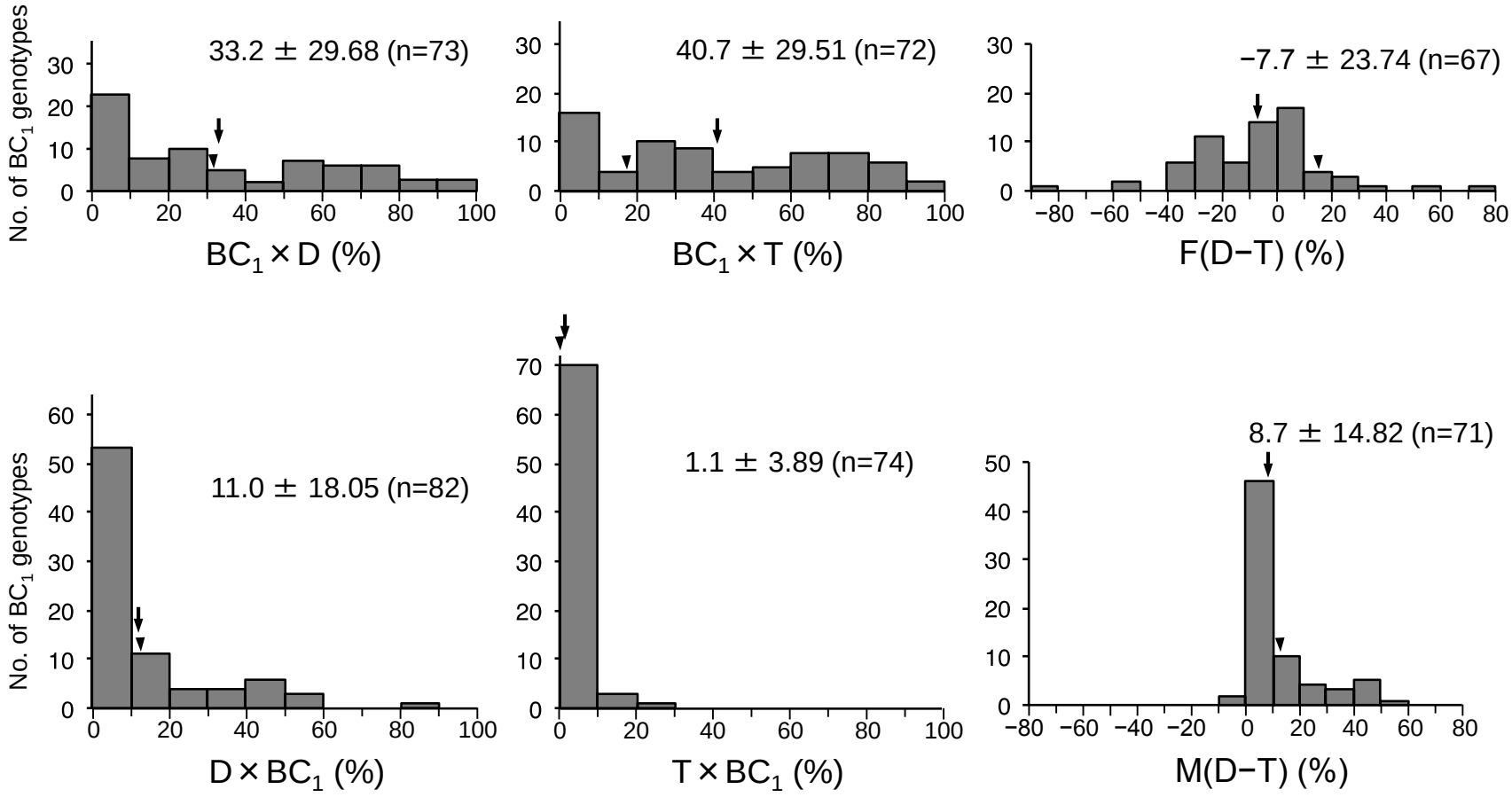
Legend of Figures

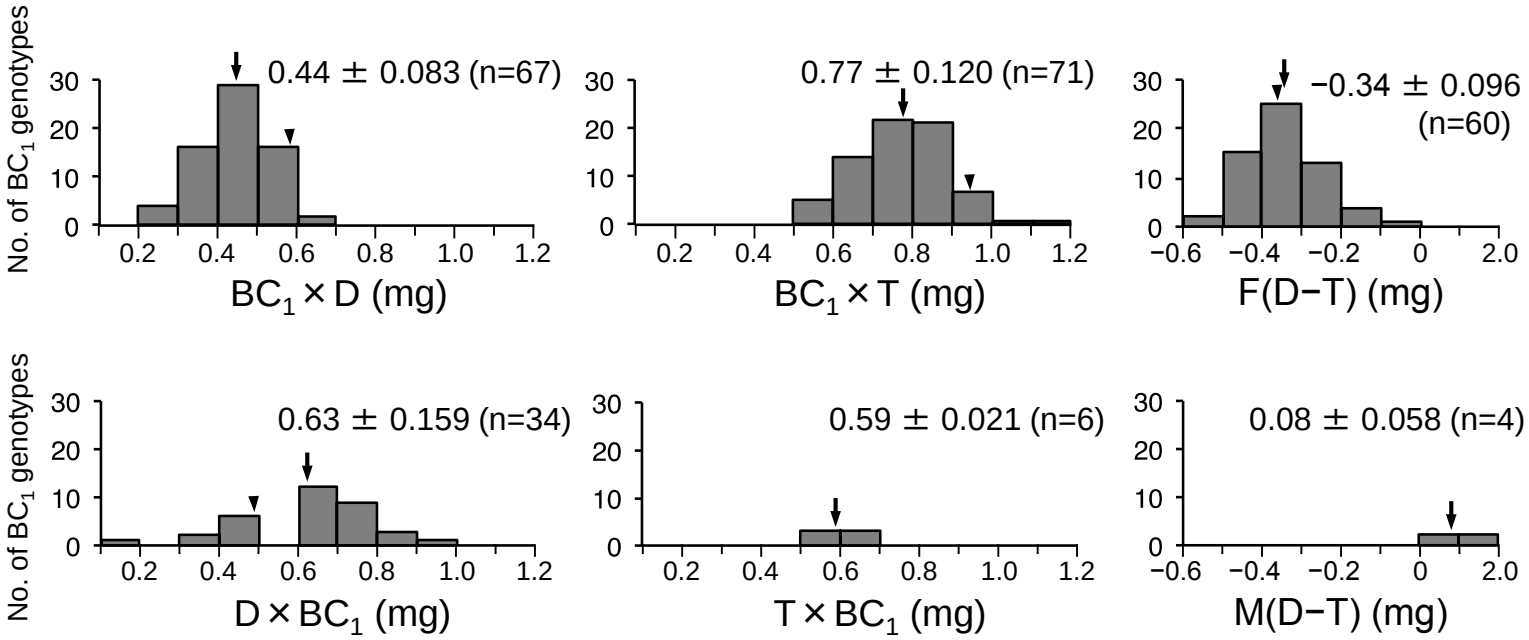
Fig. 1 Histograms showing the berry-setting rate (%) in the BC_1 plants reciprocally crossed with D and T parents and their differential responses. The mean ± standard deviation and the number of BC_1 plants analyzed (n) are represented in each histogram. Arrows indicate the means of the BC_1 plants. Arrowheads indicate the values of the F_1 parent

