**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.

**Biomass**

* Plant height
* Branch number/plant

**Growth rate**

* R01\_raceme\_start:
* R02\_mean\_rate:
* R03\_max\_rate:
* R04\_DAS\_of\_max\_rate:
* R05\_main\_ext\_period:
* R06\_50ht\_period:
* R07\_75ht\_period:
* R08\_50ht\_rate:
* R09\_75ht\_rate
* R10\_max\_height:
* R11\_max\_height\_DAS:
* R12\_extension\_days\_to\_max:

**Reproductive development**

* first\_flowers\_DAS:
* peak\_flowers\_DAS:
* flowering\_days\_to\_peak:
* total\_flowering\_days:
* flowering\_finished:
* pixel.day\_peak:
* pixel.days\_to\_peak:
* total\_pixel.days:
* mean\_pixel.days\_to\_peak:
* BBCH51
* BBCH61

**Seed yield**

* wt/pod = single pod weight
* pod wt =Pod weight/plant = total plant pod weight
* Pods/plant:
* Pods/branch:
* Main = pods on the main stem
* Weight/pod:
* seed wt\_10p\_g
* seeds\_per\_pod
* TGW\_g

**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

**Biomass**

* Plant height
* Branch number/plant

**Growth rate**

* R01\_raceme\_start:
* R02\_mean\_rate:
* R03\_max\_rate:
* R04\_DAS\_of\_max\_rate:
* R05\_main\_ext\_period:
* R06\_50ht\_period:
* R07\_75ht\_period:
* R08\_50ht\_rate:
* R09\_75ht\_rate
* R10\_max\_height:
* R11\_max\_height\_DAS:
* R12\_extension\_days\_to\_max:

**Reproductive development**

* first\_flowers\_DAS:
* peak\_flowers\_DAS:
* flowering\_days\_to\_peak:
* total\_flowering\_days:
* flowering\_finished:
* pixel.day\_peak:
* pixel.days\_to\_peak:
* total\_pixel.days:
* mean\_pixel.days\_to\_peak:
* BBCH51
* BBCH61

**Seed yield**

* wt/pod = single pod weight
* pod wt =Pod weight/plant = total plant pod weight
* Pods/plant:
* Pods/branch:
* Main = pods on the main stem
* Weight/pod:
* seed wt\_10p\_g
* seeds\_per\_pod
* TGW\_g

**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.

**Biomass**

* Plant height
* Branch number/plant

**Growth rate**

* R01\_raceme\_start:
* R02\_mean\_rate:
* R03\_max\_rate:
* R04\_DAS\_of\_max\_rate:
* R05\_main\_ext\_period:
* R06\_50ht\_period:
* R07\_75ht\_period:
* R08\_50ht\_rate:
* R09\_75ht\_rate
* R10\_max\_height:
* R11\_max\_height\_DAS:
* R12\_extension\_days\_to\_max:

**Reproductive development**

* first\_flowers\_DAS:
* peak\_flowers\_DAS:
* flowering\_days\_to\_peak:
* total\_flowering\_days:
* flowering\_finished:
* pixel.day\_peak:
* pixel.days\_to\_peak:
* total\_pixel.days:
* mean\_pixel.days\_to\_peak:
* BBCH51
* BBCH61

**Seed yield**

* wt/pod = single pod weight
* pod wt =Pod weight/plant = total plant pod weight
* Pods/plant:
* Pods/branch:
* Main = pods on the main stem
* Weight/pod:
* seed wt\_10p\_g
* seeds\_per\_pod
* TGW\_g

**Comparison group 4: F1 allohexaploids**

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."*

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

**Biomass production**

All of the nine F1 allohexaploid genotypes showed an increased plant height compared to the mid parent value and four of them outperformed both parental lines. This pattern was observed in the manual measurements as well as in the imaging analyses.

Across all F1 allohexaploid genotypes branch number per plant was between the parent genotypes, in the majority of cases closer to the lower performing parent. Only one genotype (N5C2J2.N5C2J2) showed more branching than both of the parents.

**Growth rate**

The increased maximum height of the allohexaploids was partly due to higher growth rates. Compared to the mid parent value growth rates to reach 50 and 75% of maximum raceme height were enhanced in almost all allohexaploids. Four and three were growing faster than both of their parents during the period up to 50 and 75% of maximum height, respectively.

Growth periods in terms number of days to reach 50 and 75% of maximum height do not show a uniform trend. Most allohexaploids were between the parental genotypes. Only one had a longer and one a shorter growth period. The duration of the whole growing phase from start of raceme extension to reaching maximum height was even more variable and hence did not show any clear pattern.

**Reproductive development**

Days to flowering

* first\_flowers\_DAS:
* peak\_flowers\_DAS:
* flowering\_days\_to\_peak:
* total\_flowering\_days:
* flowering\_finished:
* pixel.day\_peak:
* pixel.days\_to\_peak:
* total\_pixel.days:
* mean\_pixel.days\_to\_peak:
* BBCH51
* BBCH61

**Seed yield**

In seven of the nine F1 allohexaploid genotypes the number of pods per plant was above the mid parent value. Three genotypes performed above both parental lines, only one below.

The number of seeds per pod was variable: Six of nine allohexaploid genotypes were above the mid parent value, four of these were higher than both parents. On the other hand side, there were also two allohexaploids showing lower seed numbers compared to both parents.

* wt/pod = single pod weight
* pod wt =Pod weight/plant = total plant pod weight
* Pods/plant:
* Pods/branch:
* Main = pods on the main stem
* seed wt\_10p\_g
* seeds\_per\_pod
* TGW\_g