Comparative alloplasmic effects of *Brassica napus* and *B. juncea* on seed characteristics of *B. carinata*

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Abstract

The alloplasmic effect of the cytoplasm of *Brassica napus* (2n=38 AACC) and *B. juncea* (2n=36 AABB) on the seed characteristics of *B. carinata* (2n=34 BBCC) was performed in this study. Alloplasmic lines of *B. carinata* were developed through *B. napus*×*B. carinata* and *B. juncea*×*B. carinata* hybridization followed by repeated backcrossing to *B. carinata* as a recurrent male parent up to the BC_5 generation. The BC_5 generations with the cytoplasm of *B. napus* and *B. juncea* have been designated as N-BC_5 and J-BC_5, respectively. Reciprocal crosses of *B. carinata*×N-BC_5 and *B. carinata*×J-BC_5 were made to produce four sets of BC_6F_1 populations. The silique setting rate, total silique number per plant, seed weight changement, seed dormancy and fatty acid were put into investigation. Plants with cytoplasm of *B. napus* had less dormancy, much higher seed weight, lower silique set percentage, lower silique number than the corresponding plants with cytoplasm of *B. carinata*. Plants with cytoplasm of *B. juncea* had higher seed weight, lower silique number than the corresponding plants with cytoplasm of *B. carinata*. The results suggest that cytoplasm is involved in the development of the seed, alien cytoplasm from farther *B. napus* showed a stronger disturbing effect on *B. carinata* than that of *B. juncea*. The results also suggest that cytoplasm is involved in the seed dormancy, alien cytoplasm from *B. napus* made the seed with less dormancy than the corresponding plants with cytoplasm of *B. carinata*. The alloplasmic effect on fatty acid was also discussed.

Key words: Alloplasmic effect, *Brassica* ssp., fatty acid, seed dormancy

Introduction

Nuclear substitution method was suggested and used in cytoplasm research by Kihara (1948) and later was used for the alloplasmic effect. Alloplasmic effect study started very early by Kihara and much research has been done on wheat and also some research has been done on vegetable.

Brassica is an major group of crops, which not only used as vegetable and oil but also as condiments. Interspecific or generic hybridization is common and many crosses were made to use the alloplasm to improve some morphological and physiological characteristics. The utilization of alien genes for the improvement of oilseed *Brassica* species, such as *B. napus*, *B. juncea* and *B. carinata*, has played an important role for many years. Various characteristics have already been mobilized from wild and cultivated relatives, including male sterility, disease and insect resistances and oil quality. The need to know more has prompted interested in studying alloplasmic genetic mechanism in growth and development. Seed dormancy is a complex phenomenon and is an important persistence mechanism for many annual weeds (Baskin & Baskin, 1998). Fatty acid is one major limiting factor for a wider usage of *Brassica* ssp. Until now no report has dealt with the alloplasmic effect of *B. napus* and *B. juncea* on *B. carinata.* The species *B. napus* and *B. juncea* are related to *B. carinata*. *B. napus* contains the genomes A and C, and *B. juncea* contains the genomes A and B, while *B. carinata* contains the genomes B and C (UN, 1935). In order to study alloplasmic effect of *B. napus* and *B. juncea* on *B. carinata*, alloplasmic lines of *B. carinata* was developed by substituting its nucleus into the cytoplasm of *B. napus* and *B. juncea*, respectively, using bud pollination. This paper reports the alloplasmic effect of *B. napus* and *B. juncea* on seed characteristics of *B. carinata* such as silique setting rate, total silique number per plant, seed weight changement, seed dormancy and fatty acid.

