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Full Length Research Paper

Studies on some morphological characters and yield attributes of synthetic *Brassica* hexaploids and their parents

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Allopolyploidy plays an important role in plant evolution and confers better advantages on crop growth and breeding compared to low ploidy levels. The present investigation aimed to study the synthetic hexaploid *Brassica* (AABBCC genome) developed from *Brassica cainata* (BBCC genome) and *Brassica rapa* (AA genome) compared to their parental genotypes considering some morphological characters, yield attributes and yield. Increased vegetative growth in hexaploids over their parents was observed. Hexaploids exhibited wider ranges with higher coefficients of variations compared to the parents for all characters studied. Hexaploids had taller plants, higher number of siliqua and larger flowers than those of both parents. Though average pollen fertility in the hexaploids was lower than both parents but out of 420 hexaploids, seventeen had comparatively higher percentages of pollen fertility as well as siliqua setting. Number of seeds per siliqua and siliqua length were found to be lower than their parents. Mean seed weight per plant in the hexaploids was lower than the parents though the hexaploids had higher number of siliquae along with higher seed weight. Days to flowering and maturity in the hexaploids were in between the parents and closer to the *B. carinata* parent. Five hexaploid plants from BARIBatishak-1 × Carinata-1 and two from each of Carinata-1 × BARIBatishak-1 and Agrani × Carinata-1 were found to be promising because they have higher seed yield per plant than their parents. Those nine selected high yielding hexaploid plants could be evaluated in the subsequent generations to select the stable and desirable *Brassica* hexaploids.

Key words: *Brassica* hexaploid, morphological characters, yield attributes.

INTRODUCTION

Polyploidization is one of the major forces in plant evolution and speciation (Soltis and Soltis, 1995, 2000; Ramsey and Schemske, 1998; Bennett, 2004; Blanc and Wolfe, 2004; Adams and Wendel, 2005; Paterson, 2005; Leitch and Leitch, 2008; Gaeta and Chris, 2010). Generally, polyploids seem to be more tolerant to different biotic and abiotic stresses than diploid pro-genitors due to enhanced production of various secondary metabolites (Lewis, 1980; Levin, 1983). In nature, it has also been observed that polyploids have a more vigorous growth habit, greater dry matter production and wider adaptation than lower ploidy counterparts (Arnold, 1997). Common wheat is a fine example of hexaploid having wider adaptation, better quality and higher yielding capacity than tetraploid durum wheat (Gooding and Davies, 1997; Leitch and Leitch, 2008). The hexaploid triticale (AABBRR), a successful allopolyploid crop species was developed artificially (Ammar et al., 2004). This hexaploid triticale has wider adaptation with higher seed yield potential than its parents, tetraploid wheat

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and diploid rye (Guesdes-Pinto et al., 1996).

There is an increasing interest in the development of trigenomic *Brassica* hexaploid species with genome complement AABBCC. Among the different *Brassica* species, *Brassica carinata* (Genome, BBCC) and *Brassica rapa* (Genome, AA) have been cultivated for thousands of years (Gomez-Campo and Prakash, 1999) as important oil seed crops. These two species are characterized by their high yield of seeds with high oil content and by certain species traits, such as self-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 may combine the traits of the parental progenitors as well as can provide a number of biotic and abiotic stresses, increased yield in terms of seed production and higher oil content. *Brassica* hexaploids were therefore, developed from *B. carinata* and *B. rapa* (Malek, 2006, unpublished). We report here a comparison of synthetic hexaploid *Brassica* (AABBCC) with their parental genotypes considering some morphological characters and yield attributes.

MATERIALS AND METHODS

Synthetic *Brassica* hexaploids developed from the triploid *Brassica* hybrids (ABC) (Malek et al., 2006, unpublished) along with their parental genotypes were the plant materials. These hexaploids were developed from triploid hybrids (Genome, ABC) through the application of colchicine. Parental genotypes were Carinata-1 of *B. carinata* (Genome, BBCC), Agrani, Safal and Sampad and BAU-M/91 of *B. rapa* var. yellow sarson (Genome, AA), Tori-7 of *B. rapa* var. toria (Genome, AA), BARIBatishak-1 of *B. rapa* spp. *chinensis* (Genome, AA) and BARIChinashak-1 of *B. rapa* spp. *pekinensis* (Genome, AA).

