Introduction

Rapeseed, *Brassica napus*, is an important oleiferous crop. The major producers of rapeseed are Canada, China, India and EU countries. Amphidiploid *B. napus* (AACC, 2n = 38) resulted from spontaneous hybridization between its diploid progenitors, *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18) (U 1935). In South Asian countries, oils from mustard seed (*B. rapa*, *B. juncea* and *B. carinata*) are important sources of vegetable fat. In Bangladesh, *B. rapa* is the main crop producing species of *Brassica*, but its yield is the lowest in the world (FAOSTAT 2013). Low yields are the result of a short growth period that is characteristic in the mustard-rice cropping pattern, as well as insufficient agricultural practices, and use of low yield cultivars. In Bangladesh, mustard (*B. rapa*) is well suited as a catch crop in cropping patterns with rice var. Aman (autumn)–Mustard–rice var. Boro (winter). The growth period of mustard must be shorter to include Boro rice in the cropping pattern. At present, the leading genotype, Tori-7 (*B. rapa*), is preferred by mustard growers, yet it suffers low yield due to the reasons mentioned above.

Rapeseed (*B. napus*) produces a higher yield than *B. rapa* because of a higher photosynthetic rate compared to the two parental species and vigorous growth owing to fix heterosis effect (Tsunoda 1980). Introduction of spring type *B. napus* to the subtropical region is a conceivable choice. However, ordinary spring type *B. napus* cultivars, e.g., Swedish cultivar ‘Olga’, do not flower or need an extremely long time to flower in subtropical regions because of their strict requirement for long days prior to flowering. This cannot be satisfied in Bangladesh, where day length is 11 h–12 h (Akbar 1987, Zaman 1989). In order to produce short duration *B. napus* cultivars, two different approaches have been undertaken at the Oilseed Research Centre, Bangladesh Agricultural Research Institute (BARI). One of those approaches is resynthesis of *B. napus*. Interspecific crossing between early flowering variants of *B. rapa* and *B. oleracea* resulted in hybrids that were resynthesized through ovary culture from interspecific crosses in which *B. rapa* cultivars were reciprocally crossed with *B. oleracea*. From five different combinations, 17 hybrid plants were obtained in both directions. By self-pollinating the F₁ hybrids or introgressing them with cultivated *B. napus*, resynthesized (RS) F₃ and semi-resynthesized (SRS) F₂ generations were produced, respectively. In field trial in Bangladesh, the RS *B. napus* plants demonstrated variation in days to first flowering ranging from 29 to 73 days; some of which were similar to cultivated short duration *B. napus*, but not cultivated short duration *B. rapa*. The RS and SRS *B. napus* lines produced 2–4.6 and 1.6–3.7 times higher yields, respectively, as compared to cultivated short duration *B. napus*. Our developed RS lines may be useful for rapeseed breeding not only for subtropical regions, but also for areas such as Canada and Europe where spring rapeseed production can suffer from late spring frosts. Yield and earliness in RS lines are discussed.

**Key Words:** *Brassica napus*, interspecific hybridization, ovary culture, resynthesis, semi-resynthesis, short duration.

Note

Production of high yield short duration *Brassica napus* by interspecific hybridization between *B. oleracea* and *B. rapa*

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*Brassica napus* is a leading oilseed crop throughout many parts of the world. It is well adapted to long day photoperiods, however, it does not adapt well to short day subtropical regions. Short duration *B. napus* plants were resynthesized through ovary culture from interspecific crosses in which *B. rapa* cultivars were reciprocally crossed with *B. oleracea*. From five different combinations, 17 hybrid plants were obtained in both directions. By self-pollinating the F₁ hybrids or introgressing them with cultivated *B. napus*, resynthesized (RS) F₃ and semi-resynthesized (SRS) F₂ generations were produced, respectively. In field trial in Bangladesh, the RS *B. napus* plants demonstrated variation in days to first flowering ranging from 29 to 73 days; some of which were similar to cultivated short duration *B. napus*, but not cultivated short duration *B. rapa*. The RS and SRS *B. napus* lines produced 2–4.6 and 1.6–3.7 times higher yields, respectively, as compared to cultivated short duration *B. napus*. Our developed RS lines may be useful for rapeseed breeding not only for subtropical regions, but also for areas such as Canada and Europe where spring rapeseed production can suffer from late spring frosts. Yield and earliness in RS lines are discussed.

