Amelioration of genetic diversity and its assessment in 
*Brassica napus* – *carinata* introgression lines

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**Abstract.** One of the breeding goals in *Brassica napus* has been to facilitate introgression of novel traits from wild or closely related species through inter- and intra-specific crosses. The present study is based on the evaluation of *B. napus – carinata* introgression lines (ILs) for introgressed morphological and genetic diversity in comparison to the parental *B. napus* lines. A set of 81 *Brassica napus – carinata* ILs, developed previously following a back cross strategy, was assessed for introgressed genetic diversity by comparing them with the 15 *B. napus* recipient parents under two environments (E1: timely sown and E2: late sown) for phenotypic expression of 13 morpho-physiological traits. Trait averages as well as trait variations were generally higher for ILs, indicating introgression of variability in the desired direction. In comparison to the parents, the ILs were observed to be early to flower, early to mature, higher yielding, had more silique on the main axis and the silique were longer. ANOVA revealed highly significant differences amongst genotypes for 10 traits of which 4 traits, viz., days to 50% flowering, days to 100% flowering, plant height and pod length were highly significant in both the environments. SNP-based chromosome-wise gene diversity, as estimated using software SELECTIONTOOL, indicated high estimates for individual chromosomes in ILs as compared to the parental lines. The phenotypic variability estimated for majority of the traits in ILs is being utilized for developing new high yielding rapeseed varieties.

1. **Introduction**

The allopolyploid *Brassica napus* L. (AACC, 2n= 38) is considered to have originated ~7500 years ago [1] by way of natural hybridizations between *Brassica rapa* (AA, 2n = 20) and *Brassica oleracea* (CC, 2n= 18) [2,3]. However, these dual impediments of polyploidy and domestication, coupled with intensive breeding, especially for canola quality, have tenably culminated in a very narrow genetic base of the crop [4,5]. This is apparent by the absence of exploitable variation as well as declining response to selection for several traits of breeding value and agronomic interest. Furthermore, *B. napus* does not have any known wild forms. Therefore, one of the breeding goals is to enhance variability through inter- and intra-specific hybridizations with progenitor or related species.

In advanced breeding lines, morphological, phenological and agronomical traits have customarily been used as a benchmark for the detection of directly introgressed novel variation. However, new
genetic variations are also produced in early generations after inter- and intra-specific hybridizations [6,7] which too are of equal interest. There is ample documentation to prove that inter-specific hybridization (including allopolyploidy) leads to genome rearrangements [8,9], elimination of parental DNA sequences and gene conversions [10], transposon activation/transposon-induced insertional mutagenesis [11,12] and epigenetic changes [13-17], which in turn translate to phenotypic changes [18,19].

The B-genome containing Brassica species (B. nigra, B. carinata and B. juncea) are a treasure trove of valuable agronomic traits and of these, the allotetraploid B. carinata (BBCC; 2n = 34) is important as a potential source for introgression of desirable variability. The present investigation was thus planned to assess the B. napus - carinata introgression lines (ILs) for introgressed morphological and genetic diversity in comparison to the parental B. napus lines.

2. Materials and methods

2.1. Plant material

The germplasm, developed previously [20] following a back cross strategy (Figure 1), comprised of 81 Brassica napus – carinata ILs and 15 distinct cultivars of B. napus that were initially hybridized with B. carinata cv. PC5 (Appendix Table A). The study was conducted under two environments [timely-sown {first week of October (E1)} and late-sown {last week of November (E2)}] during the winter season of 2012-2013. Since varied field measurements necessitated a different layout plan for each environment, therefore the experiment was carried out in an alpha lattice design with two replications. Thus, E1 had 12 blocks while E2 had 16 blocks in each replication. Each genotype, randomized for each replication using software CropStat v.7.2, was sown in a 2.5 m x 1.8 m plot having 4 rows, 45 cm apart with plant-to-plant spacing of 15 cm. The crop was cultivated following recommended cultural practices for irrigated conditions.

2.2. Phenotyping for quantitative traits

The data were recorded for 13 agro-morphological traits, namely: days to 50% flowering (DTF50%), days to 100% flowering (DTF100%), number of leaves before bolting (LN), plant height in cm (PH), raceme height in cm (RH), number of primary branches per plant (PB), number of secondary branches per plant (SB), number of pods on main axis (PMA), pod length on main axis in cm (PL), seeds per pod (S/P), seed yield in g (SY), biological yield in kg (BY) and harvest index (HI). Data on DTF50% (when ≥ 50% of the plants have initiated flowering), DTF100% (when ≥ 90% of the plants have initiated flowering), SY and BY were recorded on plot basis. For remaining characters, the data were recorded from five random competitive plants from the two middle rows.

