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Development of hexaploid *Brassica* (AABBCC) from hybrids (ABC) of *Brassica carinata* (BBCC) × *B. rapa* (AA)

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Abstract

The aim of this research was to develop *Brassica* hexaploids from triploid hybrids using colchicine and to study the performances of the developed hexaploids. Triploid hybrids (genome, ABC) developed from crosses between *Brassica carinata* (genome, BBCC) and *B. rapa* (genome, AA) were used as source materials for chromosome doubling through colchicine treatment to develop *Brassica* hexaploids (genome, AABBCC). Modified injection method was found to be more effective than cotton plug method for doubling the chromosome numbers. On an average, the use of 0.10, 0.15 and 0.20% colchicine gave 36.4, 56.0 and 71.8% success in chromosome doubling by modified injection method compared to 27.4, 38.0 and 39.1% success by cotton plug method. First generation (H_1) hexaploids produced larger buds and flowers than those of both parents and triploid hybrids. The untreated triploid hybrids did not produce seeds due to having sterile pollens only while hexaploids produced fertile pollens and seeds. On an average, 59% of second generation (H_2) hexaploid seeds were euploid, where the rest of 41% seeds were aneuploid. Among the H_2 hexaploid lines, four were selected as they produced significantly higher seed yield/plant (11.57-12.4 g) compared to their parental genotypes (8.13-9.93 g). These four H_2 hexaploid lines can be used for family line development in the subsequent generations to select stable and desirable *Brassica* hexaploids.

Keywords: *Brassica*, chromosome doubling, hexaploid, fertility, yield attributes.
Abbreviations: H_1- first generation; H_2- second generation; BARI- Bangladesh Agricultural University; BINA- Bangladesh Institute of Nuclear Agriculture.

Introduction

Polyploidy has been regarded as a major breeding procedure in evolution and speciation in plants (Adams and Wendel, 2005; Leitch and Leitch, 2008; Gaeta and Chris, 2010). Generally, polyploids are more resistant/tolerant to different biotic and abiotic stresses due to enhanced production of various secondary metabolites (Lewis, 1980; Levin, 1983). So, artificial polyploidization can enhance evolution of new crop species in short time and may offer novel avenues for phenotypic response through selection of new and useful genotypes (Leitch and Bennet, 1997; Wright et al., 1998).

In crop plants, artificially developed hexaploid triticale has been found to be more adaptable with higher seed yield potential than its parents, tetraploid wheat and diploid rye (Guesdes-Pinto et al., 1996). Though hexaploids have many advantages as crop plants, but no species of the genus *Brassica* are natural hexaploids. Therefore, combination of three *Brassica* genomes (A, B and C) in the form of hexaploid may widen the environmental ranges of adaptation and selection for yield and quality in a wide range of environments. Artificially developed *Brassica* hexaploids may also be benefited from intersubgenomic heterosis (Zou et al., 2010). In Brassicaceae family, artificially developed allohexaploids with genome, AABBCC showed promising performances in important agronomic traits (Li et al., 2004; Gaeta et al., 2007; Molla et al., 2007; Prakash et al., 2009; Pradhan et al., 2010; Tian et al., 2010), but none of them has been established as crop species for cultivation. Seven germplasm materials of *B. carinata* were first introduced in Bangladesh through *Brassica* Breeding Project of Bangladesh Agricultural University (BAU) and were found to be promising in yields but had long maturity period (Rahman and Quddus, 1986). Since then there has been attempts to develop hexaploids using *B. carinata* and *B. rapa* (Molla et al., 2007; Rahman et al., 2006). Rahman et al. (1997) reported the results of crosses between *B. rapa* materials (cv. 381050) and *B. carinata* to develop hexaploid. They also subsequently crossed H_1 hexaploid as mother and diploid BAU-M/91 of *B. rapa* as pollen donor to determine the crossability. The hexaploids were better for number of seeds per siliqua and single seed weight than their parents (*B. rapa* cv. 381050 and BAU-M/91). Short duration gene of BAU-M/91 was found to be active in crossing with hexaploid. Under the above context, there is an increasing interest in the development of *Brassica* hexaploid species with genome composition of AABBCC. In *Brassica* species, *B. carinata* (genome, BBCC) and *B. rapa* (genome, AA) have been in cultivation for thousands of years (Gomez-Campo and Prakash, 1999) as important oilseed crops. These two species are characterized by their higher seed yield with high oil content with certain specific traits, such as self-

Table 1. Effect of colchicine in chromosome doubling using two methods in F_1 plants of different cross combinations.