**Key Words:** *Brassica napus*, interspecific hybridization, ovary culture, resynthesis, semi-resynthesis, short duration.
Plant materials

Parental materials were Tori-7 (toria) and BARI Sarisha 14 (yellow sarson), two short duration B. rapa cultivars, a Chinese genotype of B. oleracea var. alboglabra (B. alboglabra) and a rapid cycling line CrGC3-1 of B. oleracea. Tori-7 and BARIS-14 were developed through resynthesis and introgression programs respectively (Akbar et al. 2009). Short duration rapeseed cultivars are also important in northern rapeseed growing areas in Sweden and Canada where early frost can damage rapeseed crops; short duration turnip rape (B. rapa) is the primary crop grown in these production areas (Falk 2009, Rahman et al. 2011).

There are few studies reporting resynthesis of short duration RS B. napus despite the agricultural importance of short duration B. napus. Although BARIS-7 and BARIS-8 were selected for earliness in BARI, their degree of earliness was not equal to the extreme earliness seen in existing B. rapa cultivars such as Tori-7. One of the reasons for this is the limited number of resources for resynthesis of B. napus (Akbar 1987, 1989). Therefore, in the present study we sought to improve yield as well as earliness in B. napus adapted to short day length conditions. To do this, we attempted to resynthesize B. napus by crossing short duration B. rapa with newer cross parents such as rapid cycling B. oleracea and yellow seeded B. rapa.

Materials and Methods

Parental materials were Tori-7 (toria) and BARI Sarisha 14 (yellow sarson), two short duration B. rapa cultivars, a Chinese genotype of B. oleracea var. alboglabra (B. alboglabra) and a rapid cycling line CrGC3-1 of B. oleracea. Tori-7 and BARIS-14 were provided by the BARI Seed Bank, Bangladesh. CrGC3-1 was obtained from the Crucifer Genetic Cooperative (CrGC), University of Wisconsin, Madison, WI, USA. Cultivated B. napus, BARIS-7 and BARIS-8 were collected from BARI. Rapid cycling B. napus CrGC 5-1 was collected from CrGC. Kirariboshi is a winter type B. napus which was obtained from the National Agriculture and Food Research Organization/NARO Tohoku Agricultural Research Center, Japan. Tori-7 was partially self-compatible and the remaining A and C genome parents were self-compatible.

The RS F1 plants were self-pollinated to produce subsequent progenies, F2 and F3. At the same time, RS F1 plants were crossed with cultivated B. napus to obtain progeny SRS F1. The SRS F1 plants were then self-pollinated to obtain their corresponding F2 Progeny (Fig. 1).

Crossing, embryo rescue and chromosome doubling

Seeds were sown in 42 cell plastic trays (cell size: 3.5 × 3 cm) using vegetable soil (Yasaibaido No. 1, Honen Agri Co. Ltd., Japan). 10-day old seedlings were then transferred to plastic pots (12 cm). Seedlings and plants were grown in a greenhouse at Niigata University from 2010 to 2012. Crossing work was carried out in three seasons (except summer) under greenhouse conditions where the lowest temperature was approximately 15°C. Emasculation was conducted on floral buds one day prior to flowering. The emasculated buds were immediately dusted with fresh pollen grains collected from male parents. Pollinated flowers were isolated in thin paper bags. Ovaries bearing ovules were collected 16–20 d after pollination (DAP) for F1 embryo rescue.