2.3. DNA extraction and quantification

Following standard procedure [21], DNA was isolated from 3-4 juvenile leaves from nearly a month-old seedling of each genotype that were frozen immediately after collection till further processing. Using liquid nitrogen, the leaves were homogenized to a fine powder and pre-warmed (65°C) modified CTAB extraction buffer (900 µl) with high concentrations of NaCl and Poly-Vinyl Pyrrolidone (PVP) for removal of polysaccharides [22] and polyphenols [23], respectively, was added. After incubating the sample at 65°C for 45 min with intermittent gentle shaking, chloroform: isoamyl alcohol (1:2) mixture (800 µl) was added and the sample then shaken thoroughly to ensure complete phase emulsification. Following centrifugation (12000 rpm for 20 min), the supernatant was decanted and subsequent addition of ice-cold ethanol (600 µl) lead to DNA precipitation, which was looped out and transferred to a fresh tube. The pellet was washed twice (70% ethanol) to remove residual CTAB and air-dried (37°C) overnight. Dissolved DNA (≥100 µl 1x TE buffer) was treated with RNase (incubation at 37°C for 1 hr) and its quality and quantity assessed using “Eppendorf BiophotometerPlus”.

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Figure 1. Development of *Brassica napus-carinata* introgression lines (ILs). Hybridizing diverse *B. napus* genotypes to *B. carinata* cv. PC5; backcrossing *F*₁s to respective *B. napus* parent; selfing BCs for 2 generations; again, backcrossing BC₁S₂ to respective *B. napus* parent; selfing BC₂ and practising selection in each subsequent generation.
2.4. SNP genotyping
SNP genotyping requires high-quality DNA; of the 81 ILs and 15 parents, only 69 ILs and 5 parents (marked with an asterisk (*) in Appendix Table A) yielded such high-quality DNA, which was lyophilized and shipped to The CILR Laboratory, University of Queensland, Australia for genotyping. A 60k SNP array (Illumina Infinium), carrying 52,157 SNPs for all the A- and C-genome chromosomes of *B. napus*, was used to assess the quantum of gene diversity in the ILs. All protocols were run as per the manufacturer’s specifications. An Illumina HiScanSQ scanned the chips and data were visualized using Genome Studio V2011.1 (Illumina, Inc., San Diego, CA, USA).

2.5. Statistical analysis
The phenotypic data were analyzed with SAS v.9.3 (SAS Institute Inc., Cary, NC). The presence of statistically significant differences among the genotypes for each trait was checked with the GLM procedure. Contrast analysis was performed to check for significant variation in the ILs as compared to the parents by conducting all simulations in R version 3.0.0 [24]. Software package SELECTIONTOOL [25] was utilized for estimating the quantum of gene diversity introgressed into the lines.

3. Results

3.1. Phenotypic variations of quantitative traits in introgression lines
The phenotypic data with respect to the thirteen traits recorded under two environments, viz. E1 (timely-sown) and E2 (late-sown) are depicted in Figure 2. The data show wide variation for genotype means over the two environments. Averages as well as trait variations were generally higher for environment E1 as compared to environment E2. Comparison of the means of parental lines and introgression lines indicated introgression of variability in the desired direction. As compared to the parental lines, introgression lines were observed to be early to flower (109.8 vs. 117.1 days in E1 and 103.6 vs. 111.5 days in E2); were taller (183.3 cm vs. 162.8 cm in E1 and 174.9 cm vs. 161.1 cm in E2); had more siliquae on the main axis (68.6 vs. 59.3 in E1 and 70.6 vs. 61.5 in E2); exhibited longer pod length in E1 (7.0 cm vs. 6.8 cm) but were at par with the parents in E2 (6.9 cm vs. 6.9 cm); yielded higher in E1 (1.3 kg/plot vs. 0.9 kg/plot) but were at par with the parents in E2 (0.7 kg/plot vs. 0.8 kg/plot). The seed size trait could not be evaluated since the E1 seed was accidently mixed. In such a scenario, the test weight (1000 seed weight) of parents and ILs used for sowing the trial was evaluated. It was found that the seed size of the parents ranged from 2.00 gm to 3.68 gm, giving a mean weight of 3.04 gm (Figure 2). The seed size of the ILs ranged from 3.11 gm to 5.06 gm, giving a mean weight of 3.76 gm. This trait has not been included in the analysis.
3.2. Analysis of variance
The Analysis of variance (ANOVA) for the phenotypic trait data of the 96 test genotypes grown under two environments is given in Table 1. Highly significant differences (α=0.01) were found amongst genotypes with respect to 4 quantitative traits; viz. DTF50%, DTF100%, plant height and pod length in both the environments. Differences amongst the genotypes were also highly significant (α = 0.01) for seed yield and harvest index under E1 and for number of pods on main axis under E2. The genotypes were at par with each other for the rest of the traits in either of the environment; exceptions being the number of primary branches per plant under E1 and seed yield per plot under E2, both of which traits were significant at α = 0.05. Pooled analysis of variance suggested significant differences across the two environments. Genotype x environment effects were highly significant (α=0.01) for DTF50%, DTF100%, pod length and seed yield.

