Journal of Horticultural Research 2013, vol. 21(2): 109-113
DOI: 10.2478/johr-2013-0029

THE INITIAL ANALYSIS OF THE ABILITY FOR THE GENERATIVE PROPAGATION AND THE LEVEL OF SELF-INCOMPATIBILITY OF INBRED LINES OF CHINESE CABBAGE (*BRASSICA RAPA* ssp. *PEKINENSIS*)

Research note

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Received: September 17, 2013; Accepted December 14, 2013

ABSTRACT

In the years of 2011 and 2013, seventeen inbred lines of Chinese cabbage were evaluated according to the ability for the generative propagation and the expression of self-incompatibility. Most of the lines pollinated in the greenhouse at the open flower stage were characterized by an intermediate level of self-incompatibility and formed less than one seed/silique; the other lines were highly self-incompatible or self-compatible. The lines pollinated at the green bud stage were highly diversified according to the ability for seed development in both years of propagation; however, most of the lines formed sufficient amount of seeds for use as parental components. The average effectiveness of generative propagation after pollination at the open flower and green bud stages was higher in 2011 than in 2013, probably due to different weather conditions in these years influencing the seed development or inbreeding depression.

Key words: Chinese cabbage, seed development, self-incompatibility, pollination, breeding

INTRODUCTION

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) in the last decades became one of the most popular vegetable crop for Polish horticulture and for consumers all over the year. Chinese cabbage in Poland is produced on the acreage of 5 to 7 thousands of hectares and depends entirely on the foreign F_1 cultivars. For this reason, in the Research Institute of Vegetable Crops (now the Research Institute of Horticulture), the new research program started in 2008. The main focus was put on searching for new and genetically diversified populations of Chinese cabbage containing desirable morphological traits, having higher level of resistance for the most important biotic and abiotic stresses, and suitable for breeding purposes. One of the most important problems connected with the production of F_1 hybrids of cabbage is adaptation of an effective, inexpensive and reliable system of generative propagation (Faulkner et al. 1977; Hodgkin 1980a, b; Ockendon 1973, 1975). For breeding purposes, inbred lines of Chinese cabbage should possess high level of self-incompatibility or male sterility, high seeding index, good combining ability and genetic stability in consecutive generations (Dickson 2007; Kalia 2009). Self-incompatibility system is still broadly used by many seed companies because of the reasonably higher yield of seeds in comparison with the male sterility system. However, the lower stability of self-incompatible lines may cause a problem with a contamination of selfed seeds in the F_1 generation (Nishio & Sato 2003). Chinese cabbage, as other species from genus *Brassica*, is characterized by sphorophytic, multiallelic inheritance of self-incompatibility (Thompson 1957). Self-incom-patibility in *Brassica* species is controlled by a single polymorphic S-locus. The three highly polymorphic S-locus genes – *SRK* (S receptor kinase) encodes a membrane-spanning serine/threonine kinase that

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determine the S-haplotype specificity of the stigma; *SLG* (S locus glycoprotein) encodes a form of specific stigma protein and *SP11* (S locus protein 11) encodes a small cysteine-rich protein that determines the S-haplotype of pollen (Takayama et al. 2000). Overcoming the expression of self-incompatibility for propagation of parental lines as components for producing F_1 hybrids requires in cabbage special techniques such as pollination at the green bud stage or treatment with NaCl or CO_2 (Ockendon 1973, 1975; Tingdong et al. 2006; Wang et al. 2010).

The aim of the study was the evaluation of a level of self-incompatibility and the possibility of overcoming self-incompatibility in seventeen Chinese cabbage inbred lines with a goal to select lines usable for production of F_1 seeds.