Materials and methods

Table 1 shows the materials and designations. Abyssinian mustard (*Brassica carinata* A. Br., BBCC: 2n=34), Chuseinatane (*B. napus* L. AACC: 2n=38) and Hakarasina (*B. juncea* (L.) Czern & Coss AABB: 2n=36) were used in the experiment. All the materials, except *B. carinata* was gotten from Kyushu University, Japan, were obtained by the plant breeding lab. of the faculty of agriculture, Ehime University, Japan. For clarity of discussion *B. carinata, B. napus and B. juncea* have been assigned the capital letters C, N and J, respectively. Alloplasmic lines of *B. carinata* were developed through *B. napus*×*B. carinata* and *B. juncea*×*B. carinata* hybridization followed by recurrent backcrossing to *B. carinata* as a recurrent male parent up to the BC_5 generation. The BC_5 generations with the cytoplasm of *B. napus* (N-cytoplasm) and the cytoplasm of *B. juncea* (J-cytoplasm) have been designated as N-BC_5 and J-BC_5, respectively. Reciprocal crosses were produced between the BC_5 generations and *B. carinata* and reciprocal crosses nc-6,cn-6, jc-6 and cj-6 were subjected to the investigation. The investigation methods are as following:

1. Seed germination: germination was carried under 25°C for ten days, seeds were taken from 35 to 105 days after bud-pollination, 10 days interval. 100 seeds per cross, 50 seeds per Petri dish using two layers of 90mm filter paper. Of the open

pollination seeds are preserved in harvested fruits without separation after freshly harvested, 7 days interval, 500 seeds per population, five times performed.

2. Seed weight: based on 750 seeds from 15 plants every ten days after flowering day from 25 to 85 days.

3. Fatty acid analysis according to the protocol of Momotaz et al (2000). For each accession, 10 single selfed seeds, sampled from three plants, were analyzed three times and the average data were put to statistical analysis. Fatty acid content was expressed as percent of total fatty acid content. Three sets of reciprocal crosses and three plants every cross were used.

4. A pair wise *t* test was used in the trial.

Table 1 Experiment materials and designations

Materials and lines	Generation	Experiment designations
Chuseinatane (*B. napus*L L.)		N
A byssinian mustand (*B. carinata* A. Br.)		C
Hakarasinan (*B. juncea* (L.) Czern & Coss)		J
(N×C)×C×C×C×C×C	BC_5	N-BC_5
(J×C)×C×C×C×C×C		J-BC_5
N-BC_5×C, J-BC_5×C	BC_6F_1	nc-6,jc-6
C×N-BC_5, C×J-BC_5		cn-6, cj-6

Results

1.Alloplasmic effect on silique setting rate, silique number, seed weight and seed dormancy

The silique set percentage had difference between J- (jc-6) and C-cytoplasm (cj-6) plants, it was 77.6% and 96.9***%, respectively (t=10.4, p=0.001), while it was 39.8% and 95.6***% (t=23.3, p=0.001) in the plants with N (nc-6) and C-cytoplasm (cn-6). The total silique number was 60 and 234*** (p=0.001) in the plants with N and C-cytoplasm, respectively, but no difference was found between plants with J (207/plant) and C-cytoplasm (261/plant). Seed weight was measured among the different cytoplasm plants outside of vinyl house. (Fig.1). At the beginning the seeds weight was higher, then the weight became lower and stable, the seed weight of N-cytoplasm was higher than that of C-cytoplasm. There was significant difference between them.

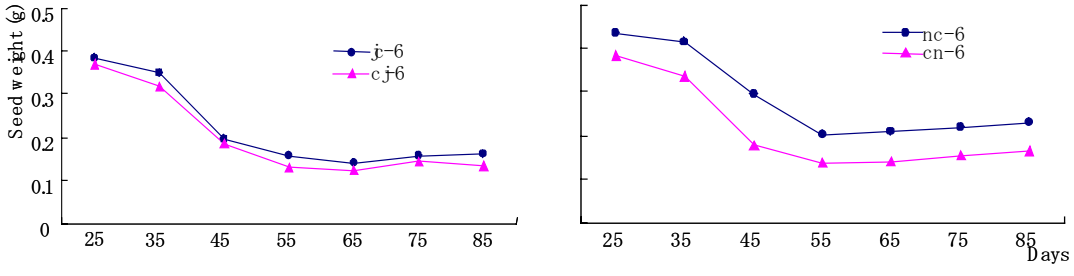

Fig. 1 The difference of seed weight changement between plants with J and C-cytoplasm or with N and C-cytoplasm. (The weight of 50 seeds)

There was no difference in the seed dormancy of seeds between J- and C-cytoplasm (Fig. 2). After pollination at 35days higher seed germination was found. But it went into dormancy quickly at 45days almost no germination was found, after 65days germination rise up gradually. Less dormancy was found in plants with N-cytoplasm compare with that of C-cytoplasm. It revealed that the dormancy was affected by nuclear and cytoplasm together. This also can be manifested in Table 2. After harvest the seeds of N-cytoplasm had less dormancy than that of C-cytoplasm and no difference was found between the plants with J-and C-cytoplasm.