Growing of plant materials

Brassica hexaploids and parental genotypes were grown following plant progeny rows with single replicate. The experiment was conducted in the experimental field of Bangladesh Institute of Nuclear Agriculture (BINA) (24°75″ N and 90°50″ E), Mymensingh, Bangladesh during Rabi season (November to March).

Pollen fertility study

For pollen fertility study, 1% acetocarmine was used. 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.

Data recording

Different morphological characters and yield attributes like plant height, number of primary branches per plant, siliqua and beak length, length and breadth of petal, length of main fruiting axis, siliqua density, siliqua angle with axis, pollen fertility (%), siliqua setting (%), number of siliquae per plant, number of viable and sterile seeds per siliqua, 100-seed weight, seed yield per plant, days to flowering and maturity, from randomly selected 30 hexaploid plants of each cross combination and 10 plants of each parental genotypes. Measurement of mean, range and coefficients of variation (CV%) of each character were calculated following the formulae suggested by Burton (1952).

RESULTS AND DISCUSSION

Performance of the hexaploids from Carinata-1 × BARIBatishak-1 and Carinata-1 × Agrani along with their parents considering some morphological characters, yield attributes and seed yield/plant are presented in Tables 1 and 2, respectively. It is mentionable that the observations from the crosses, Carinata-1 × BARIBatishak-1 and Carinata-1 × Agrani were also applicable to the other five crosses viz., Carinata-1 × Safal, Carinata-1 × BAU-M/91, Carinata-1 × Sampad, Carinata-1 × Tori-7 and Carinata-1 × BARIChinashak-1. So, data on the performance of hexaploids from these five crosses with their reciprocals and parents are not presented.

Morphological characters

Increased vegetative growth and development of hexaploid plants over their corresponding F_1s was observed (Figure 1A to B). This increased growth in the amphidiploids over their corresponding F_1 agreed to the earlier results (Chrungu et al., 1999; Choudhary et al., 2000). Color of stem and leaf of hexaploids was similar to that of *B. carinata* parent while leaf shape and dentition was similar to the *B. rapa* parents. Leaf size of hexaploid was observed larger than that respective triploid hybrid ((Figure 2A to D) and, flower buds and flowers of the hexaploids were larger than those of both parents (Figure 3A to D). Seed coat color of the hexaploids derived from the cross of reddish-black seeded Carinata-1 with four yellow seeded genotypes of *B. rapa* was similar to Carinata-1. Hexaploids derived from the cross between Carinata-1 and BARIBatishak-1 and their reciprocal, seed coat color varied from black to reddish black. This variation in seed coat color in hexaploids might be due to genic interaction (Meng et al., 1998). Colour of siliqua surface of hexaploids was similar to that of Carinata-1 (Figure 4B to C).

Hexaploids exhibited wider ranges with higher coefficients of variation (CV%) compared to the parents for all characters studied. Plant height of hexaploids was higher and had vigorous growth over the parents (Figure 4A). The present result agreed to the earlier result of Meng et al. (1998). Petal length and width, the indicator of flower size, were higher than the parents. McNaughton

Table 1. Performance of the hexaploids on some morphological characters, yield attributes and yield compared to their parents Carinata-1 and BARIBatishak-1 (BBS-1).