Cross combinations	Number of plants treated and success using cotton plug method			Number of plants treated and success using modified injection method		
	Colchicine (%)			Colchicine (%)		
	0.10	0.15	0.20	0.10	0.15	0.20
Carinata-1 × Agrani	12 (25.0)	7 (57.1)	9 (55.6)	13 (30.8)	7 (57.1)	7 (71.4)
Reciprocal	8 (37.5)	7 (71.4)	10 (40.0)	11(36.4)	8 (62.5)	8 (75.0)
Carinata-1 × Safal	13 (15.4)	19 (15.8)	13 (30.8)	8 (25.0)	6 (50.0)	12 (50.0)
Reciprocal	18 (16.7)	12 (25.0)	9 (33.3)	13 (15.4)	8 (50.0)	9 (55.6)
Carinata-1 × BAU-M/91	9 (33.3)	9 (55.6)	10 (40.0)	9 (33.3)	10 (50.0)	9 (55.6)
Reciprocal	10 (30.0)	9 (44.4)	13 (38.5)	9 (22.2)	7 (42.9)	8 (62.5)
Carinata-1 × Sampad	13 (38.5)	15 (33.3)	7 (28.1)	11 (36.4)	11 (63.6)	7 (85.7)
Reciprocal	11 (36.4)	16 (37.5)	16 (25.0)	6 (33.3)	7 (57.1)	7 (71.4)
Carinata-1 × Binasarisha-6	10 (30.0)	11 (36.4)	9 (44.4)	7 (42.9)	10 (50.0)	8 (87.5)
Reciprocal	8 (25.0)	8 (50.0)	15 (40.0)	5 (40.0)	9 (44.4)	8 (75.0)
Carinata-1 ×BARI Sarisha-6	7 (28.6)	9 (44.4)	8 (62.5)	6 (50.0)	7 (71.4)	9 (77.8)
Reciprocal	8 (25.0)	7(42.9)	15(33.3)	6 (50.0)	8 (37.5)	8 (62.5)
Sub-total of *B. carinata* × *B. rapa* var. 'yellow sarson' and reciprocal	127 (27.6)	129 (38.8)	134 (38.1)	104 (32.7)	100 (52.0)	100 (68.0)
Carinata-1 × Tori-7	3 (33.3)	2 (50.0)	-	-	-	-
Reciprocal	-	4 (25.0)	-	-	-	-
Sub-total of *B. carinata* × *B. rapa* var. 'toria' and reciprocal	3 (33.3)	6 (33.3)	-	-	-	-
Carinata-1 × BARI Chinashak-1	7 (28.6)	9 (55.6)	9 (55.6)	7 (57.1)	6 (83.3)	8 (100)
Reciprocal	9 (33.3)	9 (44.4)	10 (50.0)	6 (50.0)	6 (66.7)	8 (100)
Sub-total of *B. carinata* × *B. rapa* spp. *pekinensis* and reciprocal	16 (31.2)	18 (50.0)	19 (52.6)	13 (53.8)	12 (75..0)	16 (100)
Carinata-1 × BARI Batishak-1	9 (22.2)	8 (50.0)	8 (25.0)	8 (50.0)	7 (71.4)	8 (62.5)
Reciprocal	7 (28.6)	8 (37.5)	8 (37.5)	7 (42.9)	6 (66.7)	7 (71.4)
Sub-total of *B. carinata* × *B. rapa* spp. *chinensis* and reciprocal	16 (25.0)	16 (43.8)	16 (31.2)	15 (46.7)	13 (69.2)	15 (66.7)
Grand Total	164 (27.4)	179 (38.0)	169 (39.1)	132 (36.4)	125 (56.0)	131 (71.8)

Note: Success has been shown in percentage within parenthesis.