3.3. Genetic diversity in B. napus - carinata introgression lines
In a population under Hardy-Weinberg equilibrium, the proportion of heterozygous genotypes expected for a given locus is characterized by gene diversity or expected heterozygosity [26]. Accordingly, an unbiased estimator of gene diversity was devised for random samples of unrelated, non-inbred individuals [27]. However, when inbred lines are included, this unbiased estimator of gene diversity exhibits a downward bias [28,24]. Therefore, in a sample of diploid individuals, the expected value of gene diversity was derived to account for the effects of inbreeding [28,29], thereby producing an unbiased estimator that makes use of the mean inbreeding coefficient across sampled individuals. This
inbreeding coefficient of an individual for a randomly chosen locus is defined as the probability that the two alleles of the individual are inherited identically by descent from a common ancestor. This estimator was extended to account for the bias produced in samples containing close relatives [24], which is implemented in the SELECTIONTOOL software.

### Table 1. Analysis of variance for 13 morphological traits studied in the 81 introgression lines and 15 parents of *B. napus.*

| Source   | df | DTF50% | DTF100% | LN | PH | RH | PB | SB | PMA | PL | S/P | SY | BY | HI  |
|----------|----|--------|---------|----|----|----|----|----|-----|----|-----|----|----|-----|
| Rep      | 1  | 56.33  | 39.422  | 0.594| 1951.77**| 23.840| 3.783| 113.62**| 2506.64*| 0.003| 47.084| 0.119| 9.707| 0.0026|
| Blk (rep)| 22 | 28.926 | 24.458  | 0.590| 139.260| 31.490| 0.964| 5.455 | 135.730| 0.205| 10.427| 0.103| 3.749| 0.0007|
| Trt      | 95 | 219.95**| 186.24**| 0.447| 263.74**| 82.53* | 1.11* | 4.906 | 142.53* | 0.095| 14.533| 0.046| 1.714| 0.0007|
| Error    | 73 | 25.623 | 28.507  | 0.276| 68.580 | 38.398| 0.608| 4.784 | 69.300 | 0.095| 14.533| 0.046| 1.714| 0.0007|

| Source   | df | DTF50% | DTF100% | LN | PH | RH | PB | SB | PMA | PL | S/P | SY | BY | HI  |
|----------|----|--------|---------|----|----|----|----|----|-----|----|-----|----|----|-----|
| Rep      | 1  | 10.55  | 57.42   | 2.30 | 5.92 | 81.64 | 0.04 | 25.23 | 21.00 | 0.02 | 1.47 | 0.12 | 35.54**| 0.0047|
| Blk (rep)| 30 | 4.57   | 7.39    | 0.81 | 52.85 | 28.26 | 0.42 | 6.95 | 72.60 | 0.04 | 21.00 | 0.02 | 35.54**| 0.0047|
| Trt      | 95 | 24.56**| 47.72** | 0.97 | 212.08**| 49.99 | 0.41 | 3.90 | 145.76**| 0.29**| 20.14 | 0.049| 1.54 | 0.0012|
| Error    | 65 | 3.23   | 5.97    | 0.83 | 85.04 | 35.06 | 0.27 | 5.47 | 56.85 | 0.15 | 10.38 | 0.03 | 1.41 | 0.0007|