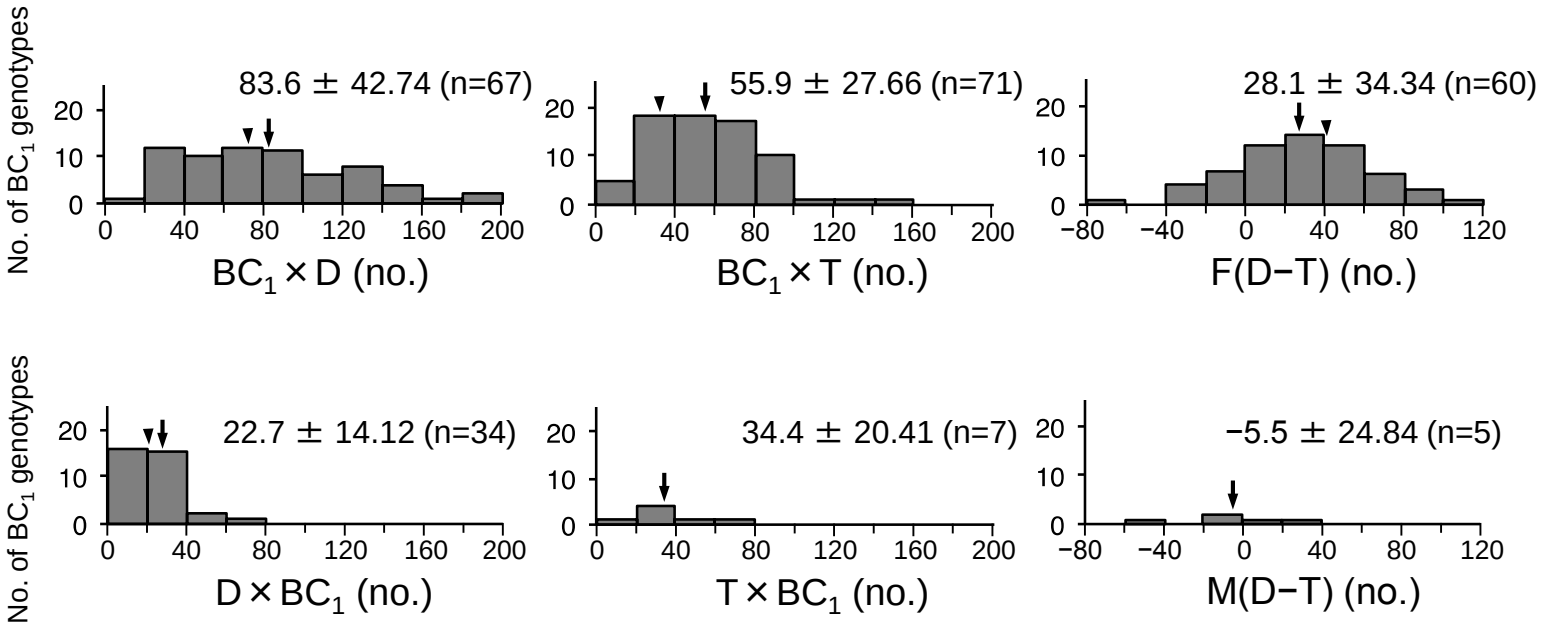
Fig. 2 Histograms showing the mean weight (mg) of a seed obtained in the BC_1 plants reciprocally crossed with D and T parents and their differential responses. The mean ± standard deviation and the number of BC_1 plants analyzed (n) are represented in each histogram. Arrows indicate the means of the BC_1 plants. Arrowheads indicate the values of the F_1 parent (the pollen of the F_1 did not produce any seed in the cross with T)