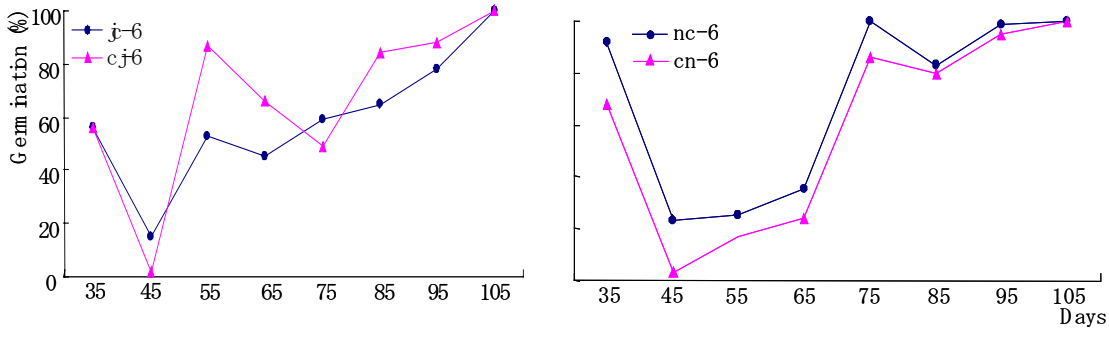

Fig. 2 The difference of seed germination between plants with J and C-cytoplasm or with N and C-cytoplasm

Table 2 The comparison of germination (%) between J-, N- and C-cytoplasm

lines	The days after harvest				
	7days	14days	21days	28days	35days
jc-6	75.4	46.6	78.3	86.2	92.6
cj-6	64.8	46.0	72.2	88.6	94.4
nc-6	69.0	57.2	88.8	88.7	94.3
cn-6	62.2	40.4*	71.8**	87.6	84.2**

2. Alloplasmic effect on the fatty acid

Fatty acid was analyzed among the plants with different cytoplasm using the reciprocal crossed and selfed seeds.

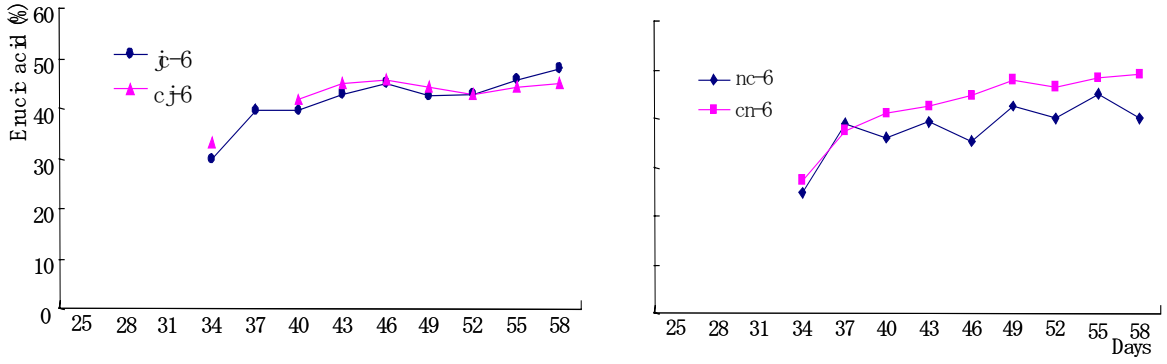

Fig. 3 The difference of erucic acid between plants with J and C-cytoplasm or with N and C-cytoplasm

Table 3 Mean±standard deviation of fatty acid composition(%) of alloplasmic and euplasmic lines

lines	C16:1	C18:1	C18:2	C18:3	C20:1	C22:1
jc-6	3.39±0.39	9.63±0.65	17.15±1.88	15.13±0.88	6.25±0.93	48.4±1.63
cj-6	2.97±0.32	9.50±1.54	17.78±2.21	14.22±1.02	6.35±0.53	49.17±2.35
nc-6	2.84±0.98	11.45±1.33	20.10±1.57***	13.90±0.69*	6.33±0.87	45.37±0.83***
cn-6	2.85±0.22	10.82±0.72	16.97±0.83	14.78±0.56	6.40±0.56	48.19±0.49