Ovary culture was carried out according to the method reported by Inomata (1977). The harvested ovaries were surface-sterilized with a 70% ethanol for 3 min and subsequently treated with calcium hypochlorite solution containing approximately 1% chlorine for 15 min and rinsed twice with double sterile water. The ovaries were placed on MS (Murashige and Skoog 1962) medium aseptically supplied with 5% sucrose and 0.8% agar, adjusted to pH 5.8. Plastic petri dishes 90 × 15 mm were used for the cultures and placed in a growth chamber maintained at 24°C with a 12 h photoperiod. Ovaries were kept on the medium for 2–6 months until embryos were fully germinated and rooted. The seedlings were transplanted into six-inch pots containing vermiculite soil : regular soil (1 : 1) and covered with transparent polyethylene sheet. The plants were then placed in a growth chamber maintained at 24°C with a 12 h photoperiod. At two weeks, after proper hardening, the plants were transferred to a greenhouse. In order to restore seed fertility in the F1 hybrids, colchicine solution 0.05% was applied on leaf axils of each hybrid to double the chromosome number as per previous report (Chen et al. 1988). As an alternative method for chromosome doubling, nitrous oxide gas treatment for 30–48 h at 6 atm was also applied to F1 hybrids which were undergoing meiosis (Nukui et al. 2011). For chromosome observation, root tips from the RS F2 young seedlings were fixed in acetate alcohol (1:3) for 2 h at room temperature. The tips were hydrolyzed in 1N

![Fig. 1. Schematic diagram showing the development process of short-duration resynthesized (RS) and semi-resynthesized (SRS) B. napus (AACC).](image-url)
Production of high yield short duration *Brassica napus*

HCl for 30 sec at 60°C and stained with 1% acetocarmine solution, then squashed in 45% acetic acid. For measurement of pollen fertility, pollen grains of F2 plants and their parents were stained with 1% acetocarmine solution and fully stained pollen grains were scored as fertile; unstained pollen grains were scored as sterile.

**Different trait evaluation**

The open-field trial was laid out in a completely randomized block design with three replications (plots), from November 1, 2012 to March 30, 2013 at BARI, Gazipur, Bangladesh. Unit plot size for each experiment was 10 m × 2.5 m. The distances between plant-plant, line-line and plot-plot were 10, 40 and 100 cm, respectively. The recommended agricultural practices and pest control measures were applied when necessary for normal growth of the plants. Data were recorded for 18 traits related to flowering, morphology, yield components and oil content. Days to 1st flowering were recorded when the first plant flowered in each plot; 50% and 100% flowering dates were recorded when 50% of plants and 100% of plants in each plot flowered, respectively. Days to maturity were recorded at the 70–80% siliqua ripening stage. 10–50 plants per line in each plot were grown for phenotypic evaluation. Data for morphological characteristics, such as floral petal color, leaf shapes, leaf color, plant height and vigorous growth. All F1 hybrids were pollen-sterile, indicating that the plants were true F1 hybrids and not false hybrids derived from female parents as matromorphous or by mere chance as a result of incomplete emasculation. Chromosome doubling of the axillary floral buds the F1 hybrids induced fertile flowers and by self-pollination F2 seeds were successfully produced in TCr-2, TCr-3, TCr-4 and AlBA-16, as well as in the 7 plants of the CrT-lines. Chromosome counting confirmed that the three F2 plants in the TCr-lines were 2n = 38 chromosome species. The pollen fertilities of the confirmed F1 plants derived through resynthesis were found to be 81.1–97.4% which demonstrated similarity to the natural *B. napus* pollen fertility of 87.2–98.2% (Table 2). Nevertheless, seeds per pod in some of the F3 lines were few, especially in the CrT-lines. Due to low seed fertility, the CrT-lines, with the exception of CrT-14, were omitted in the field trial.