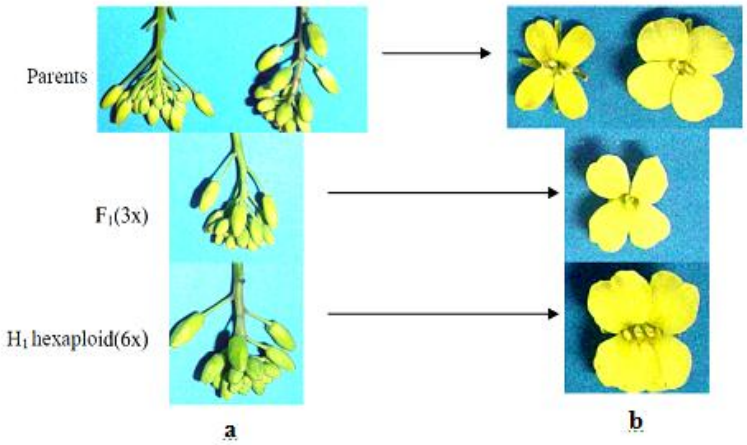

Fig 1. Inflorescence with flower buds (a) and flowers (b): smaller flower buds and flowers in triploid F_1 (3x) and larger flower buds and flowers in H_1 hexaploid (6x) from cross, BARI Chinashak-1 × Carinata-1.

incompatibility, early maturity and disease resistance in *B. rapa* (Ren et al., 2000), and resistance against drought and pod shattering and a better performance under saline and late sowing conditions in *B. carinata* (Getinet et al., 1996). So, synthesis of a new hexaploid *Brassica* species from *B. carinata* and *B. rapa* with genome AABBCC constitution may combine the traits of the parents to progenitors. These progenitors can provide a number of tolerances against biotic and abiotic stresses along with increased seed yield and higher oil content.

In the present study, we report here a potential approach to develop hexaploid *Brassica* (AABBCC) from trigenomic triploid hybrids (ABC) developed by crossing tetraploid *B. carinata* (BBCC) with diploid *B. rapa* (AA). In addition, characteristics of the H_1 and H_2 hexaploids in relation to fertility and important yield attributes are also reported.

Results

Colchicine treatment and development of H_1 hexaploids

In general, growth and development was strongly inhibited in the colchicine treated plants. Growth and development was started after three to four weeks of treatment but it was very slow even then. In cotton plug method, new shoots emerged from the colchicine treated leaf axils having thick and deep

Table 2. Mean and range of pollen fertility, siliqua setting and seeds per siliqua in H_1 hexaploid *Brassica* plants of different cross combinations.

Cross combinations	Pollen fertility (%)		Siliqua setting (%)		Seeds/siliqua (no.)	
	Mean	Range	Mean	Range	Mean	Range
Carinata-1 × Agrani	62	14.0-82.0	60	30.0-88.0	2.9	1.25-5.00
Reciprocal	57	11.5-86.4	58	26.0-92.0	3.0	1.20-5.33
Carinata-1 × Safal	54	13.5-85.0	51	19.0-84.0	2.2	0.80-5.33
Reciprocal	56	24.0-81.7	49	22.5-60.6	2.5	1.50-4.20
Carinata-1 × BAU-M/91	53	17.2-82.5	63	10.5- 87.0	3.2	0.90-5.25
Reciprocal	55	14.0-87.0	66	25.0-90.3	3.8	1.10-7.36
Carinata-1 × Sampad	43	14.0-61.0	43	22.0-63.0	2.4	0.80-4.50
Reciprocal	40	10.4-66.3	50	29.0-75.0	2.5	1.30-4.20
Carinata-1 × Binasarisha-6	50	12.0-78.5	55	19.8-83.0	2.1	0.90-3.60
Reciprocal	47	12.5-73.2	51	27.0-73.0	2.3	1.10-4.00
Carinata-1 × BARI Sarisha-6	49	23.2-75.7	50	16.7-76.0	2.2	0.70-4.10
Reciprocal	46	16.4-77.3	47	29.0-60.0	2.5	0.90-4.20
Carinata-1 × Tori-7	50	22.0-72.7	57	25.0-80.0	2.1	0.75-4.20
Reciprocal	48	47.6	44	44.0	3.4	3.40
Carinata-1 × BARI Chinashak-1	63	13.0-85.5	78	50.7-93.0	5.7	3.87-8.20
Reciprocal	64	21.0-80.3	69	31.0-90.4	5.4	3.64-7.40
Carinata-1 × BARI Batishak-1	62	12.5-86.4	73	41.5-92.0	4.8	3.12-6.5
Reciprocal	68	21.0-90.6	71	35.0-91.2	5.0	3.40-6.30