*,**,*** mean significant at p=0.05,0.01,0.001

The results showed that the seeds of N-cytoplasm with higher linoleic acid and lower erucic acid compared with that of C-cytoplasm (Fig. 3, Table3, some data can not be shown duo to limited space). No difference was found between the seeds with J and C-cytoplasm

Discussion

The significantly total siliqua and silique setting rate difference between N- and C-cytoplasm was due to the abnormal flower in the plants with N-cytoplasm, which we already reported in other paper. The difference between N- and C-cytoplasm was higher than what between J- and C-cytoplasm revealed than that N-cytoplasm affected the silique setting of substituted *B. carinata* too much than what J-cytoplasm did.

Seed size is one of the most obvious differences among various plant organs in *Brassica* species. Among diploid *Brassica* species in general, seed size ranks in reduced order from *B. oleracea* (2n=18, CC), *B. rapa* (2n=20, AA) and *B. nigra* (2n=16, BB) and seed sizes of the amphidiploid species *B. napus* (2n=38, AACC), *B. carinata* (2n=34, BBCC) and *B. juncea* (2n=36, AABB) are usually intermediate between their respective diploid progenitors. The difference of seed weight (Fig. 1) between plants with J and C-cytoplasm was lower than that between N and C-cytoplasm. The results confirmed that the seed weight was control not only by nuclear but also by cytoplasm. N-cytoplasm afftected the seed weight much more than what J-cytoplasm did, suggest that the B genome affect the seed of *B. carinata* too much than C genome did, *B. Juncea* had the B genome in the cytoplasm which consistent with the B genome in the nuclear of *B. carinata*, so keep the seed at the stable level, but the *B. napus* has no B genome in the cytoplasm instead of it was C genome, so the balance of *B. carinata* was disturbed and the effect of C genome was expressed. This is in agreement with the result of Song et al. (1988) and Liu Ai-hua et al (2006), that *B. nigra*, *B. juncea*, *B. carinata* belong to 'Nigra' lineage and *B. napus* belong to 'Rape' lineage.

From the results (Fig. 2) the seeds which harvested in immature state showed less dormancy, the dormancy became deeper as the maturity of seeds proceeded and over-mature seeds were again exhibited to be less dormant. N-cytoplasm affect the seeds to less dormancy.

B. carinata is used for oil in some area, it has several desirable agronomic characteristics compared to other *Brassica* crops such as resistant to drought, pod shattering, disease and pest but the limiting factors for a wider usage of this specie have been the naturally high levels of erucic acid in its seed. Fatty acids are formed by stepwise biosynthetic pathway in which oleic

acid either undergoes desaturation to form linoleic acid and then linolenic acid or there is further chain elongation to form eicosenoic acid and then erucic acid. Ecker R (1993) used the *Sinapis alba* L. as the material and confirmed that the composition of the fatty acids was controlled mainly by the nuclear genes of the embryo. Del Río et al (2003) used two *Brassica* carinata lines with low (about 10%) and zero erucic acid and obtained that no maternal or cytoplasmic effects for C22:1 content. From the results of this study, elucidated that the fatty acid path way was control not only by the nuclear but also the cytoplasm, the alien cytoplasm can affect the direction of pathway, the genetic farer one N-cytoplasm expressed the stronger effect than what genetic nearer J-cytoplasm did. Such variation could be accounted for different genotypes of materials involved in the research. In the trial motioned before the euplasmic and autoplasmic plants were used, whereas in this study, the alloplasmic plants were put into.

Conclusions

The comparative effect on seed dormancy and fatty acid was studied, the results offers the opportunity to breed low dormancy and low erucic acid varieties in *Brassica*. The further research should to do using the different fatty acid content and the difference genetic distance materials to confirm the present results. Alloplasmic breeding might be a potential way for shifting the fatty acid content frontiers below the level of current cultivar.

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