**Flowering and maturity**

RS F1 plants, along with their parents and cultivated *B. napus*, were grown under short-day length (11–12 h) conditions in the cropping season in Bangladesh (Fig. 2a and Supplemental Table 1). The donors of the A genome, from Tori-7 and BARIS-14, were found to be earliest for 1st flowering at 22.3 d and 30.0 d respectively. Among with

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**Table 1. Crossability of interspecific crosses between *Brassica rapa* and *B. oleracea***

<table>
<thead>
<tr>
<th>Cross combination (♀ × ♂)</th>
<th>Flower Pollinated (a)</th>
<th>Ovary set&lt;sup&gt;a&lt;/sup&gt;(rate, %)</th>
<th>Plantlets regenerated</th>
<th>Hybrids (b)</th>
<th>Cross ability (b/a, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tori-7(AA) × CrGC3-1(CC)</td>
<td>260</td>
<td>191 (73.5)</td>
<td>3</td>
<td>3</td>
<td>1.2</td>
</tr>
<tr>
<td>CrGC3-1 (CC) × Tori-7 (AA)</td>
<td>949</td>
<td>713 (75.1)</td>
<td>16</td>
<td>13</td>
<td>1.4</td>
</tr>
<tr>
<td>BARI Sharisha-14 (AA) × <em>B. alboglabra</em> (CC)</td>
<td>40</td>
<td>2 (5.0)</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>B. alboglabra</em> (CC) × BARI Sharisha-14 (AA)</td>
<td>294</td>
<td>74 (25.2)</td>
<td>1</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>CrGC3-1 (CC) × BARI Sharisha-14 (AA)</td>
<td>138</td>
<td>108 (78.2)</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>1681</td>
<td>1088 (64.7)</td>
<td>20</td>
<td>17</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> All ovaries set after pollination were used for culture.
the C genome donor parents, CrGC3-1 of *B. oleracea* was found to be early at 37.3 d while *B. alboglabra* was the latest, requiring 79.5 d during the trial. Of the RS *B. napus* plants, TCr-2 was the earliest, requiring 29.3 d to 1st flowering. TCr-4 required 35.0 d and TCr-3 and AIBA-16 required 42.7 d and 41.3 d, respectively. The entry CrT-14 required 79.5 d during the trial. Of the RS lines, CrGC5-1 was found to be early at 37.3 d while the C genome donor parents, CrGC3-1 of *B. oleracea* was latest, requiring 47.5 d to 1st flowering.

Parental lines showed extensive variation in days to maturity—80–82 d in *B. rapa*, 95–99 d in BARIS-7 and -8, 137–143 d in *B. oleracea* and 159 d in *B. napus* Kirariboshi, indicating that *B. rapa* Tori-7 and BARIS-14 could be characterized as very early, whereas CrGC5-1 and *B. alboglabra* required a long time for ripening. Days to maturity in RS *B. napus* ranged from 112–138 d, and the time for SRS *B. napus* was shorter by comparison. In comparison with its cultivars, SRS *B. napus* had a delayed maturity of 9 d later than cultivated *B. napus* (BARIS-7, BARIS-8) and 25 d later than cultivated *B. rapa* (Tori-7 and BARI-14). Greenhouse trials for RS F3 and SRS F2 were also conducted in Japan. The flowering response in RS F3 lines grown during winter in Japan ranged from 35–42 d (Supplemental Fig. 1). This confirmed the earliness of the RS F3 lines under day length (12 h) conditions in Japan.

### Yield

In *B. rapa*, seed yield per plant was 6.7 g and 5.3 g for Tori-7 and BARIS-14, respectively (Fig. 2b and Supplemental Table 1). In *B. oleracea*, CrGC3-1 and *B. alboglabra* produced 6.4 g and 13.5 g seed yield per plant, respectively. *B. napus*, BARIS-7 and BARIS-8, produced higher yields of 9.0 g and 8.9 g. The RS *B. napus* lines, with the exception of AIBA-16, had a lower number of seeds per silique, ranging from 9.9–13.0 (Supplemental Tables 1, 2). Nevertheless, the yield per plant was higher, due to an increased number of pods per plant. AIBA-16 showed the highest yield of 41.4 g due to an increase in seeds per pod (24.3) as

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**Table 2.** Characteristics of resynthesized (RS *B. napus*) F2 and semi-resynthesized (SRS *B. napus*) F2 along with their parents grown in the short-day climate of Bangladesh (Mean and ±SE)