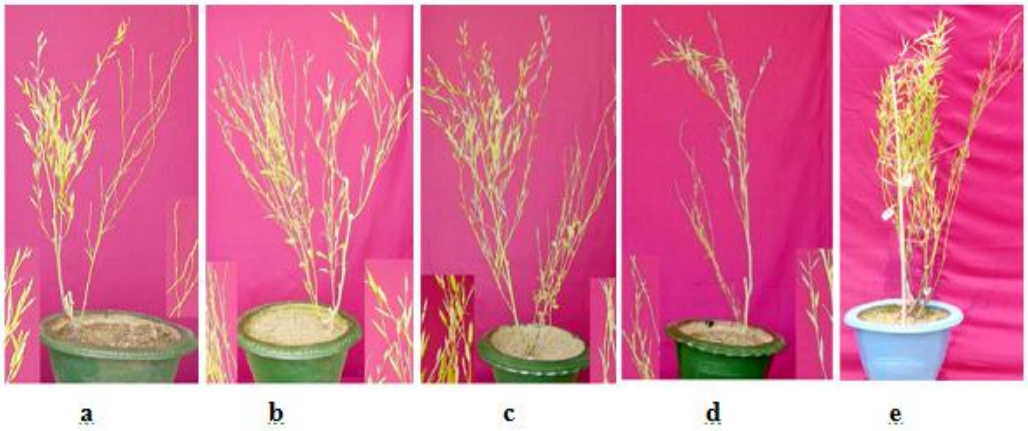

Fig 2. H_1 hexaploid shoots with siliquae and triploid shoots without siliquae developed from individual triploid hybrid (3x) of crosses, **a**: Carinata-1 × Agrani, **b**: Sampad × Carinata-1, **c**: Binasarisha-6 × Carinata-1, **d**: BARI Sarisha-6 × Carinata-1 and **e**: Carinata-1 × BARI Chinashak-1.

green leaves indicated the first symptom of induction of chromosome doubling i. e., development of H_1 hexaploid shoots from the trigenomic hybrids. In modified injection method, some of the treated racemes dried up. In some cases, all the flower buds were found to shed from the treated racemes, new racemes emerged and continued to develop, and produced hexaploid flowers.

Results on effectiveness of colchicine treatment to induce chromosome doubling in triploid hybrids of different cross combinations of both the methods have been presented in Table 1. When results of the two methods were compared, it was revealed that modified injection method was more effective than the cotton plug. In the modified injection method, maximum average chromosome doubling efficiency with 0.20% colchicine was 71.8% but with 0.10 and 0.15% colchicine, the efficiencies were 36.4 and 56.0%, respectively. In cotton plug method, on an average, 0.20% colchicine produced 39.1% success for chromosome doubling, which was closely followed by the application of 0.15% colchicine, with 38.0% success and only 27.4% success with 0.10% colchicines. Doubling of chromosome number in colchicine treated triploid hybrids was first confirmed by morphological appearances.

Performances of H_1 hexaploids compared to triploids

The chromosome doubled H_1 hexaploid shoots were more vigorous and produced larger flower buds, flowers compared to parents and triploid hybrids (Fig. 1). The H_1 hexaploid shoots produced full size anthers and filaments and; thus, produced fertile pollens. On the other hands, triploid hybrids produced shriveled, pale colour and pointed tip anthers having reduced filaments and produced sterile pollens. The H_1 hexaploid shoots developed from treated leaf axils triploid hybrids produced siliquae and seeds, while siliquae were not set in untreated triploid shoots (Fig. 2).