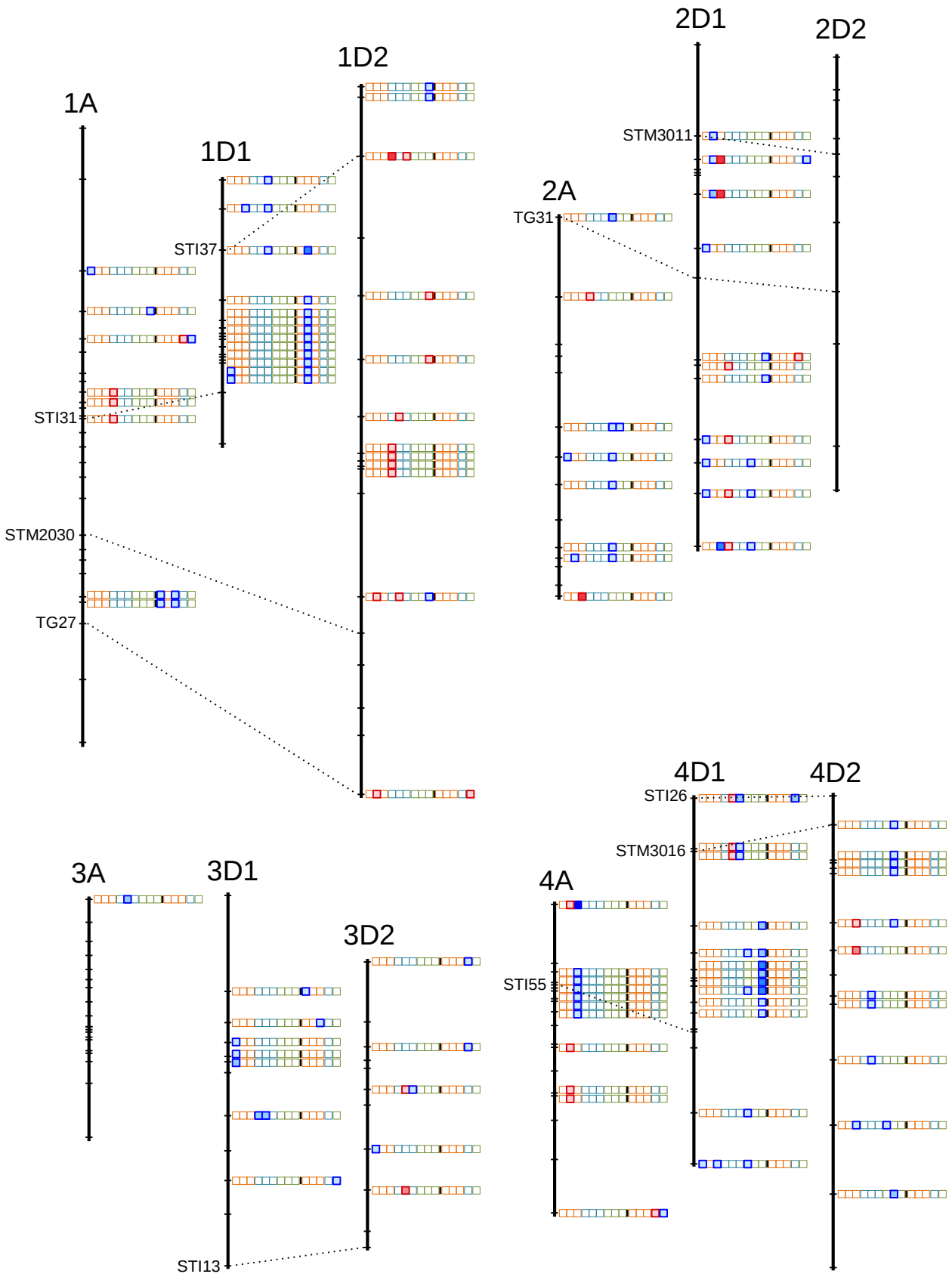
Fig. 3 Histograms showing the seed number/berry in the BC_1 plants crossed reciprocally with D and T parents and their differential responses. The mean ± standard deviation and the number of BC_1 plants analyzed (n) are represented in each histogram. Arrows indicate the means of the BC_1 plants. Arrowheads indicate the values of the F_1 parent (the pollen of the F_1 did not produce any seed in the cross with T)