<table>
<thead>
<tr>
<th>Parental species and resynthesized <em>B. napus</em></th>
<th>Pollen viability (%)</th>
<th>Plant height (cm)</th>
<th>Pod/plant</th>
<th>Seed/pod</th>
<th>1000 seeds (g)</th>
<th>Oil (%)</th>
<th>Erucic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RS <em>B. napus</em> F3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCr-2</td>
<td>97.4 ± 1.2</td>
<td>139 ± 3.8</td>
<td>526 ± 75.6</td>
<td>11.9 ± 0.2</td>
<td>4.5 ± 0.1</td>
<td>42.1</td>
<td>40.4</td>
</tr>
<tr>
<td>TCr-3</td>
<td>92.2 ± 4.8</td>
<td>104 ± 3.7</td>
<td>536 ± 109.0</td>
<td>13.0 ± 0.6</td>
<td>4.4 ± 0.1</td>
<td>41.8</td>
<td>43.6</td>
</tr>
<tr>
<td>TCr-4</td>
<td>91.1 ± 1.7</td>
<td>121 ± 3.4</td>
<td>324 ± 93.0</td>
<td>11.8 ± 1.1</td>
<td>4.5 ± 0.2</td>
<td>42.0</td>
<td>47.8</td>
</tr>
<tr>
<td>CrT-14</td>
<td>86.0 ± 1.5</td>
<td>104 ± 22.7</td>
<td>447 ± 203.1</td>
<td>9.9 ± 1.8</td>
<td>4.7 ± 0.0</td>
<td>–</td>
<td>43.4</td>
</tr>
<tr>
<td>AIBA-16</td>
<td>81.1 ± 2.2</td>
<td>119 ± 8.9</td>
<td>611 ± 204.9</td>
<td>24.3 ± 1.5</td>
<td>3.9 ± 0.0</td>
<td>42.5</td>
<td>40.5</td>
</tr>
<tr>
<td><strong>SRS <em>B. napus</em> F2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BARIS-7 × TCr-4</td>
<td>92.8 ± 2.1</td>
<td>94 ± 2.2</td>
<td>232 ± 26.1</td>
<td>18.0 ± 0.5</td>
<td>4.5 ± 0.1</td>
<td>42.4</td>
<td>47.9</td>
</tr>
<tr>
<td>BARIS-8 × TCr-4</td>
<td>95.1 ± 1.8</td>
<td>135 ± 7.9</td>
<td>443 ± 176.1</td>
<td>21.0 ± 1.5</td>
<td>3.6 ± 0.3</td>
<td>41.7</td>
<td>47.0</td>
</tr>
<tr>
<td>CrGC5-1 × TCr-3</td>
<td>87.0 ± 1.5</td>
<td>134 ± 4.1</td>
<td>550 ± 236.0</td>
<td>11.8 ± 1.9</td>
<td>3.6 ± 0.1</td>
<td>42.5</td>
<td>34.3</td>
</tr>
<tr>
<td>CrGC5-1 × TCr-4</td>
<td>92.9 ± 1.5</td>
<td>144 ± 3.6</td>
<td>532 ± 121.1</td>
<td>12.9 ± 1.5</td>
<td>3.6 ± 0.2</td>
<td>42.9</td>
<td>–</td>
</tr>
<tr>
<td>Kirariboshi × TCr-2</td>
<td>93.6 ± 2.5</td>
<td>147 ± 6.0</td>
<td>501 ± 84.7</td>
<td>12.5 ± 1.0</td>
<td>2.9 ± 0.1</td>
<td>42.4</td>
<td>–</td>
</tr>
<tr>
<td>Kirariboshi × TCr-4</td>
<td>97.9 ± 1.2</td>
<td>134 ± 7.0</td>
<td>344 ± 33.0</td>
<td>13.8 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>42.8</td>
<td>27.2</td>
</tr>
</tbody>
</table>