Characters		Carinata-1	Hexaploids (Carinata-1 × BBS-1)	Hexaploids (BBS-1 × Carinata-1)	BBS-1
Plant height (cm)	Mean	181	234	237	139
	Range	163 – 200	215 – 256	220 – 263	125 – 160
	CV(%)	6.2	5.3	5.5	8.2
Petal length (cm)	Mean	1.40	1.50	1.47	0.99
	Range	1.35 – 1.44	1.42 – 1.54	1.40 – 1.54	0.95– 1.05
	CV(%)	2.0	2.2	3.0	2.1
Petal width (cm)	Mean	0.79	0.92	0.93	0.58
	Range	0.74 – 0.81	0.85 – 1.00	0.88 – 1.00	0.53 – 0.66
	CV(%)	3.2	4.4	3.5	3.4
Pollen fertility (%)	Mean	90	78	79	90
	Range	87 – 93	65 – 90	67 – 92	82 – 94
	CV(%)	3.0	7.7	7.4	4.4
Siliqua setting (%)	Mean	96	90	91	89
	Range	91 – 99	75 – 98	77 – 98	82 – 93
	CV(%)	2.8	6.8	6.7	3.6
Primary branches/plant (no.)	Mean	11.7	7.7	7.5	5.2
	Range	9 – 14	6 – 9	6 – 9	4 – 7
	CV(%)	12.8	13.8	16.3	21.8
Length of main fruiting axis (cm)	Mean	26	54	59	61
	Range	22 – 30	44 – 62	50 – 70	49 – 70
	CV(%)	10	8.1	9.8	10.4
Siliqua density (per cm)	Mean	0.83	0.75	0.76	1.82
	Range	0.75 – 1.00	0.65 – 0.80	0.65 – 0.94	1.67 – 2.00
	CV(%)	10.5	6.3	8.7	8.3
Siliqua angle with axis	Mean	35	71	79	61
	Range	32 – 37	65 – 78	70 – 90	58 – 65
	CV(%)	4.8	5.6	5.4	4.2
Siliqua length (cm)	Mean	3.45	3.11	3.27	3.66
	Range	3.33 – 3.77	2.77 – 3.74	2.86 – 4.07	3.10 – 4.21
	CV(%)	4.8	7.6	10.0	6.8
Beak length (cm)	Mean	0.49	0.77	0.90	1.26
	Range	0.40 – 0.53	0.33 – 0.93	0.73 – 1.13	1.10 – 1.41
	CV(%)	9.0	10.0	11.6	9.3
Siliquae/plant (no.)	Mean	235	290	292	168
	Range	206 – 270	209 – 402	230-437	138 – 193
	CV(%)	9.1	20.5	20.1	10.7
Sterile seeds/siliqua (no.)	Mean	-	2.80	3.04	-
	Range	-	1.4 – 6.0	1.3 – 4.8	-
	CV(%)	-	42.6	31.0	-

Table 1. Continued.

Seeds/siliqua (no.)	Mean	14.7	7.9	7.7	26.0
	Range	12 – 17	4.6 – 10.8	4.7 – 11.0	23 – 32
	CV(%)	9.5	22.0	20.0	10.4
100-seed wt. (g)	Mean	0.267	0.338	0.363	0.162
	Range	0.253 – 0.278	0.295 – 0.393	0.300 – 0.429	0.153 – 0.172
	CV(%)	3.0	8.9	9.1	4.3
Seed yield/ plant (g)	Mean	8.66	6.67	6.60	7.07
	Range	7.36 – 10.10	3.84 – 10.40	4.15 – 12.40	5.81 – 8.13
	CV(%)	11.0	24.0	32.7	10.7
Days to flowering	Mean	63	62	62	47
	Range	-	57 – 63	55 – 62	-
	CV(%)	-	3.2	3.4	-
Days to maturity	Mean	133	128	129	105
	Range	-	125 – 130	126 – 130	-
	CV(%)	-	2.2	2.1	-

Table 2. Comparison of some morpho-physiological characters and seed yield and yield components of hexaploids with their parents, Carinata-1 and Agrani.

Characters		Carinata-1	Hexaploid (Carinata-1 × Agrani)	Hexaploid (Agrani × Carinata-1)	Agrani
Plant height (cm)	Mean	181	272	238	116
	Range	163 – 200	245 – 291	191 – 268	100 – 125
	CV(%)	6.3	5.9	8.6	6.8
Petal length (cm)	Mean	1.40	1.47	1.51	1.08
	Range	1.35 – 1.44	1.40 – 1.55	1.40 – 1.59	1.00 – 1.15
	CV(%)	2.0	3.1	3.0	2.1
Petal width (cm)	Mean	0.79	0.90	0.87	0.43
	Range	0.74 – 0.81	0.76 – 0.93	0.75 – 0.93	0.40 – 0.47
	CV(%)	3.2	4.0	5.6	3.3
Pollen fertility (%)	Mean	90	61	72	92
	Range	87 – 93	35 – 75	53 – 85	87 – 95
	CV(%)	3.0	16.0	12.0	3.5
Siliqua setting (%)	Mean	96	68	92	93
	Range	93 – 99	43 – 92	71 – 97	90 – 97
	CV(%)	2.8	25	6.4	2.7
Primary branches/plant (no.)	Mean	11.7	8.3	8.4	8.8
	Range	9 – 14	6 – 10	6 – 10	6 – 10
	CV(%)	12.8	17.2	17.8	15.8
Length of main fruiting axis (cm)	Mean	26	74	69	40

Table 2. Continued.