Percent pollen fertility and siliqua setting, and number of seeds per siliqua in the H_1 hexaploids varied within the same as well as different cross combinations (Table 2). Among the cross combinations, hexaploids of Carinata-1 × Agrani, Carinata-1 × Safal, Carinata-1 × BAU-M/91, Carinata-1 × BARI Chinashak-1, Carinata-1 × BARI Batishak-1 and all of their reciprocals had comparatively higher pollen fertility than other combinations. Hexaploids from Carinata-1 × Agrani showed pollen fertility range from 14.0 to 82.0% with an average of 62% while in its reciprocal, the range was 11.5-86.4% with the mean of 57%. Hexaploids from carinata-1 ×

Table 3. Somatic chromosome number in H_2 hexaploid seeds.

Hexaploids from different cross combinations	Root tips examined (no.)	Euploid seeds (no.) (2n = 54)	Aneuploid seeds (no.) (54 < 2n < 54)	Euploid (%)	Aneuploid (%)
Carinata-1 × Agrani	20	13	7	65	35
Reciprocal	20	12	8	60	40
Carinata-1 × BAU-M/91	18	10	8	56	44
Reciprocal	19	9	10	47	53
Carinata-1 × Tori-7	20	8	12	60	40
Reciprocal	22	10	12	45	55
Carinata-1 × BARI Chinashak-1	25	16	9	64	36
Reciprocal	20	14	6	70	30
Carinata-1 × BARI Batishak-1	25	17	8	68	32
Reciprocal	18	13	5	72	28
Total	207	122	85	59	41

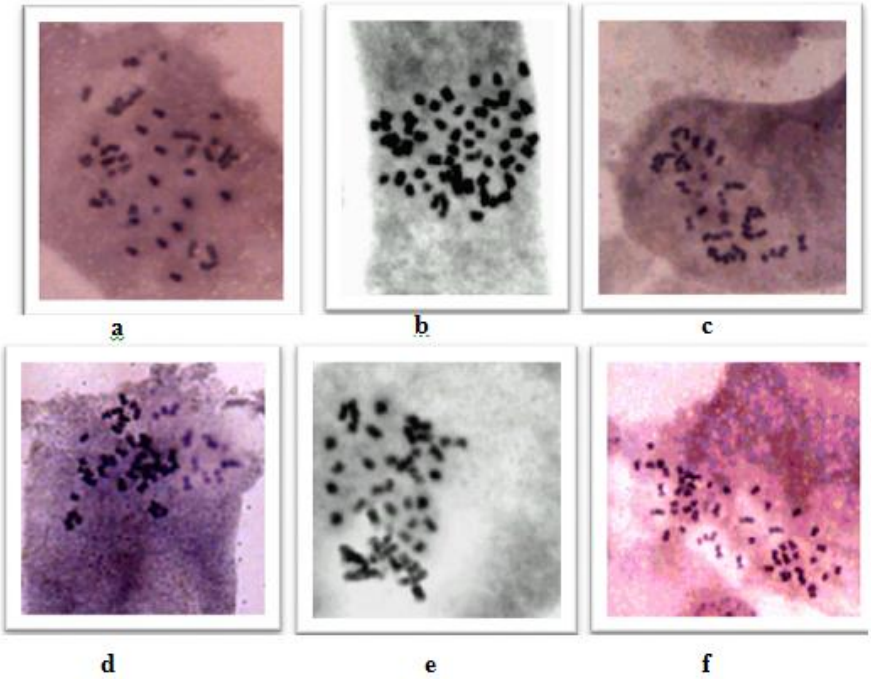

Fig 3. Variation in chromosome number in H_2 hexaploid seeds of cross Agrani × Carinata-1, **a**: euploid with 54 chromosomes and **b-f**: aneuploids with 45 to 57 chromosomes other than 54.

BARI Batishak-1 showed pollen fertility range of 12.5-86.4% with the mean of 62%, while hexaploids from its reciprocal had a range of 21.0-90.6% with the mean of 68%. Hexaploids obtained from the crosses, Carinata-1 × Agrani, Carinata-1 × BAU-M/91, Carinata-1 × BARI Chinashak-1, Carinata-1 × BARI Batishak-1 and all of their reciprocals produced comparatively higher percentages of siliqua setting, the values of which ranged from 25 to 93%. In respect to seed setting per siliqua, hexaploids from Carinata-1 × BARI Chinashak-1 and Carinata-1 × BARI Batishak-1 and reciprocals of both produced comparatively higher number of seeds per siliqua (3.12-8.20) than others.