MATERIAL AND METHODS

Seventeen inbred Chinese cabbage lines with diversified morphological and agrobotanical characters were evaluated in the Department of Genetics, Breeding and Biotechnology of the Research Institute of Horticulture (formerly the Research Institute of Vegetable Crops) in Skierniewice, Poland, in 2011 and 2013. Each inbred line was represented by three or four plants selected in the field in a vegetative stage on the base of the head yield, uniformity, desired morphological traits, quality and resistance to the most common bacterial, fungal and physiological diseases such as black-spot (*Alternaria* spp.), bacterial soft-rot (*Pectobacterium carotovorum*, *Pseudomonas* spp.), black rot (*Xanthomonas campestris*), bacterial leaf spot (*Pseudomonas syringe* pv. *maculicola*) and tip-burn. Each line was propagated by lateral shots, rooted in the greenhouse and vernalized during winter season at the temperature of 10 °C from December to the end of February under natural photoperiod. During vernalization, plants were protected against pathogens and fertilized according to the requirements and recommendations for this species. In the beginning of March, the vernalized plants were transplanted into 5 litre-large plastic pots and placed at the ground in the greenhouse at 15-22 °C. Blooming of plants lasted from the first week of March until the end of May. To evaluate the ability of each line for the generative propagation 10-20 green buds per plant of a size 3-5 mm was self-pollinated. The evaluation of the self-incompatibility level was made by analysis of seed setting by self-pollinated 10-20 freshly opened flowers per plant. Each of the flower stacks assigned for the generative propagation was isolated with transparent film and paper. The harvest of matured seed stacks started 40-50 days after pollination and lasted from the second decade of May until beginning of July. Seeds were dried, extracted and cleaned, and the average number of seeds per silique and the total number of seeds per plant were counted separately for each line.

The level of self-compatibility was shown as the ability for seed setting by flowers self-pollinated at open flower stage. According to Wallace (1979a, b), self-compatibility is full when seed setting is at least six seeds per silique. Lines that are capable of forming an average of at least one seed per silique are defined as partially self-compatible and lines that formed an average of less than one seed per silique after pollination with own pollen at the open flower stage are defined as partially self-incompatible.

RESULTS AND DISCUSSION

Analysis of the ability for the generative propagation showed high level of diversity between seventeen Chinese cabbage lines evaluated in the greenhouse in the years 2011 and 2013. All plants were characterized by normally developed, fertile flowers without visible deformations of generative organs. The average number of seeds obtained after pollination at the open flower stage was higher in 2011 (48.62 seeds per 60 flowers of one plant) than in 2013 (6.04 seeds) (Table 1). In 2011, two lines (WS 330, MR 31) were characterized by relatively low ability for the generative propagation and developed less than 10 seeds per 60 flowers after pollination at open flower stage, while the highest number of seeds was obtained for CH 41 line (288.0) (Table 1). In 2013, only five lines developed more than 10 seeds per plant (BK 41, BK 336, 433-40, CH 41, Y 2/1), while four lines (MR 32, Y 297,

WS 330, WS 610) did not develop seeds after pollination at open flower stage (Table 1). In 2011, 14 lines pollinated at open flower stage formed less than one seed per silique and were characterized by partial self-incompatibility while three other lines (MR 32, MK 4/1 and CH 41) that developed 1.11 to 2.12 seeds per silique were partially self-compatible. In 2013, almost all lines showed high or partial self-incompatibility with the exception of BK 336 line that was partially self-compatible and developed 1.03 seeds per silique. The average self-incompatibility level for all Chinese cabbage lines were comparable in 2013 (0.30 seeds per silique) and 2011 (0.54 seeds per silique). Four lines (MR 31, RK 41, WS 330, WS 610) that showed relatively strong and stable level of self-incompatibility in both years of propagation could be used for breeding as maternal components for the experimental F_1 hybrids. The other lines that differed according to the level of self-incompatibility in both years are probably characterized by low level of intraline uniformity or they possess weak and/or unstable s-alleles (Ockendon 1973). The results are in accordance with those of the other researchers that described self-incompatibility trait for cabbage plants as relatively dependent on environmental effects such as temperature and humidity (Dickson 2007; Faulkner et al. 1977; Hodgkin 1980a, b). However, the lines with weak self-incompatibility alleles could be used as paternal components for the creation of the new cultivars and for the breeding programs with the use of cytoplasmic male sterility.

Table 1. Number of seeds produced by self-pollinated inbred lines of Chinese cabbage at the open flower stage. Shadowed cells represent lines of high level of self-incompatibility and the effective possibility for generative propagation

