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ENVIRONMENTAL FACTORS AND GENOTYPIC VARIATION OF SELF-INCOMPATIBILITY IN *BRASSICA OLERACEA* L. VAR. *CAPITATA*

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Variation in the level of self-incompatibility (SI) was quantified in 14 Polish lines of cabbage (*Brassica oleracea* L. var. *capitata*) pollinated in various temperature/humidity conditions. Two methods were used to measure the expression of incompatibility: counts of seed set and counts of pollen tubes penetrating the styles 48 h after self-pollination. The level of self-incompatibility varied distinctly depending on the cultivar (from 0 to 65% seed-containing siliques). The genetic background of the material determined the influence of environmental factors on SI. Lower temperature (12°C) and higher humidity (90%) positively affected bud pollination, possibly due to a less stressful protocol. The observed great genotypic variation suggests it may be difficult to find a universal method suitable for breaking the self-incompatibility barrier for the species. The method may have to be tailored to the particular genotype.

Key words: Bud pollination, cabbage, humidity, seed production, self-incompatibility, temperature.

INTRODUCTION

Cabbage is one of the most economically important members of the *Brassica*. As in other Brassicaeae species, cabbage favors cross-fertilization over self-fertilization, by means of a sophisticated self-incompatibility (SI) system whereby stigma epidermal cells recognize and reject self-pollen. To breed hybrid varieties, however, self-incompatible inbred parent lines have to be selfed. To produce selfed progeny, green bud pollination, although laborious and time-consuming, is still the method most frequently used. The results of alternative methods such as electric aided pollination (Roggen et al., 1972), thermally aided pollination (Roggen and Van Dijk, 1976), CO_2 treatment (Nakanishi and Hinata, 1975; Taylor, 1982), salt treatment (Fu et al., 1992) or okadaic acid treatment (Scutt et al., 1993; Rundle et al., 1993) have not yet been confirmed across a wide genotype range. Reported successes have involved only single clones or lines. As a first step we estimated the levels of self-incompatibility (SI) in fourteen Polish lines of cabbage (*Brassica oleracea* L. var *capitata*), using two methods for quantifying SI: counting the seeds that developed to maturity, and counting the pollen tubes that penetrated the style 48 h after self-pollination. As it has been repeatedly demonstrated for *Brassica* species that the degree of SI is affected by environmental factors (Nakanishi and Hinata, 1975; Carter and McNeilly, 1975; Visser, 1977; Taylor, 1982), the effects of temperature and humidity on seed production were also studied.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

Lines of *Brassica oleracea* L. var. *capitata* (P 1116, P 703, P 601, 7023b, 7024b/8, 15425, 15428/14, 378/4, 378/8, 1344, 705, 263, K-48, 26/2) were obtained from "Polan" Horticulture, Plant Breeding

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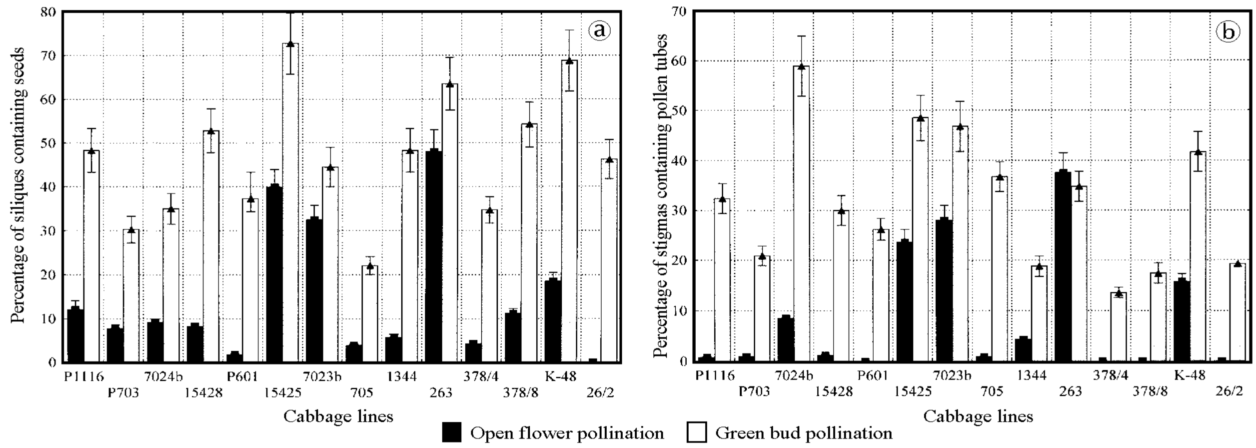