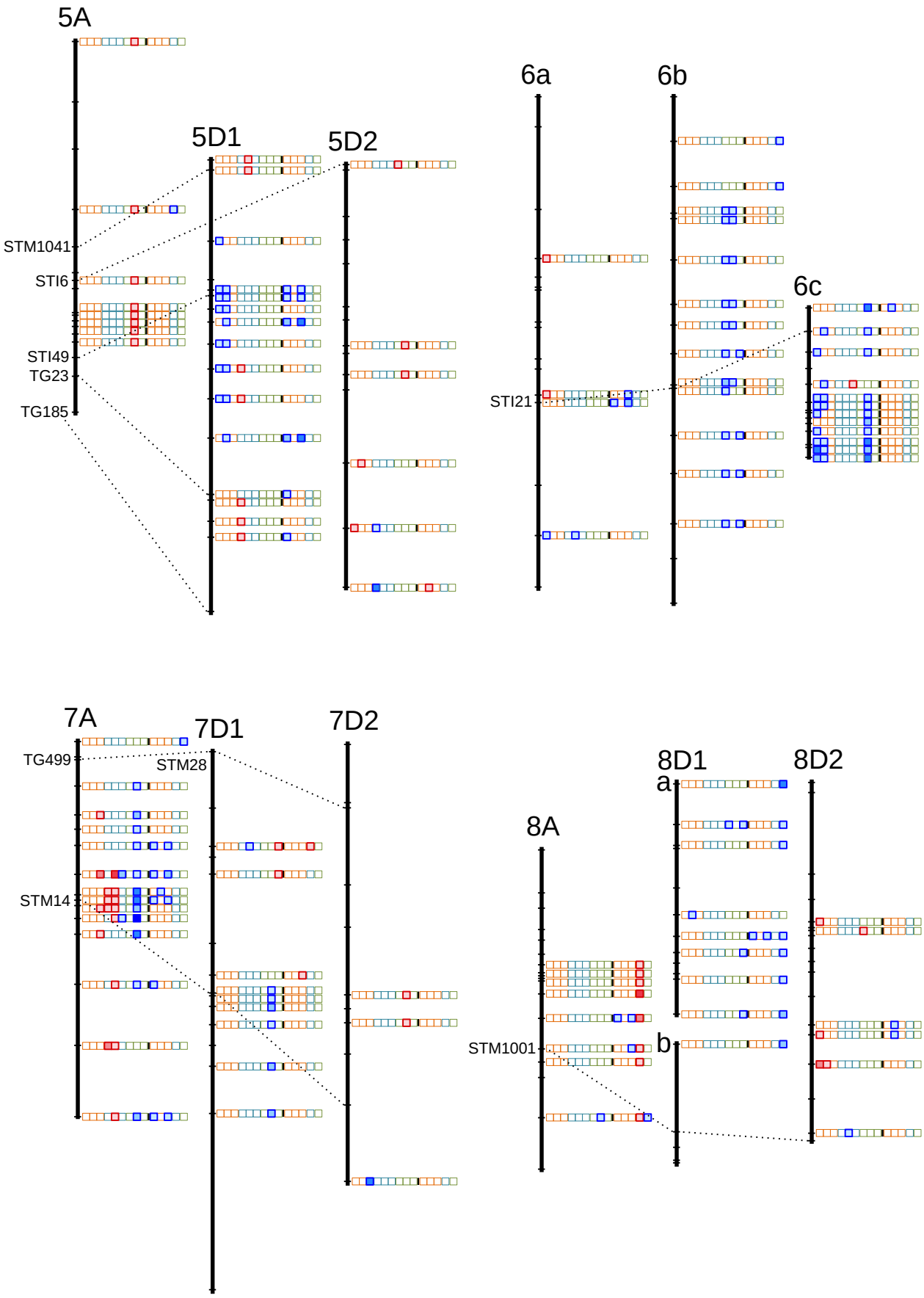
Fig. 4 Crossability-related QTLs detected by a single-marker analysis. The *S. demissum* genetic map, composed of 36 chromosomes and one unassigned linkage group (=chromosome 13) (Ono et al. 2016), was used with slight modifications (see Materials and methods). Detected QTLs are shown in a “trait evaluation array” with different color depth indicating significance levels