Parental species

<table>
<thead>
<tr>
<th>Parental lines</th>
<th>Pollen viability (%)</th>
<th>Plant height (cm)</th>
<th>Pod/plant</th>
<th>Seed/pod</th>
<th>1000 seeds (g)</th>
<th>Oil (%)</th>
<th>Erucic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tori-7 (AA)</td>
<td>96.1 ± 0.0</td>
<td>69 ± 1.9</td>
<td>201 ± 24.3</td>
<td>15.3 ± 0.3</td>
<td>2.2 ± 0.0</td>
<td>40.6</td>
<td>43.2</td>
</tr>
<tr>
<td>BARIS-14 (AA)</td>
<td>91.3 ± 1.4</td>
<td>73 ± 1.2</td>
<td>75 ± 2.0</td>
<td>26.0 ± 0.6</td>
<td>2.7 ± 0.0</td>
<td>42.8</td>
<td>52.8</td>
</tr>
<tr>
<td>CrGC3-1 (CC)</td>
<td>92.2 ± 2.5</td>
<td>55 ± 2.8</td>
<td>164 ± 8.3</td>
<td>8.9 ± 0.5</td>
<td>4.4 ± 0.1</td>
<td>41.3</td>
<td>53.3</td>
</tr>
<tr>
<td><em>B. alboglabra</em></td>
<td>99.0 ± 0.6</td>
<td>139 ± 3.2</td>
<td>267 ± 48.9</td>
<td>13.4 ± 4.9</td>
<td>4.3 ± 0.0</td>
<td>41.4</td>
<td>46.6</td>
</tr>
<tr>
<td>CrGC5-1 (AACC)</td>
<td>87.2 ± 0.3</td>
<td>139 ± 3.8</td>
<td>343 ± 59.3</td>
<td>17.0 ± 0.8</td>
<td>2.9 ± 0.1</td>
<td>42.3</td>
<td>33.6</td>
</tr>
<tr>
<td>BARIS-7 (AACC)</td>
<td>98.2 ± 0.5</td>
<td>88 ± 1.4</td>
<td>113 ± 11.3</td>
<td>21.1 ± 0.6</td>
<td>3.7 ± 0.1</td>
<td>41.6</td>
<td>40.2</td>
</tr>
<tr>
<td>BARIS-8 (AACC)</td>
<td>91.4 ± 1.2</td>
<td>93 ± 1.9</td>
<td>112 ± 2.8</td>
<td>23.3 ± 0.4</td>
<td>3.4 ± 0.2</td>
<td>40.5</td>
<td>51.2</td>
</tr>
<tr>
<td>Kirariboshi (AACC)</td>
<td>95.2 ± 4.8</td>
<td>96.4 ± 5.1</td>
<td>72 ± 26.7</td>
<td>5.0 ± 0.0</td>
<td>1.5 ± 0.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

LSD (0.05) 6.1 19.0 318 4.3 0.4

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'a' TCr (Tori-7 × CrGC3-1), CrT-14 (CrGC3-1 × Tori-7), AIBA (*B. alboglabra* × BARIS-14), BARIS (BARI sarisha).

a LSD (0.05); least significant difference at P = 0.05.
well as increased pods per plant (611). The relatively higher yield was also achieved in remaining RS *B. napus* lines, TCr-2 (28.3), TCr-3 (31.4), TCr-4 (17.8) and CrT-14 (24.5), indicating that the four RS lines, with the exception of TCr-4, showed a 97–363% significantly higher yield compared to the mean yield of 9.0 g per plant seen in the check cultivars (BARIS-7 and BARIS-8). In SRS *B. napus*, the F2 lines of BARIS-8 × TCr-4 showed the highest yield at 33.4 g, followed by CrGC5-1 × TCr-4 (23.8 g); the lowest yield recorded was seen in the F3 lines of Kirariboshi × TCr-4 (14.7 g). SRS *B. napus* showed a 64–273% higher yield in comparison to the mean yield of the check cultivars. The late ripening RS lines tended to produce higher yields than the early ripening lines and there was a weak association between days to maturity and yield in the parents and the tested lines, although the correlation coefficient was not significant (r = 0.36) (Fig. 3). The two exceptional cultivars of CrGC 3-1 and Kirariboshi were omitted because they had low seed fertilities of 8.9 and 5.0 seeds/pod, respectively. Such low fertility was likely the result of undetermined environmental factors such as immaturity due to late flowering or genetically controlled sterility.