	Range	22 – 30	60 – 91	48 – 82	36 – 45
	CV(%)	10.0	14.3	12.1	7.7
Siliqua density (per cm)	Mean	0.83	0.80	0.93	0.92
	Range	0.75 – 1.00	0.60 –0.90	0.82–1.00	0.82–1.10
	CV(%)	10.5	11.5	6.7	8.3
Siliqua angle with axis	Mean	35	50	49	57
	Range	32 – 37	45 – 58	45 – 60	50 – 60
	CV(%)	4.8	8.2	9.6	5.8
Siliqua length (cm)	Mean	3.45	2.47	2.88	4.27
	Range	3.33 – 3.77	1.84 – 3.30	2.07 – 3.60	3.81 – 4.92
	CV(%)	4.8	18.3	10.2	6.3
Beak length (cm)	Mean	0.49	0.84	0.90	1.57
	Range	0.40 – 0.53	0.65 –1.13	0.67–1.10	1.31–1.75
	CV(%)	9.0	14.0	12.0	10.7
Siliquae/plant (no.)	Mean	235	371	437	110
	Range	206 – 270	278 – 465	218 – 608	84 – 120
	CV(%)	9.1	19.1	27.4	10.7
Sterile seeds/ siliqua (no.)	Mean	-	3.0	3.3	-
	Range	-	1.2– 4.6	2.1 – 5.0	-
	CV(%)	-	31.0	21.6	-
Seeds/siliqua (no.)	Mean	14.7	2.7	4.5	23.5
	Range	12 – 16	1.7 – 5.0	3.0 – 6.8	19 – 26
	CV(%)	8.4	36.1	25.0	9.2
100-seed wt. (g)	Mean	0.267	0.450	0.423	0.322
	Range	0.253 – 0.278	0.400 – 0.496	0.302 – 0.503	0.307 – 0.336
	CV(%)	3.0	8.44	11.3	2.8
Seed yield/plant (g)	Mean	8.66	4.50	6.65	7.65
	Range	7.36 – 10.10	2.10 – 6.60	3.50 – 10.30	6.22 – 8.90
	CV(%)	11.0	37.8	28.8	10.0
Days to flowering	Mean	63	47	45	37
	Range	-	43 – 50	42 – 48	-
	CV(%)	-	4.0	3.7	-
Days to maturity	Mean	133	118	114	97
	Range	-	114 – 123	112 – 118	-
	CV(%)	-	2.6	2.4	-

(1973) and Meng et al. (1998) also observed larger flowers in synthetic *Brassica* hexaploids than their parents. Average pollen fertility in hexaploids was found to be lower than the parents. But it was observed that some of the hexaploids from Carinata-1 × BARIBatishak-1 and Carinata-1 × BARIChinashak-1 and their reciprocals,