Chromosome number in H_2 hexaploid seeds

The H_2 hexaploid seeds were examined cytologically for counting somatic chromosome number of five crosses with their reciprocals and the results are presented in Table 3. Results showed that on an average, 59% of seeds were euploid with 54 chromosomes as expected for hexaploids. The rest of 41% seeds were aneuploids, which had more or less number of chromosomes than the euploid and the numbers ranged from 45 to 57 (Fig. 3).

Performances of H_2 hexaploids

Performances of H_2 hexaploids from selected cross combinations having comparatively better performances on the basis of their yield attributes and seed yield/plant are presented in Table 4. Among the hexaploids, four lines produced significantly higher seed yield/plant (11.57-12.40 g) than the parental genotypes. Thus, these four H_2 lines are expected to be evaluated in the subsequent generations to select stable and desirable *Brassica* hexaploids.

Discussion

In the present study, inhibited growth and development in colchicine treated plant tissues in *Brassica* hybrids was in agreement with those of McNaughton (1973a) and Aslam et al. (1990). The results of higher success rate in modified injection method over cotton plug are in close agreement with the results of Currah and Ockendon (1987) in haploid

Table 4. Yield attributes and seed yield/plant of some selected high yielding H_2 hexaploid lines and their parents.

Hexaploid lines and parents	Siliquae/plant (no.)	Seeds/siliqua (no.)	1000-seed weight (g)	Seed yield/plant (g)
BBS1 × C1-1	275cd	11.00e	4.13cd	12.40a
BBS1 × C1-2	270de	10.80e	4.21c	12.11a
BBS1 × C1-3	263de	10.80e	4.15cd	11.85a
BBS1 × C1-4	271de	10.60e	4.06cd	11.57a
BBS1 × C1-5	246e	10.70e	3.98d	10.34b
C1 × BBS1-1	260de	10.70e	3.75e	10.40b
C1 × BBS1-2	258de	10.80e	3.68e	10.23b
AG × C1-1	368a	6.20g	4.53ab	10.30b
AG × C1-2	363a	6.30g	4.60a	10.17b
C1 × BCS1-1	302b	7.20f	4.20c	9.23cd
C1 × BCS1-2	296bc	7.00f	4.40b	9.16cd
Carinata-1	253de	14.70d	2.71f	9.93bc
BBS1	185f	26.00a	1.67g	8.13e
Agrani	98g	25.00b	3.71e	8.90de
BCS1	165f	22.20c	1.80g	6.52f
F test	**	**	**	**
SE (±)	8.20	0.21	0.06	0.31
CV (%)	5.50	2.83	2.73	6.14

In a column, figures having the same letter(s) do not differ significantly at $p \leq 0.05$ by DMRT; ** indicates significant at 1% level of probability.
Note: BBS1 × C1 = BARI Batishak-1× Carinata-1; C1 × BBS1 = Carinata-1 × BARI Batishak-1; AG × C1 = Agrani × Carinata-1; C1 × BCS1 = Carinata-1 × BARI Chinashak-1; BBS1 = BARI Batishak-1and BCS1 = BARI Chinashak-1.

brussels sprout. Different methods of colchicine application having different rates of success to induce chromosome doubling are also reported earlier and showed similarity with the present results (Aslam et al., 1990; Shi et al., 2002).

The results indicated that chromosome doubling rate varied with the concentrations of colchicine, which showed close agreement with the results of other researchers (Aslam et al., 1990; Mollers et al., 1994). It has also been established earlier that the effective concentration of colchicine may vary for different species and among different strains within the same species under various environmental conditions and it was also observed in case of treatment duration and stages of plant growth and development (Levin, 1983; Hague and Jones, 1987). In the present study differences were also observed in the developed hexaploids from the cross combinations.