The oil content in seeds was approximately 40% in all tested parental and hybrid lines, which makes them sufficient for use in oil production (Table 2). The seeds contained a remarkable 27.2–53.3% erucic acid; other important characteristics are presented in Supplemental Table 3.
what extent the C genome of *B. alboglabra* contributes to earliness in *B. napus*, however, this study could demonstrate that the C genome of *B. alboglabra* did not have any negative effect on earliness.

Akbar (1989) produced RS *B. napus* by crossing *B. rapa* Tori-7 (toria), Kalyania (brown sarson) and Sonali sarishra (yellow sarson) with *B. alboglabra* and cauliflower cultivars as *B. oleracea* parents. He carried out the field trial for earliness evaluation in Bangladesh and demonstrated early flowering in his developed RS lines, indicating that the parents used were able to transmit sufficient short day adaptability to their descendants. In the present study, yellow seeded *B. rapa* BARIS-14 and rapid cycling *B. oleracea* CrGC3-1 were used as parents. These two variants have never been used as parents to produce RS *B. napus* in previous studies. In the winter of 2012–2013 in Bangladesh, the days to 1st flowering (37.3 d) for CrGC3-1 was much earlier than that for *B. alboglabra* (79.5 d) and days to 1st flowering of the resultant hybrid plants (TCr-2, 3, 4, CrT-14) ranged from 29.3–47.5 days. Although there was a big difference in the flowering time of *B. alboglabra* and CrGC3-1, the hybrids derived from the crosses using CrGC3-1 had limited achievement of earliness. As a result, compared to cultivated BARIS-7, our RS and SRS lines showed a similar level of early flowering, but late maturity due to the extended period between flowering and maturity. The late maturity had a negative effect for earliness but contributed to the high yield by increasing yield components such as pods per plant and weight per 1000 seeds. BARIS-7, which was selected over seven years from the initial RS lines produced by Akbar (1989), showed earliness, but low yield, whereas our developed lines achieved moderate earliness and high yield. This difference may be due to differences in the varieties of parents used in each study. Akbar (1987) found that some of the reselected successive F₃ lines exhibited transgressive earliness in comparison to F₂ lines; this was consistent with our results (data not shown). Similarly, Rahman et al. (2011) reported that days to flowering progressively decreased with repeated selection for earliness across self-pollinated generations (F₃ to F₆). Successive selection in the next generation could reduce the number of days to maturity and ensure optimal high yield adaptability to existing ecological systems where the crop is produced.

**Discussion**

**Ovary culture**

Embryo, ovule and ovary culture are used as major embryo rescue techniques in interspecific hybridization of *Brassica* species where hybrid embryos cease their growth due to lack of appropriate endosperm (Chen et al. 1988, Inomata 1993, Nishi et al. 1959, Olsson 1960, Takeshita et al. 1980). Previous studies reported that ovary culture was useless in crosses of *B. oleracea* (♀) × *B. rapa* (♂), but effective in the reciprocal cross (Hossain et al. 1989, Inomata 1977, 1978, Takeshita et al. 1980). However, Song et al. (1993) successfully resynthesized *B. napus* in both crossing directions by using *B. oleracea* CRGC 3-1 as a parent. In this study, we also confirmed that CrGC3-1 had higher crossability with *B. rapa*.