| Source   | df | DTF50% | DTF100% | LN | PH | RH | PB | SB | PMA | PL | S/P | SY | BY | HI  |
|----------|----|--------|---------|----|----|----|----|----|-----|----|-----|----|----|-----|
| Env      | 1  | 4565.08**| 2821.91**| 26.51**| 2785.66**| 2094.56**| 10.14**| 20.83 | 352.83 | 0.12 | 190.19**| 15.46**| 75.06**| 0.17**|
| Rep      | 1  | 57.82  | 96.00   | 0.28 | 874.23 | 96.86 | 2.30 | 15.88 | 1034.38| 0.026| 32.60 | 0.23 | 41.19 | 0.01|
| Blk      | 15 | 18.08  | 18.10   | 0.76 | 110.65 | 16.38 | 0.37 | 9.13  | 99.65  | 0.30 | 11.94 | 0.23 | 7.70  | 0.001|
| Trt      | 95 | 181.81**| 184.03**| 0.90 | 469.80**| 101.78**| 1.09**| 5.34  | 252.45**| 0.74 | 17.67 | 0.12**| 2.19  | 0.002**|
| Env x Trt| 95 | 72.84**| 60.46** | 0.62 | 58.96  | 32.69 | 0.68 | 5.54  | 67.25  | 0.22**| 14.26 | 0.096**| 1.62 | 0.001|
| Error    | 176| 14.84  | 16.81   | 0.58 | 83.03 | 36.36 | 0.52 | 5.79  | 79.10  | 0.16 | 11.90 | 0.04 | 1.63  | 0.001|

| Source   | df | DTF50% | DTF100% | LN | PH | RH | PB | SB | PMA | PL | S/P | SY | BY | HI  |
|----------|----|--------|---------|----|----|----|----|----|-----|----|-----|----|----|-----|
| Rep      | 1  | 57.82  | 96.00   | 0.28 | 874.23 | 96.86 | 2.30 | 15.88 | 1034.38| 0.026| 32.60 | 0.23 | 41.19 | 0.01|
| Blk      | 15 | 18.08  | 18.10   | 0.76 | 110.65 | 16.38 | 0.37 | 9.13  | 99.65  | 0.30 | 11.94 | 0.23 | 7.70  | 0.001|
| Trt      | 95 | 181.81**| 184.03**| 0.90 | 469.80**| 101.78**| 1.09**| 5.34  | 252.45**| 0.74 | 17.67 | 0.12**| 2.19  | 0.002**|
| Env x Trt| 95 | 72.84**| 60.46** | 0.62 | 58.96  | 32.69 | 0.68 | 5.54  | 67.25  | 0.22**| 14.26 | 0.096**| 1.62 | 0.001|

*: Significant at α = 0.05; **: Significant at α = 0.01
Figure 3. Enhanced genetic diversity of the introgression lines as compared to the parental lines, highlighted with the use of SELECTIONTOOL software. (A-genome chromosomes are represented by numbers 1-10 while C-genome chromosomes are represented by numbers 11-19). (a) Parental lines and (b) Introgression lines.

Figure 3 (obtained by using SELECTIONTOOL software) depicts the estimates of chromosome-wise gene diversity for parents as well as for ILs. The estimates for individual chromosomes were high in ILs as compared to the diversity present in individual chromosomes of the parental B. napus lines. This was especially so for A-genome chromosomes of the ILs; for the C-genome chromosomes, enhanced gene diversity was recorded only for chromosome 19 (C9). Increased genetic diversity could partly be attributed to B-genome translocations/introgressions replacing chunks of chromosomes of the recipient B. napus as also observed with SSR and GISH analyses [30]. During the course of the present investigation, we have observed that most of the ILs carried B-genome segments from B. carinata [30], but this diversity of B-genome introgressions could not be accounted for by A-/C-genome SNPs (Unpublished), since the B-genome SNPs were unavailable at that point of time.

4. Discussion

The existence of variability is imperative for any breeding program. A number of studies have shown that B. napus possesses a narrow genetic base [31-36,15], which has been further restricted through aggressive plant breeding efforts towards canola quality [37]. This is apparent by the absence of exploitable variation as well as declining response to selection for several traits of breeding value and agronomic interest.