In the present study, the bigger flowers were produced in *Brassica* hexaploids than their parents and respective F_1, which is in agreement with the findings of Meng et al. (1998), Li et al. (2004) and Tian et al. (2010). The increased growth, observed in the amphidiploids over their corresponding F_1s, was due to higher ploidy level in agreement with earlier results of Vyas et al. (1995), Chrungu et al. (1999) and Choudhary et al. (2000). Lower pollen fertility in the synthetic amphidiploids developed from the wide hybrids of Brassicaceae family through interspecific hybridization was reported by other researchers (Akbar, 1989; Song et al., 1993; Rahman, 2001; Pradhan et al., 2010; Tian et al., 2010). Lack of balance between nucleus and cytoplasm could result in sterility and account for variation in fertility in synthetic amphidiploids (Caspari, 1948; McNaughton, 1973b; Chrungu et al., 1999). Tian et al. (2010) reported that reduction in fertility of the hexaploid progeny might be resulted due to many factors. The most important of which might be irregular chromosome pairing followed by abnormal or unequal segregation. Formation of univalents or multivalents in the polyploid may have contributed to unequal segregation at anaphase-I of meiosis and consequently to a decrease in fertility (Qian et al., 2005; Tel-Zur et al., 2005). In the present study, the wide range of pollen fertility indicates that there were variable degrees of univalents to multivalent formation during the cell division.

Relatively high siliqua setting in the hexaploids are in agreement with the results of Tokumasu (1976), who reported 46.8-83.9% siliqua setting in synthetic *Brassicoraphanus*. Dolstra et al. (1982) found poor siliqua setting in *Raparadish* and concluded that it might be due to the genetic control. Lower number of seed setting in the hexaploid plants could be explained by lacking of balance between nucleus and cytoplasm or imbalance between parental genomes after fertilization causing endosperm abortion resulting in non-viable seeds along with viable seeds (Takeda and Takahata, 1988; Song et al., 1993; Tian et al., 2010).

In synthetic amphidiploids, simultaneous formation of aneuploids with euploids was reported as a common phenomenon by Song et al. (1993), Choudhary et al. (2000) and Tian et al. (2010). Aneuploid formation in the synthetic *Brassica* amphidiploids might be occurred due to the multivalent association at Diakinesis followed by Metaphase-I of meiosis (Sarla and Raut, 1988). This is mostly because there were no genes that preserve diploid chromosome behaviour in polyploids known as *Ph* in wheat (Riley and Chapman, 1958; Sears and Okamoto, 1958). Suppression of homologous pairing in *Brassica* was reported by Yang and Röbbelen (1994), while Busso et al. (1987) reported that there was no cytoplasmic factor for pairing regulation in *Brassica*. Affinity of allosyndetic pairing between A and C genomes was reported by Ahmad et al. (2002) and Tian et al. (2010), between B and C by Li et al. (2004) and Ge and Li (2007), and between A and B by Ahmad et al. (2002), Li et al. (2004) and Ge and Li (2007). Tian et al. (2010) reported that chromosomal instability of the artificially developed *Brassica* hexaploid progenies might be resulted from many factors. The most important of which might be irregular chromosome pairing followed by abnormal segregation.

Fertility improvement in subsequent generations in synthetic *Brassica* amphidiploids was reported in artificially produced *B. napus*, *B. juncea* and *B. carinata* by Akbar (1989) in synthetic *B. napus* (Song et al., 1993) in *Raphanofortii* (Choudhary et al., 2000) and by Momotaz et al. (1998) in allohexaploids developed from hybridization between *B. carinata* and two species of *Sinapis* (*S. arvensis* and *S. turgida*).

Materials and methods

Plant materials

Morphologically as well as cytologically confirmed trigenomic hybrids (ABC) were developed from crossing between *B. carinata* and *B. rapa* (Malek et al., 2006) and used as plant materials to induce chromosome doubling. The parental genotypes used in developing trigenomic hybrids were Carinata-1 of *B. carinata* and varieties viz., Agrani, Safal, BARI Sarisha-6, Binasarisha-6 and Sampad, and line BAU-M/91 of *B. rapa* var. yellow sarson, variety Tori-7 of *B. rapa* var. toria, variety BARI Batishak-1 of *B. rapa* spp. *chinensis* and variety BARI Chinashak-1 of *B. rapa* spp. *pekinensis*.