**Earliness**

Short duration rapeseed cultivars must possess not only no requirement for vernalization, but also short day adaptability. The trait requiring no vernalization could be easily incorporated in short duration rapeseed because selection for that characteristic is simple; i.e. evaluating flowering time in the spring to summer cropping; many spring cultivated rapeseed genotypes are also available as parents. In transferring short-day adaptability via introgression, Rahman et al. (2011) and Zaman (1989) demonstrated that the C genome of *B. alboglabra* carries early flowering allele(s) despite the fact that *B. alboglabra* is quite late flowering in comparison to *B. rapa* and *B. napus*. In this study also, *B. alboglabra* was late (79.5 d) to 1st flowering compared to the *B. rapa* parent BARIS-14 (30.0 d). However, the hybrid plant, AIBA-16, resulting from the cross of *B. alboglabra* × BARIS-14, flowered early (41.3 d), indicating that its late flowering nature was recessively inherited in AIBA-16 and earliness seen in AIBA-16 must have come from BARIS-14. Using RS *B. napus* in this study, we could not determine to Fig. 3. Relationship between yield per plant and days to maturity in RS, SRS *B. napus* and their parents. The correlation coefficient was calculated by omitting the exceptional data of two cultivars (*).
for CrT-lines produced by using CrGC3-1 as the female parent. In order to eliminate meiotic irregularities in the RS lines, selection of lines showing stable seed fertility, as well as crossing RS lines with ordinary *B. napus* cultivars were found to be very effective (Namai et al. 1980). We therefore selected TCr-lines and AIBA-16 which had high seed fertility among their developed lines and crossed our RS lines with cultivated *B. napus*. Even so, several of the selected RS and SRS lines still had a low number of seeds/pod (Table 2), which is consistent with the results of Zhao et al. (2009) who reported that SRS lines derived from a cross between RS lines and cultivated *B. napus* had fewer seeds per pod, but many more pods per plant in comparison to rapeseed cultivars. This suggests that the seeds-per-pod trait is a key component for improving yield in rapeseed breeding using RS *B. napus*. In our developed RS lines, the drawback concerning fewer seeds per pod was counterbalanced by the setting of an increased number of pods per plant, resulting in a high yield. As a result, our developed RS lines revealed significant heterosis on yield, at least in comparison to Bangladeshi rapeseed cultivars, thus confirming the usefulness of our RS lines. This is in contrast to results showing that although RS lines have significant potential in heterosis as parents for production of F1 hybrid cultivars, raw amphidiploids derived from interspecific hybridization display low yield (Gehringer et al. 2007, Jesske et al. 2013, Seyis et al. 2006, Zou et al. 2010).

It is reported that correlation between flowering time and yield was discovered in genetic analyses using the segregating population derived from spring type and winter type rapeseed cultivars and both negative and positive correlations were observed depending on population and cropping pattern (Butruille et al. 1999, Udall et al. 2006). For example, in a segregating population, early flowering plants normally set pods in moderate climate and, thereafter, late flowering plants encounter the hot summer which inhibits seed production. In such cases, flowering time and yield have been negatively correlated. In this study, the yield for the developed short duration *B. napus*, BARIS-7 and -8 was low, while late ripening RS lines tended to produce a higher yield than early ripening lines (Fig. 3). Although further studies are required to confirm this result, the positive relationship between high yields and late maturity was often found in spring rapeseeds (Butruille et al. 1999, Starmer et al. 1998) and winter rapeseeds (Habekotté 1997), thus benefiting from a longer growth period. If yield and days to maturity could be positively correlated, it would be difficult to combine short duration with high yield in rapeseed breeding; the successive selection for earliness in RS *B. napus* populations might sacrifice yield. Overcoming negative correlation between earliness and yield might be necessary to obtain high yield short duration *B. napus*.

At present, none of the *B. napus* cultivars performed in short duration and yield at a satisfactory level to replace *B. rapa* Tori-7. The artificial *B. napus* plants we developed achieved moderate earliness and higher yield in comparison with Bangladeshi rapeseed cultivars, BARIS-7 and -8. Our RS lines could potentially be used for short duration *B. napus* breeding not only for cultivars suited to subtropical regions like Bangladesh, but also for use in production areas suffering from late season frosts such as Canada and Northern Europe. In that regard, the successive selection with appropriate balance of the two important traits (earliness and yield) will be required in subsequent generations.

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