The present set of 81 B. napus-carinata introgression lines was evaluated for introgressed variability. Comparison of phenotypic means indicated introgression of desirable variability for DTF50% (less), days to maturity (less), number of siliquae on the main shoot (more), siliqua length (more) and seed yield (more). Significant differences were found amongst genotypes with respect to DTF50% and DTF100%. Ostensibly, early flowering and early maturing genotypes exhibit high performance due to lack of heat stress at grain filling stage. Early flowering B. napus genotypes are of immense relevance since they can escape terminal heat stress and also adapt to avoid pest pressure [38].

Pooled analysis suggested significant differences across the two environments thereby indicating the differential response of genotypes to environment. The lower and higher range for majority of the traits was found in the introgression lines rather than the B. napus parents. This is indicative of the
transgressive segregants that were obtained during the course of development of ILs. A collective appraisal of the data in Table 1 and Figure 2 strongly suggests that three agro-morphological traits, viz. pod length, seeds per pod and seed yield can profitably be used for selection breeding to bring about quantitative improvements in rapeseed. Higher heritability of a trait is helpful in breeding new varieties with desirable agronomic traits. The phenotypic variability estimated for a majority of traits showed that these introgression lines can indeed be utilized for developing new high yielding rapeseed varieties, especially those possessing high pod shatter tolerance.

A-genome of *B. napus* possesses more gene diversity as compared to its C-genome is very well known. A major objective of developing introgression lines of *B. napus* was to enhance gene diversity by introgressing novel variation from *B. carinata*. It appears that the enhanced gene diversity of A-genome chromosomes in the introgression lines developed in the present study possibly accrued from introgressions from B-/C-genome of *B. carinata*. Pairing and genetic exchange between A- and C-genome chromosomes of Brassica species has also been earlier reported [39].

That the alien gene introgressions can potentially increase productivity was first observed in oats [40]. Then followed the collegial association of QTL mapping and alien gene introgression, and the AB-QTL strategy [41]. The ILs described in this study ferry substituted B-genome segment(s) on the A-/C-genome of *B. napus*, which may bear crucial productivity and/or defense related QTLs, and can thus be utilized for its genetic and functional genomic analysis. Currently, the genes/QTLs controlling productivity and pod shattering in *B. napus* are being mapped using these ILs.

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Appendix

Table A. List of *B. napus– carinata* Introgression Lines (ILs) and *B. napus* parental lines used in the study; the lines marked with * were used for molecular analysis.