Fig. 1. Genotypic variation in the intensity of expressed self-incompatibility among 14 Polish cabbage lines. (**a**) Fruit set (percentage of seed-containing siliques), (**b**) Percentage of examined stigmas containing pollen tubes.

and Seed Production and examined in an experimental sequence lasting three years. The material was over-wintered in a frostproof glasshouse, and in mid April set into plastic pots and placed in a glasshouse at ~20˚C. About a week before flowering, the plants were distributed in different temperature or temperature/humidity treatments (temperature 12˚C or 23˚C, relative humidity 60% or 90%, and 16 h photoperiod). About a week after pollination, the plants were transferred to open-air conditions where they remained to harvest time.

POLLINATION PROCEDURE AND SELF-INCOMPATIBILITY ASSAY

Self-pollination was carried out during the main flowering period. Mature open flowers and green buds were pollinated by brushing the anthers of a fertile flower across the stigmatic surface of the flower. Samples of the styles were gathered 48 h after pollination (at least 10 flowers from 3 plants) and examined for pollen tube growth using fluorescence microscopy (Dyki, 1978) or left on the plants to set seed, and for the seeds to ripen.

Data are presented as percentages of styles containing pollen tubes and percentages of siliques containing seeds (fruit set).

RESULTS

Pollen tube counting as an assay for SI often does not strictly reflect the intensity of expressed compatibility (Fig. 1a,b), because it is affected by environmental factors that influence the pollen tube growth rate. That is why we chose seed production assay as our SI measure, even though it has the considerable disadvantage of requiring a duration of 60 days. There was strong genotypic variation in the SI level among the cabbage lines examined. Self-pollination on open flowers revealed 3 classes among the tested lines: almost total SI (0–5% seed-containing siliques), high SI (6–12%) and low SI (20–48%) (Fig. 1a). Green bud pollination increased the mean percentage of fertile siliques from 14.5 to 47, with the increase ranging from 15% to 50%.

TEMPERATURE EFFECT

The seed set count results (Fig. 2a,b) showed a strong temperature effect on seed production. In the case of open flower pollination, the effect of temperature was genotype-dependent: among 14 tested lines, 4 increased the percentage of fertile siliques per pollination (11% up to 44%), 3 lines were almost unaffected, and 7 decreased fertile silique production (5–65%). Analyses of green bud pollination data revealed that higher temperature halved the percentage of fertile siliques as compared with lower temperature. Such a disadvantageous effect was noted for almost all examined lines (Fig. 2b).

HUMIDITY EFFECT

The mean percentages of siliques containing seeds after self-pollination are presented versus treatment in Table 1 (open flower pollination) and