H_1 hexaploid induction with colchicine

The experiment was conducted at Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh during November to March 2003-04.

Two different methods *viz.* (a) Cotton plug and (b) Modified injection methods were used to induce chromosome doubling as follows. In each method, three different concentration of colchicine (0.10, 0.15 and 0.20 %) were applied.

Method 1: Cotton plug method: In this method, treatment was carried out on the F_1 plants when the plants were at 5 to 6 leaves growth stage. Firstly, the F_1 plants grown in pots were placed under shade. The apical shoots of each plant were then removed with a pair of sharp scissors. Then 2-3 leaf axils of the top position of each trigenomic hybrid were selected for colchicine treatment. A small cotton wool ball was placed on each of the selected leaf axils of each plant. The cotton plugs placed per leaf axil were soaked with colchicine solution of desired concentration at six hours intervals with 10 micro-litre solution each time with the help of a micro-pipette (modified version of Gland, 1981). The duration of treatment was 24 hours for all the concentrations. At the end of treatment, the plants were kept under shade for another 24 hours for proper action of colchicine.

Method 2: Modified injection method: In this method, when the flower buds in axillary racemes were emerging from the upper leaf axils, the main inflorescences of the hybrid plants were removed. Three to four racemes were kept from each selected hybrid plant, while the other racemes and side branches were removed from the plant. The upper surface near the base of the side raceme was scraped and wounded slightly with a needle. Then 10 micro-litres of aqueous solution of colchicine of desired concentration was applied to the wounded surface of each raceme with the help of a micro-pipette (modified version of Chen et al., 1988).

Any side shoots/racemes growing from the non-treated leaf axils were removed periodically since they tended to be unaffected by the treatment and assumed dominance over the treated leaf axils/side raceme (McNaughton, 1973a).

Pollen fertility study and cytological analysis

The pollen fertility was studied using 1% acetocarmine. Intensely stained and normal shaped pollen grains were scored as fertile, while the unstained and collapsed ones were scored as sterile. The ratio of stained pollen to the total was expressed as percentage of pollen fertility. Young root tips of the germinating second generation (H_2) hexaploid seeds were used to count the somatic chromosome number. Root tips were fixed in acetic alcohol (1:3) after pretreatment with saturated aqueous α-mono-bromo-napthalene solution for 2.5 hours followed by hydrolysis in 10% HCl for 12 minutes at 60° C and stained with 1% acetocarmine. The individual chromosome was counted through microscope at 100 times magnification and photographs of the chromosomes were taken from semi-permanent slides.

Growing of H_2 hexaploids with their parents and statistical analysis

Second generation (H_2) hexaploid seeds collected from H_1 hexaploid plants having higher percentages of pollen fertility and siliqua setting along with higher number of seeds/siliqua were used for growing H_2 hexaploid lines. Parental genotypes were also grown with H_2 hexaploid lines following randomized complete block design with three replications in the experimental field of BINA, Mymensingh, Bangladesh during November to March 2004-05. Different cultural practices including irrigation, application of pesticides were done properly as and when necessary for the normal growth of the plants of each plot. Data were taken with respect to number of siliquae plant/plant, number of seeds siliqua/siliqua, 1000-seed weight and seed yield plant/plant from 10 randomly selected plants from each plot.

Statistical analysis such as analysis of variance (ANOVA) was performed following Gomez and Gomez (1984) and the means were compared with Duncan's Multiple Range Test (DMRT) using the statistical computer package program MSTAT-C.

Conclusion

From the present study, a protocol has been developed for induction of H_1 hexaploid *Brassica* from triploid hybrids of crossing between *B. carinata* and *B. rapa*. Among the H_2 hexaploids, four lines with improved yield attributes and seed yield per plant have been selected for further improvement in the subsequent generations. These materials can be used as a breeding stock for crosses with other *Brassica* hexaploid population from the same or different sources. However, more research is needed to stabilize the synthesized hexaploids. On the basis of the results of both present and previous works, it could be concluded that the development of *Brassica* hexaploids for commercial cultivation is possible. This will not only widen the genetic base of the *Brassica* to fit under more stress conditions, but also help speciation with new chromosome numbers.

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