| Code   | Entry                                                                 |
|--------|-----------------------------------------------------------------------|
| *IL 1  | CHARLTON/NCA/DWARF/CARINATA HARD                                     |
| IL 2   | CHARLTON/NCA-3/SH9                                                   |
| *IL 3  | CHARLTON/NCA-3/SH8                                                   |
| *IL 4  | CHARLTON/NCA-3/SH8+                                                  |
| *IL 5  | CHARLTON/NCA-3/SH7+                                                  |
| *IL 6  | CHARLTON-MONTY/NCA-2/DT/SH 10                                        |
| *IL 7  | CHARLTON-MONTY/NCA-2/SH10                                            |
| IL 8   | MYSTIC/NCD-2/SH 10                                                   |
| *IL 9  | MYSTIC/SH9+                                                          |
| *IL 10 | MYSTIC/NCA-4/SH8+                                                   |
| *IL 11 | MYSTIC/NCA-4/SH8                                                    |
| *IL 12 | MYSTIC/NCD-2/SH7                                                    |
| *IL 13 | RAINBOW/NCA /LONG PODS/SH10                                          |
| *IL 14 | RAINBOW/NCA/SH10                                                    |
| *IL 15 | RAINBOW/NCD-4/SH9+                                                  |
| *IL 16 | RAINBOW/NCA/SH9+                                                    |
| *IL 17 | RAINBOW/NCD-4/SH9+                                                  |
| *IL 18 | RAINBOW/NCD-4/SH 9                                                  |
| *IL 19 | RAINBOW/NCA-3/SH8                                                   |
| *IL 20 | RQ-001/NCA-2/SH10                                                    |
| *IL 21 | RQ-001/NCA-2-2/SH10                                                  |
| *IL 22 | RQ-001/NCA/TRILOCULAR                                               |
| *IL 23 | RQ-001/NCA-2/SH9+                                                   |
| *IL 24 | RQ-001/NCA-2/2/SH9+                                                 |
| *IL 25 | RQ-001/NCC-2-2/SH9                                                  |
| *IL 26 | RQ-001/NCA-2/2/SH8+                                                 |
| *IL 27 | RQ-001/NCA-2/2/SH8                                                  |
| *IL 28 | RQ-011/NCB/SH10                                                     |
| *IL 29 | RQ-011/NCB/SH10                                                     |
| *IL 30 | RQ-011/NCB/SH10                                                     |
| *IL 31 | RQ-011/NCA/SH10+                                                    |
| *IL 32 | RQ-011/NCB/SH8                                                      |
| *IL 33 | RQ-011/NCB/SH8+                                                     |
| *IL 34 | RQ-011/NCA/SH9+                                                     |
| *IL 35 | RQ-011/NCB/SH9                                                      |
| *IL 36 | RR-001/NCA-4/Alt DT/SH10                                             |
| *IL 37 | RR-001/NCA-4/SH10                                                   |
| *IL 38 | RR-001/NCA-4/SH10                                                   |
| *IL 39 | RR-001/NCB/SH9+                                                     |
| *IL 40 | RR-001/NCB-4/SH9+                                                   |
| *IL 41 | RR-001/NCA/HEAVY BEARING/SH8                                        |
| *IL 42 | RR-002/NCA/SH9+                                                     |
| *IL 43 | RR-002/NCA/Alt/SH9                                                  |
| *IL 44 | RR-002/NCA/SH8+                                                     |
| *IL 45 | RR-005/NCA-1/SMALL PODS/CARINATA HARD                                |
| *IL 46 | RR-005/NCA/CARINATA HARD                                             |
| *IL 47 | RR-005/NCA-1/SH10                                                   |
| *IL 48 | RR-005/NCA-1/SH10                                                   |
| *IL 49 | RR-005/NCA-1/SH10                                                   |
* IL. 50  RR-005/NCA/SH10
* IL. 51  RR-005/NCA/SH10
* IL. 52  RR-005/NCA/SH9+
* IL. 53  RR-005/NCA-1/SH9+
* IL. 54  RR-005/NCA/SH9+
* IL. 55  RR-005/NCA/SH9
* IL. 56  RR-005/NCA/SH8
* IL. 57  RR-005/NCA-1/SH7
* IL. 58  RR-009/NCA/SH9+
* IL. 59  RR-009/NCB/SH9+
* IL. 60  RR-009/NCA-2/SH9+
* IL. 61  RR-009/NCA-1/SH9+
* IL. 62  RR-009/NCA/SH9+
* IL. 63  RR-009/NCA-1/SH9+
* IL. 64  RR-009/NCA/SH9+
* IL. 65  RR-009/NCB/SH9
* IL. 66  RR-009/NCA/SH7
* IL. 67  RR-009/NCA/SH8+
* IL. 68  RR-013/NCA/SH10
* IL. 69  RR-013/NCA-3/SH8
* IL. 70  RR-013/NCA-3/SH7
* IL. 71  SKIPTON/NCC-4-1/SH10
* IL. 72  SKIPTON/NCC-4-1/SH9+
* IL. 73  SKIPTON/NCA/SH9+
* IL. 74  SKIPTON/NCC/SH9
* IL. 75  SKIPTON/NCC-4-1/DWARF/SH8
* IL. 76  SKIPTON/NCC-4-1/INTENSE PODDING/SH8
* IL. 77  SURPASS 400/NCA/SH9+
* IL. 78  SURPASS 400/NCB/BOLD PODS/SH8
* IL. 79  SURPASS 400/NCB-3/SH9
* IL. 80  TRIGOLD x GSC-6/NCA-2-1/RADISH PODS/SH8
* IL. 81  TRIGOLD x GSC-6/NCA-2-1/DWARF/SH 8

Parent 82  CHARLTON
Parent 83  MONTY
* Parent 84  MYSTIC
* Parent 85  RAINBOW
Parent 86  RQ-001
* Parent 87  RQ-011
Parent 88  RR-001
Parent 89  RR-002
* Parent 90  RR-005
Parent 91  RR-009
Parent 92  RR-013
Parent 93  SKIPTON
* Parent 94  SURPASS 400
Parent 95  TRIGOLD
Parent 96  GSC-6 (napus parent, used as check)
* Parent 97  PC 5 (carinata parent, used for trait introgression)