Line	Year 2011		Year 2013	
	Number of seeds from 60 flowers per plant	Number of seeds per silique	Number of seeds from 60 flowers per plant	Number of seeds per silique
BK 41	46.5	0.79	34.6	0.62
BK 336	57.0	0.45	13.5	1.03
433-40	20.0	0.34	11.4	0.56
433-38	65.0	0.47	1.0	0.15
SP 2/1	29.5	0.47	2.1	0.75
HT 4/1	11.0	0.31	0.3	0.02
MR 31	8.5	0.13	1.1	0.11
MR 32	58.5	1.11	0.0	0.00
CH 41	288.0	2.12	13.0	0.43
CH 42	42.0	0.69	5.0	0.36
RK 41	10.0	0.05	2.5	0.29
Y 2/1	20.0	0.24	15.0	0.62
Y 297	17.5	0.48	0.0	0.00
MK 4/1	82.5	1.19	2.5	0.14
WS 330	5.0	0.05	0.0	0.00
WS 610	30.0	0.22	0.0	0.00
HG 4	35.6	0.69	0.7	0.06
Average	**48.62**	**0.54**	**6.04**	**0.30**

Fifteen Chinese cabbage lines (with the exception of CH 42, and 433-38) were characterized by high ability for generative propagation when pollinated at the green bud stage in both years of experiment (Table 2). Self-pollinated lines had significantly higher average seed setting in 2011 (189.11 seeds per plant) than in 2013 (72.51 seeds per plant).

The number of seeds per plant after pollination at the green bud stage in 2011 ranged from 2.0 (CH 42) to 539.0 (433-40) and from 5.5 (433-38) to 129.0 (MR 32) in 2013. The average number of seeds per silique after pollination at the green bud stage was also diversified and ranged from 0.33 (CH 42) to 15.80 (MR 31) in 2011 and from 0.69 (MK 4/1) to 12.35 (Y 2/1) in 2013.

Obtained results showed that in both years of propagation, the average number of seeds per silique was significantly higher after self-pollination at the green bud stage than after self-pollination at open flower stage (Tables 1 & 2). Self-pollination of Chinese cabbage lines at the green bud stage effectively overcame the self-incompatibility, which enables the usage of most self-incompatible lines in a generative propagation (Tingdong et al. 2006; Thompson & Taylor 1966). Different ability for the generative propagation between two years could be explained by much less favourable weather conditions during blooming season in 2013 than in 2011. It was especially connected with the low temperatures (from -5 to 0 °C) and low level of insulation in March and April. Additionally, the lower ability for generative propagation in the year 2013 could be associated with a higher level of the inbreeding depression. In the next consecutive years, the further inbreeding by sib-pollination will be performed together with an evaluation of the ability to set seeds as well as evaluation of traits at the vegetative stage. The goal is to obtain stable, self-incompatible but able for generative propagation lines with desired morphological and agro-economical characters. Selected lines will be provided as parental components in the breeding of the modern F_1 hybrids/cultivars of Chinese cabbage.

Table 2. Number of seeds produced by self-pollinated inbred lines of Chinese cabbage at the green bud stage. Shadowed cells represent lines of high level of self-incompatibility and the effective possibility for generative propagation

Line	Year 2011		Year 2013	
	Number of seeds from 60 buds per plant	Number of seeds per silique	Number of seeds from 60 buds per plant	Number of seeds per silique
BK 41	108.0	2.10	85.6	4.08
BK 336	451.0	3.58	120.2	8.98
433-40	539.0	9.13	75.7	6.83
433-38	261.0	1.89	5.5	5.50
SP 2/1	294.5	4.17	94.4	7.63
HT 4/1	129.3	3.66	101.3	7.24
MR 31	121.0	15.80	51.7	4.60
MR 32	27.5	1.09	129.0	7.74
CH 41	115	0.84	61.2	4.94
CH 42	2.0	0.33	10.3	8.50
RK 41	246.0	1.23	42.5	6.30
Y 2/1	178.0	2.14	173	12.35
Y 297	79.0	2.16	62.0	3.34
MK 4/1	239.5	3.47	29.2	0.69
WS 330	121.0	1.21	99.0	1.80
WS 610	275.0	2.02	71.0	3.84
HG 4	55.0	1.07	21.0	2.42
Average	**189.11**	**3.26**	**72.51**	**5.69**

CONCLUSIONS

1. Chinese cabbage lines collected at the Research Institute of Horticulture are highly diversified according to the level of self-incompatibility and the ability for seed setting when self-pollinated at the green bud stage.
2. Overcoming the self-incompatibility is possible through self-pollination at the green bud stage.
3. Lines presenting both the stable high level of self-incompatibility and ability to set seeds in the result of self-pollination at the green bud stage could be candidates for experimental hybridization to find components for F_1 hybrids.

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