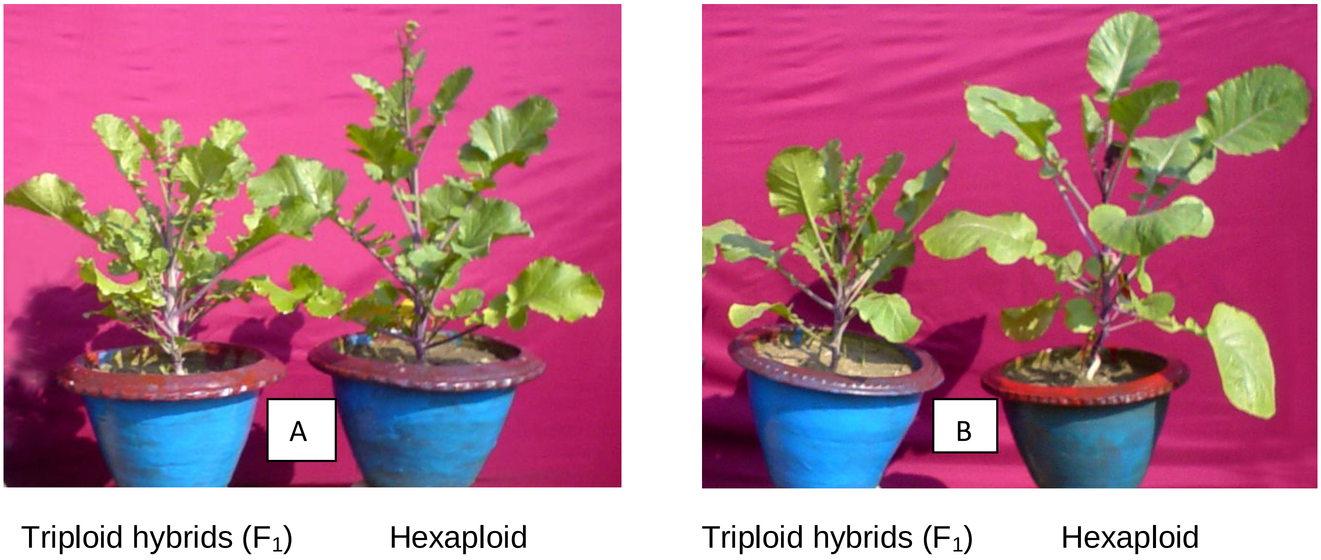

Figure 1. Hexaploid plant with vigorous vegetative growth over triploid hybrid (F1) of crosses, A: Agrani × Carinata-1 and B: BARIChinashak-1 × Carinata-1.

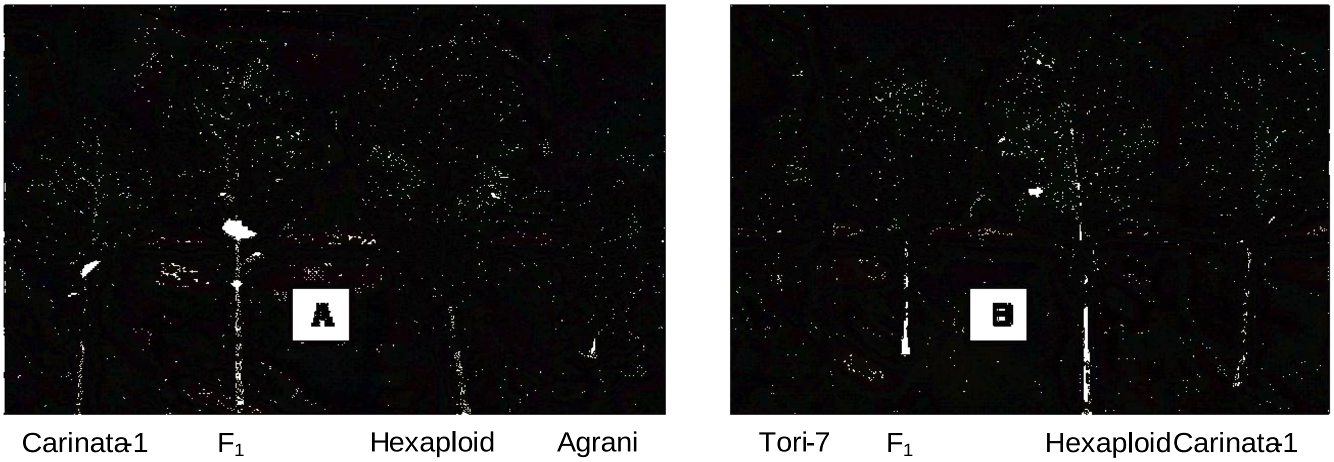

Figure 2. Leaves of parents, triploid hybrids (F_1) and hexaploid (6x), A: Carinata-1 × Agrani and B: Tori-7 × Carinata-1.

Agrani × Carinata-1 and BAU-M/91 × Carinata-1 had comparatively higher pollen fertility. Percent pollen fertility was reflected in siliqua setting that is, those C_2 plants had higher percentages of pollen fertility had also higher percentage of siliqua setting.

It was also observed that some hexaploids in all cross combinations had lower pollen fertility, which might be due to the presence of aneuploids with the hexaploids. The result shows similarity with the result of Sjödin and Glimelius (1989) in the population of synthetic *B. naponigra*. Intermediate number of primary branches per plant was found in the hexaploids from two crosses and their reciprocals (Carinata-1 × BARIBatishak-1 and Carinata-1 × BARIchinashak-1) while the rest had the lower number. Intermediate length of fruiting axis was observed in the hexaploids from Carinata-1 × BARIBatishak-1 and Carinata-1 × BARIChinashak-1 and their reciprocals and for the rest it was longer than both parents. In all hexaploids, it was observed that length of siliqua was shorter than both parents, but beak length was in between those of the parents.