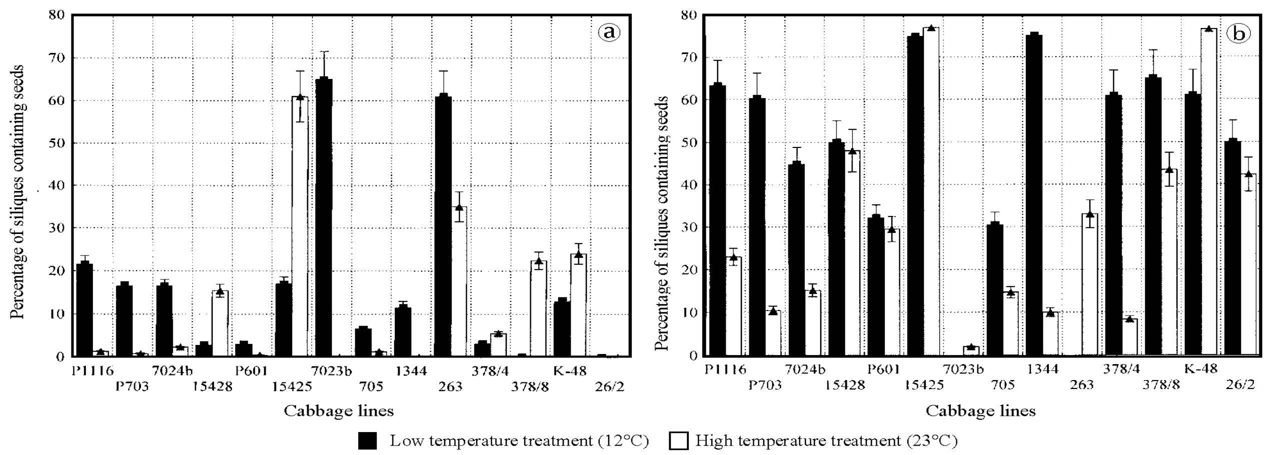

Fig. 2. Effect of temperature on self-incompatibility among 14 Polish cabbage lines. (**a**) Open flower pollination, (**b**) Green bud pollination.

Table 2 (green bud pollination). For both pollination techniques, the data revealed that the humidity effects on the frequency of seed production were less than the temperature effects. Moreover, strong genotypic variation was again observed. The results indicate that the best condition for open flower pollination for most lines was 12°C and 60% relative humidity. Only 2 of 9 lines increased their seed production under 90% relative humidity. The results on higher pollination temperature are not reliable, as most tested lines were highly self-incompatible even under elevated humidity. Two lines with low SI levels reacted the opposite way to elevated humidity (Tab. 1).

Similarly, after green bud pollination at the more favorable lower temperature (12°C), no clear effect of humidity was noted. A positive effect of elevated humidity on seed set was observed when pollination was done at 23°C (Tab. 2).

DISCUSSION

The large variation in the response of self-incompatibility to environmental factors between cabbage lines should be considered in studies on overcoming SI barriers. The strong genotypic effects and the interaction observed between different environmental factors indicate that there may be no single, universal method.

No general effect of temperature treatment was established in our cabbage population. Similar results were obtained by Nasrallah and Wallace (1968) and Visser (1973): cabbage clones with high SI remained self-incompatible across several temperature treatments. Plants with low SI produced about ten times more seed after a temperature change from 29°C to 15°C just before pollination. The same effect observed by Visser (1977) in brussels sprout clones suggested that the influence of temperature on SI is determined by the position of the S-allele in the dominance series, and by the genetic background of the material. The advantageous effect of lower temperature on green bud pollination may be attributable to more favorable conditions of pollination. The green bud pollination procedure exerts

TABLE 1. Fruit set ability (percentage of seed-containing siliques) after open flower pollination of 9 lines of *Brassica oleracea* var. *capitata* under various temperature/humidity conditions. Results are means of over 400 flowers per genotype. Mean variance does not exceed 10% of measured value

Line	Temperature			
	12°C		23°C	
	Relative humidity		Relative humidity	
	60%	90%	60%	90%
378/4	0	13	6	0
P1116	20	5	0	5
P703	14	15	0	1
705	9	2	1	1
263	82	48	3	66
P601	20	45	0	1
7023B	76	54	0	0
7024B	69	17	0	0
15425	50	21	80	53
Mean	38	27	10	14

TABLE 2. Fruit set ability (percentage of seed-containing siliques) after bud pollination of 9 lines of *Brassica oleracea* var. *capitata* under various temperature/humidity conditions. Results are means of over 550 buds per genotype. Mean variance does not exceed 10% of measured value

Line	Temperature			
	12˚C		23˚C	
	Relative humidity		Relative humidity	
	60%	90%	60%	90%
378/4	48	76	22	23
P1116	22	36	28	25
P703	73	100	9	11
705	90	39	49	62
263	99	84	30	41
P601	100	98	41	61
7023B	98	77	2	1
7024B	81	65	17	52
15425	91	78	64	82
Mean	87	81	29	40

mechanical and/or water stress, exposing the stigma to environmental factors 1–4 days before the buds should open naturally. Lower environmental temperature could be more favorable, limiting transpiration.

The same effect could be seen in the experiment on the effect of elevated humidity. The response seems to be under strong genetic control, with minor modifications exerted by other environmental factors. The advantageous effect of higher humidity on green bud pollination may have the same physiological background as suggested for temperature, limiting the stress caused by the pollination procedure.

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