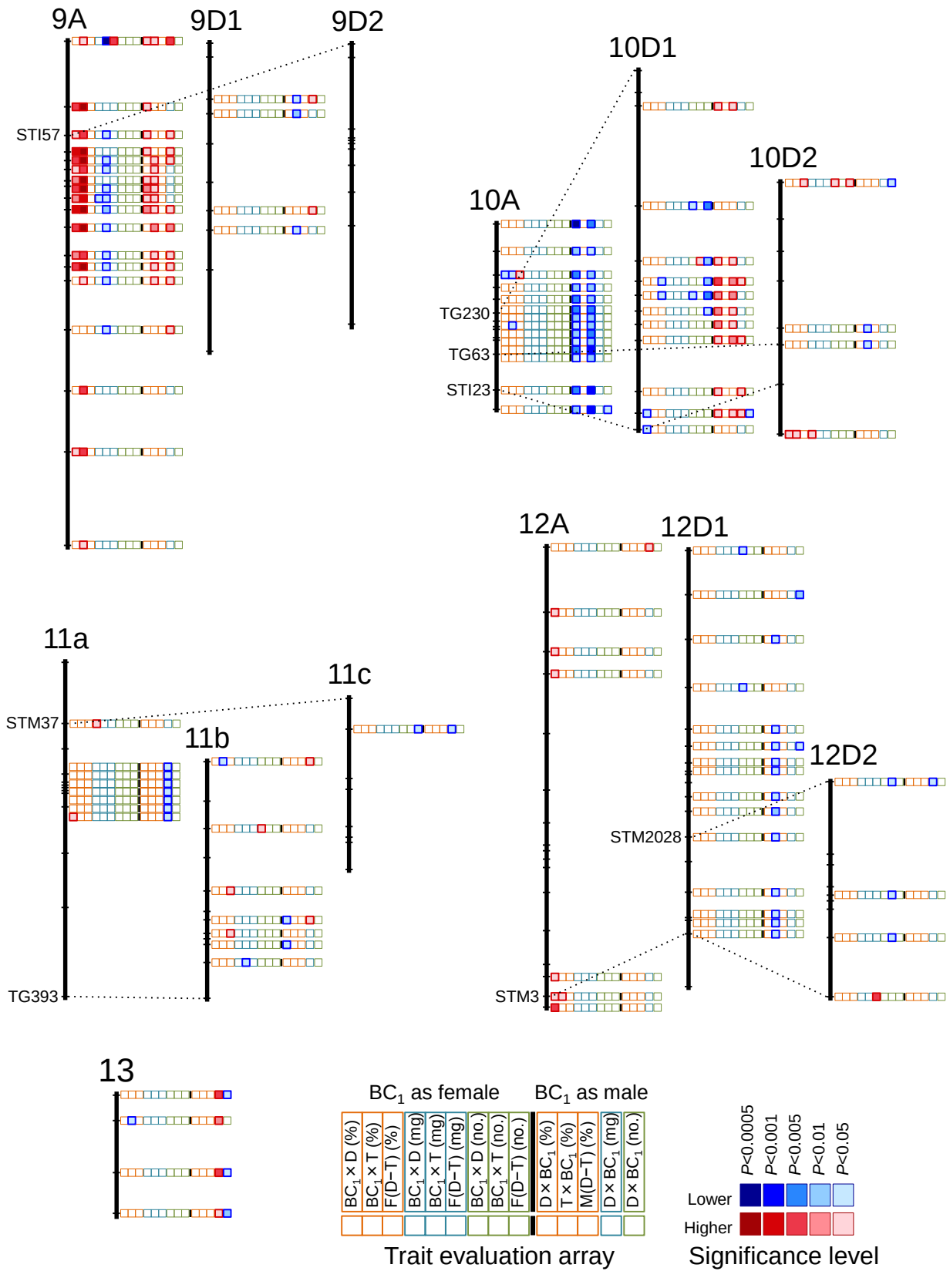

No. of BC1 genotypes
33.2 ± 29.68 (n=73)
BC1 × D (%)
40.7 ± 29.51 (n=72)
BC1 × T (%)
−7.7 ± 23.74 (n=67)
F(D−T) (%)
11.0 ± 18.05 (n=82)
D × BC1 (%)
1.1 ± 3.89 (n=74)
T × BC1 (%)
8.7 ± 14.82 (n=71)
M(D−T) (%)


No. of BC1 genotypes
0.44 ± 0.083 (n=67)
BC1 × D (mg)
0.77 ± 0.120 (n=71)
BC1 × T (mg)
−0.34 ± 0.096
(n=60)
F(D−T) (mg)
0.63 ± 0.159 (n=34)
D × BC1 (mg)
0.59 ± 0.021 (n=6)
T × BC1 (mg)
0.08 ± 0.058 (n=4)
M(D−T) (mg)


No. of BC1 genotypes
83.6 ± 42.74 (n=67)
BC1 × D (no.)
55.9 ± 27.66 (n=71)
BC1 × T (no.)
28.1 ± 34.34 (n=60)
F(D−T) (no.)
No. of BC1 genotypes
22.7 ± 14.12 (n=34)
D × BC1 (no.)
34.4 ± 20.41 (n=7)
T × BC1 (no.)
−5.5 ± 24.84 (n=5)
M(D−T) (no.)


1A
1D1
1D2
STI37
STI31
STM2030
TG27
2A
2D1
2D2
STM3011
TG31
3A
3D1
3D2
STI13
4A
4D1
4D2
STI26
STM3016
STI55


5A
5D1
5D2
STM1041
STI6
STI49
TG23
TG185
6a
6b
6c
STI21
7A
7D1
7D2
TG499
STM28
STM14
8A
8D1
a
b
8D2
STM1001


9A
9D1
9D2
STI57
10D1
10D2
10A
TG230
TG63
STI23
11a
11c
11b
STM37
TG393
12A
12D1
12D2
STM2028
STM3
13
BC1 as female
BC1 as male
BC1 × D (%)
BC1 × T (%)
F(D−T) (%)
BC1 × D (mg)
BC1 × T (mg)
F(D−T) (mg)
BC1 × D (no.)
BC1 × T (no.)
F(D−T) (no.)
D × BC1 (%)
T × BC1 (%)
M(D−T) (%)
D × BC1 (mg)
D × BC1 (no.)
Trait evaluation array
P<0.0005
P<0.001
P<0.005
P<0.01
P<0.05
Lower
Higher
Significance level