Yield attributes and yield

The most important yield attribute that is, number of

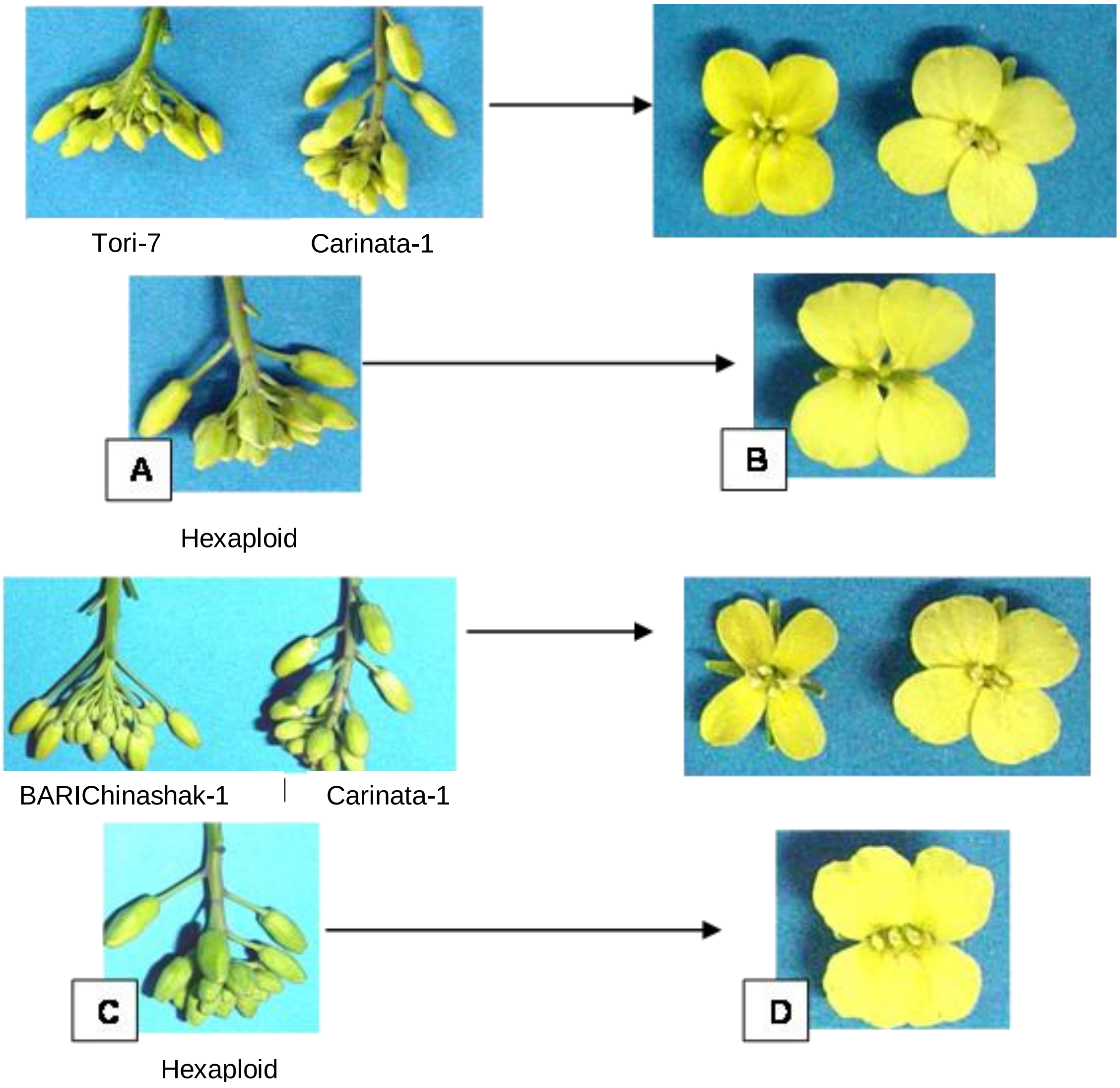

Figure 3. Inflorescence and flowers of parents and hexaploid of crosses A and B: Tori-7 × Carinata-1; C and D: BARIChinashak-1 × Carinata-1.

siliquae per plant was observed to be higher in number in hexaploids than both parents except hexaploids from Carinata-1 × Safal. Number of seeds per siliqua, another important yield attribute, was found to be lower number in all hexaploids than parents. Comparatively higher number of seeds per siliqua was observed in the hexaploids from Carinata-1 × BARIBatishak-1 and Carinata-1 × BARIChinashak-1 and their reciprocals and in Carinata-1 × BAU-M/91. The lower mean number of seeds per siliqua in the hexaploids compared to their parents might be due to the presence of sterile seeds in the siliqua. Mean weight of 100-seed in the hexaploids was higher than both parents. Higher 100-seed weight might be due to lower number of seeds per siliqua.

