**EPPN project**

**Results parent-progeny comparisons**

**Comparison group 1: Resythesized *Brassica napus***

*B. rapa* (AA, diplod, homozygous) x *B. oleracea* (CC, diploid, homozygous) = synthetic *B. napus* (AACC, tetraploid, 2 genomes, homozygous)

Complete data only available for one of the three parent-progeny sets.

**Hypothesis:**

Increasing ploidy level and heterozygosity will increase growth rate and/or biomass production in hybrids.

**Growth**

Growth was significantly enhanced in resythesized *Brassica napus* compared to its diploid parent genotypes. Synthetic *B. napus* showed maximum heights superior to the tallest parent (by 60cm on average). The increased maximum height is not associated with enhanced growth rates but with extended growth periods, exceeding the longest parental growth period by 5.6 days on average. In contrast, branching was reduced compared to the mid-parent mean.

**Fertility estimates**

Several fertility estimates were decreased in synthetic *B. napus* compared to the mid-parent mean. Total plant pod weight was on average 5.95g lower than the MPV. This may be due to a lower number of seeds per pod, which was on average reduced by 8.62 compared to the lowest performing parent, although thousand seed weight was increased (1.04g above the highest parental TSW).

Flowering in resythesized *Brassica napus* time did not differ significantly from the parents.

**Comparison group 2: Novel allohexaploids**

*B. napus* (AACC, tetraploid) x *B. carinata* (BBCC, tetraploid) x *B. juncea* (AABB, tetraploid) = NCJ hybrids (AABBCC, allohexaploid)

**Hypotheses:**

Higher ploidy levels will result in increased hybrid vigour

1. The allohexaploids will grow faster than the tetraploid and diploid parental species
2. The allohexaploids will have a higher total biomass production at flowering compared to the diploid and tetraploid species

**Growth**

Among the 17 progeny sets that emerged from seven NCJ allohexaploid genotype combinations 7/17 showed an increased and 2/17 a decreased height at harvest compared to the mid-parent mean. These differences in biomass production were highly dependent on the combination of parent genotypes: one progeny set each of genotypes **N5C2J2 and N4C2J1 and all progeny sets of genotypes N5C2J1 and N1C1J1** exceeded the mid-parent mean. Four of the latter outperformed the better parent in terms of transgressive segregation. The increased plant height at maturity was in all of these genotypes due to elevated growth rates (determined over the period up to 50 % of maximum height). In genotype N1C1J1 biomass accumulation was additionally enhanced by a prolonged growth period compared to the mid-parent mean.

The two progeny sets showing a lower height than the mid-parent mean and the smallest parent, respectively, belonged to genotype N6C2J2. The growth rate of this genotype was below the lowest parental growth rate (assessed over the period up to 75% of maximum height).

Of the two O1J3 allohexaploid progeny sets, one reached a greater height than the parents average due to a prolonged growth period exceeding the longest parental period, which compensated for reduced growth rates.

Biomass production in terms of branching was increased in all seven progeny sets of the genotypes N1C1J1 and N4C2J1. Five of them even exceeded the higher performing parent. A branch number below the average of the parental lines was only found in the genotypes N5C2J2 and N6C2J2.

**Fertility estimates**

Across the NCJ allohexaploid genotype combinations (17 progeny sets), fertility estimates were highly variable and most progeny showed significantly lower seed fertility compared to the mid-parent mean. Single pod weight was decreased in 14/17 progeny sets compared to their parents average, in 3 of them even compared to the lowest parent. The decline in pod weight can be explained by a lower number of seeds per pod (found in 12/17) and a lower seed weight (found in 11/17 progeny sets compared to their parents average). As in most progeny sets the number of pods per plant was not different from the mid-parent mean, the decrease in single pod weight was also reflected at the whole plant level (9/17). Only one progeny set showed similar seed fertility to the mid-parent mean for seed number and weight.

Days to appearance of first flowers in the NCJ allohexaploids was significantly delayed in 13/17 progeny sets (belonging to the genotypes N5C2J1, N5C2J2, N6C2J2, N1C1J1 and N4C2J1 on average by XX days across all genotypes. Four of them were even later than their latest parent. This delay was found by imaging analyses as well as by BBCH scoring.

One of the two O1J3 allohexaploid progeny sets was reduced in seed number and weight and delayed in flowering, as well.

**Comparison group 3: JC hybrids**

*B. juncea* (AABB, tetraploid, 2 genomes, homozygous**?**) x *B. carinata* (BBCC, tetraploid, 2 genomes, homozygous**?**) = JC hybrids (BBAC**?,** tetraploid, **3** genomes, homozygous)

* Effects of number of genomes. Same ploidy level.

**Hypothesis:**

Increasing heterozygosity will increase growth rate and/or biomass production in hybrids.

**Growth**

Among the tetraploid JC hybrids both progeny sets grew faster and reached a greater maximum height than their parents average. One of them also showed increased branching compared to the mid-parent mean.

**Fertility estimates**

Total plant pod weight was reduced in both progeny sets, which is in agreement with the lower number of pods per plant and the reduced thousand seed weight found in J1C2 plants. The increase in number of seeds per pod and seed weight per ten pods found in J1C1 relative to the parental average seems contradictory, but the dataset for this genotype is incomplete (e.g. little data on number of pods per plant or branch available).

Flowering time was also highly variable among the two JC hybrid genotypes: BBCH51, the stage at which the main inflorescence becomes visible from the top, was advanced in J1C1 and delayed in J1C2 compared to their mid-parent value.

**Comparison group 4: F1 allohexaploids**

A complex set of comparisons: each of the heterozygous hexaploids to their two parent hexaploid lines (and maybe all of them to the species parents, but let's see first how reasonable this is)

AABBCC (homozygous) x AABBCC (homozygous) = AABBCC (heterozygous)

**Hypotheses:**

Increased heterozygosity in the allohexaploid hybrids will result in increased hybrid vigour

1. Hybrid allohexaploids will grow faster than their homozygous allohexaploid parents
2. Hybrid allohexaploids will have a higher total biomass production at flowering than their homozygous allohexaploid parents

**Growth**

Across the nine heterozygous F1 allohexaploids there was a trend towards an enhanced growth compared to the mid-parent mean. At harvest six of nine allohexaploid progeny lines had reached a height closer to the higher performing parent (i.e. they were significantly taller than the smaller parent and not different from the taller parent). Three of them were significantly taller than their parents average. Branching in the F1 allohexaploids was within the range of the parental lines.

The increased maximum height compared to the smaller parent was more often achieved by a prolonged growth period to reach 50% of maximum height (3/6). In only one case it resulted from faster growth (between start of shoot expansion and reaching 50% of maximum height). For most progeny genotypes growth rates and periods did not differ significantly from their parents.

**Reproductive development**

Flowering time was highly variable among the nine heterozygous allohexaploid progeny lines. Nevertheless, three genotypes started flowering earlier than their parents on average and six reached BBCH61 and full flower closer to the earlier parent (significantly earlier than slower parent). One genotype flowered earlier than both parents. These observations were largely consistent in BBCH scoring and imaging analyses.

**Fertility estimates**

The heterozygous F1 allohexaploids showed a high segregation for seed number per pod. One of them was lower than both parents. The number of pods on the main stem was between parents or not different with a tendency to perform closer to the better parent.

Total plant pod weight was closer to higher performing parent in five of nine F1 allohexaploid genotypes. Thousand grain weight was highly variable but did not show transgressive segregation. Only one genotype had a lower seed weight per ten pods in comparison with both parents.