**EPPN project: Results parent-progeny comparisons**

**Resythesized *Brassica napus***

Growth was significantly enhanced in resythesized *Brassica napus* R2O4 compared to its diploid parent genotypes. Synthetic *B. napus* had an average maximum height of 179 cm, which was 60 cm above the tallest parent. The increased maximum height was associated with prolonged growth periods: shoot elongation up to 75% of maximum height was extended to 20.9 days and exceeded the longest parental growth period on average by 5.6 days. In contrast, branching was reduced compared to the mid-parent mean.Flowering time in resythesized *Brassica napus* did not differ significantly from the parents.

Several fertility estimates were decreased in synthetic *B. napus* compared to the mid-parent mean. Total plant pod weight was on average 36.1 g, which was 5.95 g lower than the mid-parent value. This may be due to the low number of seeds per pod: with an average of 9.4, the synthetic *B. napus* produced half as many seeds (8.6 fewer seeds per pod) than the lowest parental seed number per pod. In contrast, thousand seed weight with an average of 6.18 g exceeded the highest parental value by 1.04 g (~15%).

***Brassica juncea* × *B. carinata* (JC) hybrids**

The two JC hybrid genotypes (J1C1 and J1C2, one progeny set of each) both reached a lower maximum height at harvest than their parents on average: with a mean height of 108 cm they were 31 cm smaller than the mid-parent mean. This was due to lower growth rates compared to the mid-parent value (during the period up to 50% of maximum height). In addition, branching was also reduced in genotype J1C1 compared to the parental lines.

Flowering time was 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 the respective mid-parent means.

Fertility traits were also highly variable in JC hybrids. Total plant pod weight was extremely reduced in genotype J1C2 (by 29.13 g to 3.92 g) and moderately reduced in genotype J1C1 (by 11.35 g to 28.1 g) compared to the parental mean. The extremely low plant pod weight in genotype J1C2 is consistent with the lower number of pods per plant and the reduced thousand seed weight found for this genotype. Genotype J1C1, in contrast, showed a higher number of seeds per pod and an increased seed weight per ten pods compared to the parent average; pod-related data was unfortunately not available for this genotype.

**Novel allohexaploids (NCJ; *B. napus* × *B. carinata* × *B. juncea*)**

Among the 17 progeny sets from seven NCJ allohexaploid genotype combinations, eight reached a maximum height (measured by hand) above the mid-parent mean, whereas two remained below the mid-parent mean and the rest was not different. The taller progeny sets (genotypes N5C2J2, N4C2J1, N5C2J1 and N1C1J1) had an average height of 167 cm, which was 36.3 cm higher than the parental mean. Four progeny sets, belonging to genotypes N5C2J1 and N1C1J1, even outperformed the better parent (transgressive segregation). The image analyses showed mostly the same result: 7/17 progeny sets had a higher biomass production than the mid-parent mean. 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.

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 these progeny sets had 7.7 more branches than the parental mean, and five progeny sets even exceeded the higher performing parent. A lower branch number than the parental average was only found in 3/17 progeny sets of (genotypes N5C2J2 and N6C2J2).

The 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) and advanced in 2/17 progeny sets, whereas it was not different in the others. With an average of 64.2 days (between sowing and appearance of first flower pixels) the delayed progeny sets started flowering by 8.6 days later compared to the mid-parent mean. Four progeny sets lagged behind their latest flowering parent. This delay was found by imaging analyses as well as by visual BBCH scoring.

Fertility estimates were found to be reduced in relation to the parental averages across all seven NCJ allohexaploid genotype combinations. In 14/17 progeny sets single pod weight was lower than the parental mean, while it did not differ in the others. With an average of 0.038 g it was 0.028 g lower than the MPV, and three progeny sets were even below the lowest parental weight. The decline in pod weight can be explained by a lower number of seeds per pod (found in 12/17 progeny sets) and a lower seed weight (found in 11/17 progeny sets) compared to the average of the parents. 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 (from genotype XX) showed similar seed fertility to the mid-parent mean for seed number and weight.

**Novel allohexaploids (*B. oleracea × B. juncea*)**

Of the two O1J3 allohexaploid progeny sets, one grew 35.5 cm taller than the parent average and reached a maximum height of 154 cm, whereas the other progeny set was not different from the MPV. This was due to a prolonged growth period up to 75% of maximum height, which took 23.9 days and exceeded the longest parental growth period by 8.5 days on average. With regard to maximum height, the extension of growth periods compensated for relatively low growth rates close to the lower performing parent. Pod weight per plant was decreased in one of the two progeny sets (by 9.9g to 24.2g), whereas the other one was not different from the MPV. This was despite the increased pod number per plant, which could not compensate for the decline in pod weight due to a reduced seed number and weight.

**Allohexaploid hybrids (F­1­ hybrids between different allohexaploid lineages)**

Across the nine 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), the others were not significantly different from the MPV. According to the hand measurements 3/9 were by 26.3 cm taller than the mid-parent mean, with an average maximum height of 168.3 cm.

Superior maximum heights were rather due to increased growth rates than to extended growth periods. Four of the genotypes showing increased heights relative to the smaller parent also had higher growth rates in comparison with this parent. However, in relation to the mid-parent mean growth rates were mostly not significantly increased, except for one genotype. Branching in the F1 allohexaploids was within the range of the parental lines.

Flowering time was highly variable among the nine F­1 allohexaploid genotypes. Nevertheless, 3/9 genotypes started flowering earlier than their parents (on average 3.5 days compared to the mid-parent mean), whereas the others were not significantly different from the MPV. Compared to the development of each individual parent, 6/9 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.

Differences in fertility traits between the homozygous allohexaploid parents and their heterozygous F­1 progeny were highly variable among the various parent-progeny sets, although rarely significant. Pod weight per plant was not significantly altered compared to the mid parent mean in any progeny set. Nevertheless, some of the underlying traits showed shifts in a few cases, but theses were often in opposite directions. For example, seed weight per ten pods was significantly different from the mid-parent mean in three progeny sets: in 2/9 it was reduced, whereas in 1/9 it showed an increase. However, in some of the heterozygous F1 allohexaploids fertility is likely to be reduced compared to their parent average: Genotypes N5C2J2.N5C2J2 and N6C2J2.O1J3 showed a decline in seed number per pod (by 2 to 4 on average) and a decreased seed weight per ten pods (by 676.5 g to 783.5 g on average).

**General trends and comparisons across experimental progeny sets**

* Which traits were most affected by allopolyploidisation, and in which direction? (e.g. : was flowering time generally delayed, fertility generally decreased, and plant height increased?)
* What does the comparison of the novel allohexaploids to their F1 hybrids tell us about relative contributions of allelic heterosis and increase in ploidy level? (do we see any difference in which traits are affected when we go from allotetraploid to allohexaploid compared to when we make allohexaploid hybrids?)