Mean seed yield per plant in the hexaploids was lower than the parents though mean number of siliquae per plant and mean 100-seed weight was higher than the parents. Some of the hexaploids from reciprocal of Carinata-1 × Agrani and, cross Carinata-1 × BARIchinashak-1, and Carinata-1 × BARIBatishak-1 with its reciprocal had higher seed yield than both parents. Lower mean seed yield per plant in the hexaploids was due to lower number of seeds per siliqua. In all hexaploids, days to flowering and days to maturity were in between the parents and closer to the *B. carinata* parent. Mean days to flower and maturity was observed to be the lowest that is, 39 and 111 days, respectively in the hexaploids from Tori-7 × Carinata-1. Hexaploids from BAU-M/91 × Carinata-1 took comparatively lower mean days to flower (43 days) and days to maturity (113 days) followed by Agrani × Carinata-1. But hexaploids from Carinata-1 × BARIBatishak-1 and its reciprocal, days to

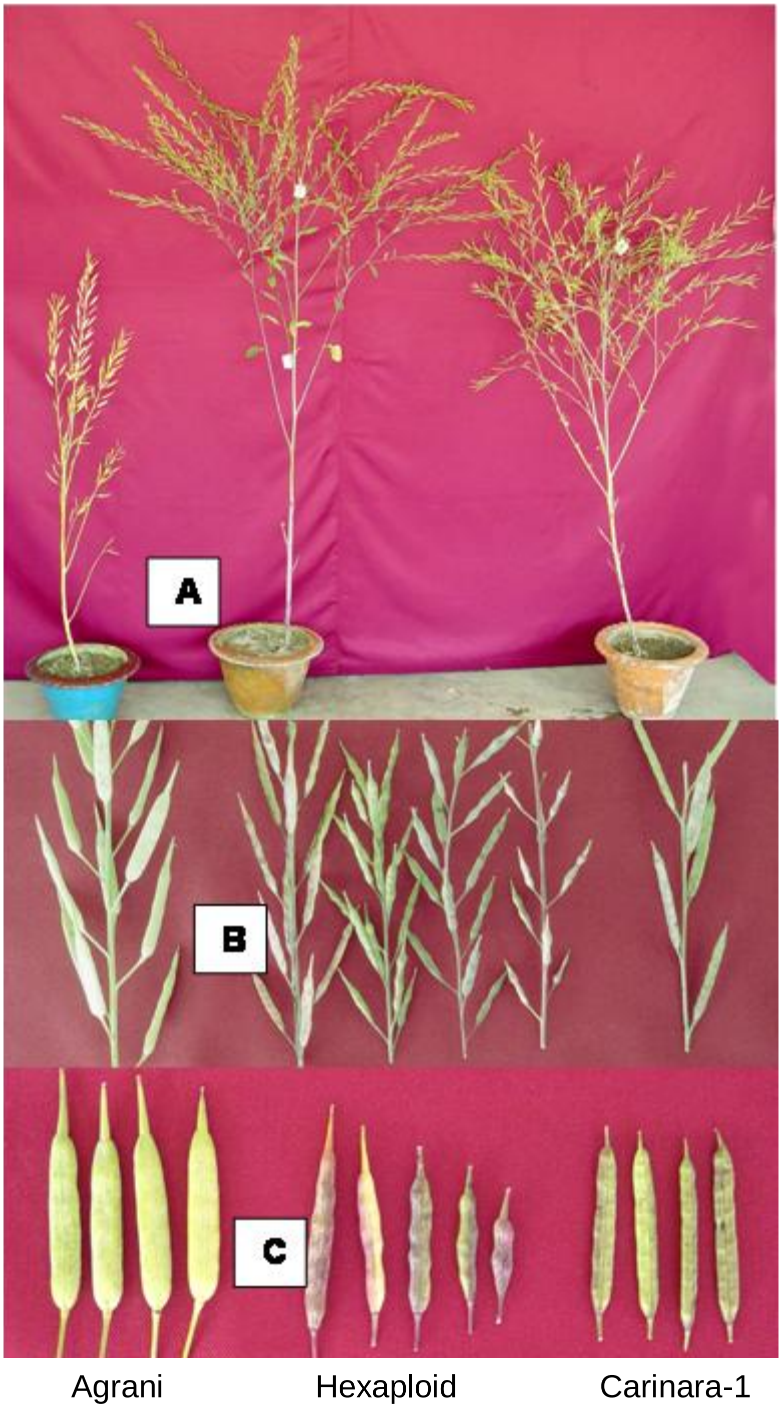

Figure 4. Hexaploid with parents of Agrani × Carinata-1, A: Hexaploid plant with parents, B: Siliqua with axis of hexaploid (H_2) and parents and C: Siliquae of hexaploid and parents.

