**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 8/17 reached a maximum height above the mid-parent mean at harvest (measurement by hand). These progeny sets belonging to the genotypes N5C2J2, N4C2J1, N5C2J1 and N1C1J1 had an average height of 167 cm, which was by 36.3 cm higher than their parents mean. Four of them (genotypes N5C2J1 and N1C1J1) outperformed the better parent in terms of transgressive segregation. In the image analyses 7/17 grew taller and genotype N7C1J1 was also among them.

The increased plant height at maturity was due to elevated growth rates relative to the mid-parent mean (determined over the period up to 50 % of maximum height). In genotype N1C1J1 biomass accumulation was additionally enhanced by a prolonged growth period.

Four of the 17 NCJ allohexaploid progeny sets grew at lower rates and remained smaller (both assessed by imaging analyses) than their smallest parent. All four were of genotype N6C2J2.

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, N4C2J1 and N7C1J1 compared to their mid-parent mean. With an average of 18.3 branches/shoot they were by 7.7 above the mean parental branch number. 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 seven NCJ allohexaploid genotype combinations, fertility estimates were highly variable and most progeny showed significantly lower seed fertility compared to the mid-parent mean. In 14/17 progeny sets single pod weight was lower than their parents mean. With an average of 0.038g it was by 0.028g below the mid parent value. Three of them were even below their 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.

**Reproductive development**

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). With an average of 64.2 days (between sowing and appearance of first flower pixels) they were on average delayed by 8.6 days compared to the mid-parent mean. Four of them were lagging behind their latest flowering 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) x *B. carinata* (BBCC) = JC hybrids (AABC, BBAC, CCAB?)

**Hypothesis:**

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

**Growth**

In the group of JC hybrids both progeny sets reached a **lower maximum height** at harvest than their parents on average. With a mean height of 108 cm they were on average 31 cm smaller than their parents. This was due to lower growth rates compared to the mid-parent mean (during the period up to 50% of maximum height). In addition, genotype J1C1 showed less branching than the parental lines.

**Fertility estimates**

Fertility traits showed a high variability in JC hybrids. Total plant pod weight was extremely reduced in genotype J1C2 (by 29.13g) and moderately reduced in genotype J1C1 (by 11.35g). The extremely low plant pod weight in genotype J1C2 is in agreement with the lower number of pods per plant and the reduced thousand seed weight found in this progeny set. In contrast, J1C1 showed a higher number of seeds per pod and an increased seed weight per ten pods compared to the mid-parent mean.

**Reproductive development**

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**

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 allohexaploid genotypes there was a tendency towards enhanced growth. In all progeny sets maximum height assessed by imaging analyses was closer to the higher performing parent (i.e. significantly above the smaller parent, not different from the taller parent). Four of these progeny sets reached a maximum height above the mid-parent mean (on average by 242.5 pixels). According to the hand measurements 3/9 were taller than their parents average (by 26.3 cm on average).

Superior maximum heights were rather due to increased growth rates than to extended growth periods. Four of the progeny sets showing increased heights relative to the smaller parent also had higher growth rates in comparison with this parent. However, compared to the mid-parent mean growth rates were mostly not significantly different, except for one progeny set.

Branching in the F1 allohexaploids was also within the range of the parental lines.

**Fertility estimates**

Parent-progeny differences in fertility traits showed a high variability between the F1 allohexaploids, although progeny were only in a few cases significantly different from the mid-parent mean. Seed weight was in some progeny sets reduced (thousand seed weight in 1/9 and seed weight per ten pods in 2/9 progeny sets) in others increased compared to the parents average (seed weight per ten pods in 1/9 progeny sets). Some of the F1 allohexaploids showed a clear reduction in fertility compared to their parents average. For example genotypes N5C2J2.N5C2J2 and N6C2J2.O1J3 showed a decline of seed number per pod (by 2 to 4 on average) and a decreased seed weight per ten pods (by 676.5g to 783.5g on average).

**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 3.5 days compared to the mid-parent mean) 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.