**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)

**Hypothesis:**

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

**Growth**

* plant height was increased in X/X, decreased in X/Y compared to their parents average
* plant height was increased in X/X, decreased in X/Y in comparison with both parents
* branch number was increased in X/X, decreased in X/Y compared to their parents average
* branch number was increased in X/X, decreased in X/Y in comparison with both parents
* growth rates to reach 50 and 75% increased in X/X, decreased in X/Y compared to their parents average
* growth rates to reach 50 and 75% was increased in X/X, decreased in X/Y in comparison with both parents
* growth periods to reach 50 and 75% increased in X/X, decreased in X/Y compared to their parents average
* growth periods to reach 50 and 75% was increased in X/X, decreased in X/Y in comparison with both parents

Reprod. development

* flowering (first\_flowers\_DAS / peak\_flowers\_DAS) advanced in X/X, delayed in X/Y compared to their parents average
* flowering (first\_flowers\_DAS / peak\_flowers\_DAS) advanced in X/X, delayed in X/Y in comparison with both parents
* BBCH51 (Hauptinfloreszenz inmitten der obersten Blätter von oben sichtbar) compared to their parents average
* BBCH51 (Hauptinfloreszenz inmitten der obersten Blätter von oben sichtbar) in comparison with both parents
* BBCH61 (ca. 10% der Blüten am Haupttrieb offen. Infloreszenzachse verlängert) compared to their parents average
* BBCH61 (ca. 10% der Blüten am Haupttrieb offen. Infloreszenzachse verlängert) in comparison with both parents
* single pod weight (wt/pod) was increased in X/X, decreased in X/Y compared to their parents average
* single pod weight (wt/pod) was increased in X/X, decreased in X/Y in comparison with both parents
* pod wt = total plant pod weight was increased in X/X, decreased in X/Y compared to their parents average
* pod wt = total plant pod weight was increased in X/X, decreased in X/Y in comparison with both parents
* Pods/plant were increased in X/X, decreased in X/Y compared to their parents average
* Pods/plant was increased in X/X, decreased in X/Y in comparison with both parents
* Main = pods on the main stem was increased in X/X, decreased in X/Y compared to their parents average
* Main = pods on the main stem was increased in X/X, decreased in X/Y in comparison with both parents
* seed wt\_10p\_g was increased in X/X, decreased in X/Y compared to their parents average
* seed wt\_10p\_g was increased in X/X, decreased in X/Y in comparison with both parents
* seeds\_per\_pod was increased in X/X, decreased in X/Y compared to their parents average
* seed wt\_10p\_g was increased in X/X, decreased in X/Y in comparison with both parents
* TGW\_g was increased in X/X, decreased in X/Y compared to their parents average
* TGW\_g was increased in X/X, decreased in X/Y in comparison with both 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.

Example: "Across the seven NCJ allohexaploid genotype combinations (17 progeny sets), most *progeny showed significantly lower seed fertility compared to the mid-parent mean (16/17 progeny sets showed significantly reduced seed weight per 10 pods, and 12/17 progeny sets showed significantly lower seed number per pod). Only one progeny set showed similar seed fertiliity to the mid-parent mean for both measured seed traits. Days to flowering in the NCJ allohexaploids was significantly delayed across all genotype combinations (although not in all progeny sets), on average by XX days across all genotypes."*

**Fertility estimates**

Across the NCJ allohexaploid genotype combinations (17 progeny sets), 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).

* TGW\_g was decreased in 11/17 compared to their parents average, in one case even below the lowest parent
* seeds\_per\_pod was decreased in 12/17 compared to their parents average, in 2 cases below the lowest parent
* not compensated for by number of pods per plant, which was within range of parents
* single pod weight (wt/pod) was increased in X/X, decreased in X/Y in comparison with both parents
* pod wt = total plant pod weight was increased in X/X, decreased in X/Y compared to their parents average
* pod wt = total plant pod weight was increased in X/X, decreased in X/Y in comparison with both parents
* Pods/plant were increased in X/X, decreased in X/Y compared to their parents average
* Pods/plant was increased in X/X, decreased in X/Y in comparison with both parents
* Main = pods on the main stem was increased in X/X, decreased in X/Y compared to their parents average
* Main = pods on the main stem was increased in X/X, decreased in X/Y in comparison with both parents
* seed wt\_10p\_g was increased in X/X, decreased in X/Y compared to their parents average
* seed wt\_10p\_g was increased in X/X, decreased in X/Y in comparison with both parents
* 13/17 delayed first\_flowers\_DAS compared to their parents average
* 12/17 delayed peak\_flowers\_DAS compared to their parents average, 6 of them even compared to the latest parent
* flowering (first\_flowers\_DAS / peak\_flowers\_DAS) advanced in X/X, delayed in X/Y in comparison with both parents
* BBCH51 (Hauptinfloreszenz inmitten der obersten Blätter von oben sichtbar) compared to their parents average
* BBCH51 (Hauptinfloreszenz inmitten der obersten Blätter von oben sichtbar) in comparison with both parents
* BBCH61 (ca. 10% der Blüten am Haupttrieb offen. Infloreszenzachse verlängert) compared to their parents average
* BBCH61 (ca. 10% der Blüten am Haupttrieb offen. Infloreszenzachse verlängert) in comparison with both parents

**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**

* plant height was increased in X/X, decreased in X/Y compared to their parents average
* plant height was increased in X/X, decreased in X/Y in comparison with both parents
* branch number was increased in X/X, decreased in X/Y compared to their parents average
* branch number was increased in X/X, decreased in X/Y in comparison with both parents
* growth rates to reach 50 and 75% increased in X/X, decreased in X/Y compared to their parents average
* growth rates to reach 50 and 75% was increased in X/X, decreased in X/Y in comparison with both parents
* growth periods to reach 50 and 75% increased in X/X, decreased in X/Y compared to their parents average
* growth periods to reach 50 and 75% was increased in X/X, decreased in X/Y in comparison with both parents

Reprod. development

* flowering (first\_flowers\_DAS / peak\_flowers\_DAS) advanced in X/X, delayed in X/Y compared to their parents average
* flowering (first\_flowers\_DAS / peak\_flowers\_DAS) advanced in X/X, delayed in X/Y in comparison with both parents
* BBCH51 (Hauptinfloreszenz inmitten der obersten Blätter von oben sichtbar) compared to their parents average
* BBCH51 (Hauptinfloreszenz inmitten der obersten Blätter von oben sichtbar) in comparison with both parents
* BBCH61 (ca. 10% der Blüten am Haupttrieb offen. Infloreszenzachse verlängert) compared to their parents average
* BBCH61 (ca. 10% der Blüten am Haupttrieb offen. Infloreszenzachse verlängert) in comparison with both parents
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* Pods/plant was increased in X/X, decreased in X/Y in comparison with both parents
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* seeds\_per\_pod was increased in X/X, decreased in X/Y compared to their parents average
* seed wt\_10p\_g was increased in X/X, decreased in X/Y in comparison with both parents
* TGW\_g was increased in X/X, decreased in X/Y compared to their parents average
* TGW\_g was increased in X/X, decreased in X/Y in comparison with both parents

**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.