flowering and maturity were near to Carinata-1 having longer days to flower (63 days) and maturity (133 days).

Among the hexaploids from all the cross combinations, five hexaploid plants from BARIBatishak-1 × Carinata-1 and two from each of Carinata-1 × BARIBatishak-1 and Agrani × Carinata-1 were found to be promising because of having higher seed yield per plant than their parents (Table 3).

Wider variation for most of the characters studied in the hexaploids from all cross combinations might be due to

Table 3. Yield attributes and seed yield per plant of selected nine hexaploids.

Hexaploid accessions	Siliqua/plant (no.)	Seeds/siliqua (no.)	100-seed weight (g)	Seed yield/plant (g)
BBS1×C1 -1	275	11.0	0.413	12.40
BBS1×C1 -2	270	10.8	0.421	12.11
BBS1×C1 -3	263	10.8	0.415	11.85
BBS1×C1 -4	271	10.6	0.406	11.57
BBS1×C1 -5	246	10.7	0.398	10.34
C1×BBS1-1	260	10.7	0.375	10.40
C1×BBS1-2	258	10.8	0.368	10.23
AG×C1-1	368	6.2	0.453	10.30
AG×C1-2	353	6.3	0.460	10.17

simultaneous presence of aneuploids and euploids. In synthetic amphidiploids, formation of aneuploid simultaneously with euploids was reported to be a common phenomenon (Sjödin and Glimelius, 1989; Song et al., 1993; Choudhary et al., 2000; Tian et al., 2010). Aneuploid formation in the synthetic *Brassica* amphidiploids might have occurred due to the multivalent association at diakinesis and metaphase-I of meiosis as reported earlier (Prakash and Hinata, 1980; Sarla and Raut, 1988; Chevre et al., 1994; Chrungu et al., 1999) as there was neither genes (*Ph*) that preserve diploid chromosome behaviour in polyploids are known in wheat (Riley and Chapman, 1958; Sears and Okamoto, 1958) for suppressing homoeologous pairing (Yang and Röbbelen, 1994) nor cytoplasmic factors for regulating pairing (Busso et al., 1987) in *Brassica*.

Differences between the hexaploids obtained from the cross and reciprocal cross for different characters might be due to maternal effect as the developed hexaploids obtained from the cross and reciprocal cross possessed different cytoplasm that is, hexaploids obtained from cross possessed cytoplasm of *B. carinata* and those obtained from reciprocal possessed cytoplasm of *B. rapa*. Lower number of seed setting in the hexaploids might be due to lower female fertility or lack of balance between nucleus and cytoplasm or between parental genomes causing endosperm abortion resulting non-viable seeds along with normal viable seeds (Tokumasu, 1976; Primard et al., 1988; Takeda and Takahata, 1988; Song et al., 1993; Tian et al., 2010). Sarla and Raut (1988) observed a wide range of variation in artificially developed 40 *B. carinata* plants for morphological and yield contributing characters and reported that those wide variations were due to variation in chromosome number that is, presence of aneuploids along with the euploids.

Failure during meiosis and gamete formation may be due to translocations between different genomes (Tel-Zur et al., 2005), which leads to multivalent pairing, and reduced pollen viability. Formation of univalents or multivalents in the polyploids may have contributed to unequal segregation at anaphase-I of meiosis and consequently to a decrease in fertility (Tel-Zur et al., 2005; Qian et al., 2005). Tian et al. (2010) reported that chromosomal instability of the artificially developed *Brassica* hexaploid progeny may result from many factors, the most important of which might be irregular chromosome pairing followed by abnormal segregation. The results of this study clearly showed that it needs further screening and evaluation in the subsequent generations for selection of desirable hexaploid *Brassica* genotypes from the nine selected high yielding hexaploid plants having improved yield attributes and seed yield.

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